Chemical Modification of Bark Tannins for Adhesive Formulation

by

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We accept this thesis as conforming
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ABSTRACT

A reaction is described that cleaves catechin (I) or conifer tannins (II) into catechol (III) and quinoline derivatives (IV and V). The reagent and conditions required for this reaction were 30% ammonium sulphite in concentrated ammonium hydroxide solution heated to 175°C for 1 to 3 hours. The optimization of these conditions is described along with a quick gas chromatography-based assay procedure for catechol. A mechanism for the reaction of catechin under these reaction conditions is proposed based on the structure of the end products, and the behavior of compounds related to the starting material. It was found that an understanding of the classic Bucherer reaction was necessary for elucidation of the cleavage reaction, so a discussion of the former is presented.

The production of catechol and other simple phenolics from tree bark tannins could be important in the utilization of the latter materials as adhesives in the forest product industry. A review of the literature on tannin-based adhesives is presented. Adhesive formulations based on the mixture of organic compounds produced from the cleavage of western hemlock (Tsuga heterophylla (Raf.) Sarg.) bark tannins were qualitatively and quantitatively evaluated for strength. Although early indications were promising, the quality of the bark used, and
its ability to produce catechol, degraded rapidly during storage. Adhesives made with bark stored for more than two months did not produce good bond strengths.
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ABBREVIATIONS

PF - Phenol-Formaldehyde
PRF - Phenol-Resorcinol-Formaldehyde
RF - Resorcinol-Formaldehyde
r.e. - resonance energy
HMWF - High Molecular Weight Fraction
LMWF - Low Molecular Weight Fraction
SS - Shear Strength
WF - Wood Failure
INTRODUCTION

We are living in a world with a growing dependance on man-made materials. Synthetic plastics are becoming increasingly important for clothing, automobiles, adhesives for building construction, etc., while the primary source of these plastics, petroleum, is becoming increasingly scarce. Recently, there has been a rebirth of interest in producing chemicals from renewable resources such as agricultural and forest residues. The oil crisis of 1973 was the primary cause of this. Government research-funding agencies have become aware that petroleum will inevitably be more expensive to produce and be in shorter supply as the world's easily accessible oil-fields dry up. Substitutes for energy and chemical products derived from petroleum will have to be found. In Canada, forest industry by-products such as lignin derivatives, foliage, and bark are obvious candidates to at least partially substitute for petroleum in energy and chemical feedstock applications. These materials can be obtained in large quantities and are a renewable resource. Fuel applications are straightforward compared to chemical applications of forest by-products. There have been elaborate schemes and processes envisaged for converting renewable organic materials into useful products or industrial chemicals, but very few applications are actually in practice today. Many factors
contribute to this state of affairs. Two important ones are the still relatively low cost of petroleum that makes competitive processes, based on renewable resources, uneconomical, and a fundamental lack of knowledge about the chemistry of the complex organic molecules that make up the biomass resource.

The work described here is concerned with the use of bark from an important local softwood, *Tsuga heterophylla* (Raff.) Sarg. (western hemlock), as a source of chemicals that could substitute for petroleum-derived phenol and resorcinol in adhesives used in the forest products industry. Western hemlock was chosen because the bark contains large amounts of tannin, a complex phenolic polymer. The chemical structure of tannin suggests that it could be used as a direct substitute for phenol or resorcinol in phenolic adhesives. This approach has worked with the tannin from some species. However, for various reasons that will be described later, softwood tannins (including western hemlock) have been successful in adhesive applications only when the bark material is used as an extender, rather than as a substitute, for phenol or resorcinol in these adhesives. Chemical modification of the softwood tannins appears to be necessary in order to use them without fortification with conventional resins.

The ultimate objective of the research presented in this thesis was to chemically modify western hemlock bark extract so it can be used commercially as an adhesive without having to add any synthetic resin as a fortifier. This was done by using a reaction that breaks up western hemlock bark tannin into
catechol and other products. In the elucidation of the mechanism of this reaction, much of the work was done with catechin, which has often been used as a simple model compound of the complex tannins. A reaction mechanism will be proposed based on the results of experiments using this, and other compounds, as models. Before the mechanistic investigations were done, the yield of catechol from catechin and western hemlock bark tannins was optimized by investigating the relationships between catechol yield and reaction temperature and time. In this way, the yield of the co-products of catechol from catechin were also maximized, allowing easier isolation of these compounds in reasonable quantities and facilitating their identification. Finally, experiments were done to evaluate the adhesive properties of the product mixture obtained from western hemlock bark tannins using this cleavage reaction.
CHAPTER I. LITERATURE REVIEW

I-A. The Nature of Western Hemlock Bark

Before a complex organic material like western hemlock bark can be utilized efficiently in any application, an understanding of its physical and chemical nature must be developed. This is the purpose of this section of the literature review.

(1) Anatomy

The mature bark of a coniferous tree has two distinct parts. Adjacent to the vascular cambium is a layer of inner living bark often called the functional phloem. This tissue performs the physiological function of carrying nutrients and hormones from the leaves to the growing xylem or immature wood cells on the inside of the vascular cambium. The outer layer of bark, or rhytidome, is composed of dead phloem cells which have no conduction function, but which help to protect the living cells of the tree from mechanical damage. Also included in the outer bark is at least one layer of tissue called the periderm (1,2) (see Figure I-1).

A tree grows in diameter by the vascular cambium dividing periclinally, producing cells to the inside which grow and differentiate into the various xylem cells. As the diameter of the xylem increases, the cambium must grow in circumference by
occasional oblique anticlinal cell division. The rates of periclinal and anticlinal cambium cell division are balanced, so the diameter growth of the xylem is in harmony with the circumferential growth of the cambium. However, the mature phloem which was originally formed by periclinal division of the cambium to the outside, has no mechanism for circumferential growth. As the stem grows, the outer bark cracks and sloughs off. In order to protect the live phloem and cambium from exposure that would lead to bacterial or fungal infection and desiccation, the periderm forms within the living bark.
The periderm is composed of three layers. The first tissue to form is the phellogen (cork cambium), which is a single layer of cells originating from phloem parenchyma cells. In a similar manner to the vascular cambium, the phellogen cells divide periclinally to form a thin layer of phelloderm to the inside (usually) and a thicker layer of phellem to the outside (always). The phellem or cork cells have no pits in their cell walls and have a high wax content. It is the cork which is the important barrier protecting the live phloem, cambium and xylem cells. When a periderm forms, the phloem cells to the outside die and become part of the outer bark or rhytidome. Several periderms may be contained in the rhytidome.

Western hemlock bark has all the features described above. Table 1-1 contains data on some important physical characteristics of western hemlock bark (3). Data from two other species are given for comparative purposes. For the relatively small western hemlock trees examined, the authors found an average total bark thicknesses of 0.252 inches. Larger trees have bark thicknesses of up to about 1 inch (4). Other information on physical properties of hemlock bark has been published (5,6). In general, the rhytidome of western hemlock is a dark reddish-brown colour with scales formed from successive periderms two to three millimeters in thickness. The cork layer is quite thin, about 20 cells in radial thickness. The inner bark is a light yellow colour when fresh, but rapidly oxidizes to red after exposure (4).

Phloem anatomy is important in chemical applications,
<table>
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<th>Species</th>
<th>D.B.H.(in.)</th>
<th>Height(ft.)</th>
<th>Bark Thickness(in.)</th>
<th>Moisture Content (% O.D.)</th>
<th>Specific Gravity (O.D. Wgt./Green Vol.)</th>
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<td></td>
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<td>8</td>
<td>47</td>
<td>0.129</td>
<td>0.071</td>
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(Average of data from samples taken at 1 and 4.5 foot heights)

Table I-1. Some Average Data of the Physical Characteristics of the Bark of Three Important B.C. Conifers (3)
because it has an influence on the chemical composition of the bark. For example, Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco.) has very thick phellem layers. Associated with the cork, are correspondingly large amounts of waxes that have been commercially extracted from this species (7). For western hemlock, the average molecular weight of the bark tannins could be different in the inner and outer bark zones. The relative proportions of the two zones in a given sample of bark may affect the suitability of the extracted polyphenolics for chemical applications.

(2) Chemical Composition

The substances that make up hemlock bark are a complex mixture. Cellulose, hemicellulose and lignin, the important constituents of wood, are also found in bark, but while they account for about 95% of the woody material, in bark they make up only about 50%. The rest of the bark material is primarily polyflavanoids. Table 1-2 shows the chemical analysis of air-dried, fresh hemlock bark as reported by Herrick (8).

There has been little work done specifically on the characteristics of lignin and cellulose from western hemlock bark. Research on other conifer species indicates that the differences between conifer bark cellulose and lignin, and those materials found in wood are relatively minor (10,11,12,13). For example, holocellulose isolated from bark often contains a
<table>
<thead>
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<th>Fraction</th>
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<tr>
<td>Cellulose</td>
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<tr>
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</tr>
<tr>
<td>Lignin</td>
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</tr>
<tr>
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<tr>
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<td>Tannin (water soluble)</td>
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<tr>
<td>Phlobaphenes</td>
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<tr>
<td>Carbohydrates (water soluble)</td>
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Table I-2. Chemical Analysis of Western Hemlock Bark (8)

significantly higher proportion of mannose (12,14), and some studies indicate the lignin of conifer barks may be more highly crosslinked than wood lignin (15,16).

Research on the chemical composition of western hemlock bark has focused on the polyphenolic portions. In general, these compounds have a structure based on the flavanoid carbon skeleton. Figure I-2 shows the ring numbering system and ring designations of the flavanoids. In the past, solubility in various solvents was used as the classification method for bark extractions. Simple flavanoids were soluble in diethyl ether, tannins were the polyphenol portion that dissolved in water and alcohol, phlobaphenes were soluble in ethanol but insoluble in water, and the phenolic acids were extractable with dilute alkali but insoluble in neutral solvents. It is apparent now, that the last three compound groups are very closely related and probably differ only in degree of polymerization or level of oxidation in the case of the phlobaphenes.
The structures of some simple flavanoids and related compounds that have been isolated from western hemlock bark are shown in Figure I-3. The ether soluble, red-purple coloring matter associated with the flavanoids has not been well characterized. Alkali fusion of this material in a eutectic mixture of potassium and sodium hydroxide gives protocatechuic acid, catechol and phloroglucinol (17), the same products obtained by fusion of tannin. Hergert has suggested that the material is probably a polymer of cyanidin (Figure I-4). His conclusions were based on the UV and IR spectra of this material and its behavior in chromatographic systems (9,19).

Structural elucidation of the major polyflavanoid components has been done using some specialized techniques. Alkali fusion, mentioned earlier, provides information on the hydroxylation patterns of the A- and B-rings found in the monomer moieties that make up the flavanoid polymer (18). Analysis of anthocyanidins, which are formed by degradation of the polyflavanoid with mineral acids in boiling alcohol
Figure 1-3. Structure of Some Flavanoids and Related Compounds Isolated from Western Hemlock Bark (9,17)
solution, can give information on the nature of the monomer, as can thioglycolysis (20). Both these techniques cleave interflavanoid bonds and give products that have structures related to the monomeric units of the polyflavanoid being degraded. As mentioned above, alkali fusion of western hemlock tannin yielded mainly phloroglucinol, catechol and protocatechuic acid, as well as small amounts of para-hydroxybenzoic acid (17). Degradation with hydrochloric acid in boiling isopropanol gave cyanidin as the main product, along with small amounts of a second compound tentatively identified as pelargonidin (9) (Figure I-5). The degradation of hemlock tannin by HSCH$_2$COOH (thioglycolysis) was studied by Sears and Casebier (21). Methylated tannin was degraded by 60% thioglycolic acid at 140° C to give two main products in approximately equal quantities (10% total yield) after further methylation. These were identified as being 2,3-cis and 2,3-trans methyl (3-hydroxy-5,7,3',4'-tetramethoxyflavan-4-yl-thio)
Figure I-5. Structures of Cyanidin and Pelargonidin

acetates (Figure I-6). This information indicates that both catechin and epicatechin moieties are present in the tannin polymer. Evidence for this monomer mixture was reported earlier by Hergert (17), who found that mild acid hydrolysis of hemlock tannin gave traces of catechin and epicatechin. The type of structure for hemlock tannin suggested by this work, is a polymer of catechin and epicatechin moieties joined by C(4)-C(8) interflavanoid bonds as shown in Figure I-7.

Molecular weight studies on acetylated tannin indicated that the number average molecular weight is 1500-2000 (4-5 flavanoid units per tannin molecule) (17). However, this representation may be somewhat simplistic. Hemingway (20) has
Figure I-6. Thioglycolysis Products of Western Hemlock Tannin

pointed out that thioglycolysis yields from polyflavanoids containing only C(4)-C(8) bonds should be almost quantitative (22). Since hemlock tannin thioglycolysis has a much lower yield, the molecule may well contain structural features not yet defined.

Recent work has suggested that there may be two types of tannins in plants; linear flavanoid polymers with mainly C(4)-C(8) interflavanoid bonds and more globular, possibly branched, polyflavanoids where a large proportion of the interflavanoid bonds are C(4)-C(6). Haslam and his group (22) have found that the dimeric procyanidins from Salix caprea L., Crataegus monogyna Jacq. and other species, had a ratio of C(4)-C(8)
Figure I-7. Structure of Western Hemlock Bark Tannin

interflavanoid bonds to C(4)-C(6) bonds of 8 or 9 to 1, while the higher molecular weight polyflavanoids have almost exclusively C(4)-C(8) bonds (23,24). Conformational studies have indicated that polymers of this type are linear, thread-like, structures with the catechol B-rings on the outside of the molecule taking a helical arrangement (22,24).

Hemingway et al. (25), working with polyflavanoids of southern pine species, have found that the tannins from these species appear to contain a much higher proportion of C(4)-C(6) bonds and in their native state probably have globular, branched structures. Their conclusions were based on the structure of isolated di- and trimeric flavanoids and partial thiolytic
degradation of tannins (26,27,28). The presence of branch points in pine tannins has not yet been proven directly, although thiolytic cleavage rates have provided indirect evidence for branching. Detailed work of this kind has not been done with western hemlock tannins, but the thioglycolysis data outlined above do show similarities to the behavior of pine tannins under these conditions. It is likely that the structure shown in Figure I-7 is too simplistic. There is probably a relatively high proportion of C(4)-C(6) bonds to the C(4)-C(8) bonds shown and possibly some branching through these positions.

Alkali fusion of the phlobaphene fraction of western hemlock bark gave essentially the same products as degradation of the tannin (17), while thioglycolysis did not yield any recognizable thioethers (20). This evidence suggests that the phlobaphenes are structurally similar to tannin but probably have the C-ring in a higher oxidation state (29). The phlobaphenes could be a higher molecular weight fraction of the polycyanidins extractable by ether, as described above, or a polyquinone structure as suggested by Herrick and Hergert (30) (Figure I-8).

The phenolic acids were originally named because of the presence of phenolic groups and a carbonyl functionality that was assumed to be due to a carboxylic acid (29). The carbonyl functionality could also be induced in water soluble tannins by treatment with base (31). The properties of the alkali soluble fraction are definitely modified by the extraction process. After isolation, most or all of the phenolic acid material is
soluble in ethanol (29,32). Thioglycolysis of hemlock bark extracted with neutral solvents yields the same products as thioglycolysis of tannin, but with a higher proportion of the epicatechin derivative (21). This information leads to the conclusion that, before extraction, the phenolic acid fraction has essentially the same structure as tannin but is not soluble in neutral solvents due to a higher molecular weight or perhaps more extensive crosslinking (17).

Some insight into the alkali induced rearrangement of tannin was provided by the work of Sears et al. (33) using catechin as a model compound. It was found that catechin undergoes a rearrangement reaction in refluxing dilute sodium hydroxide to give a compound containing an acidic enol functionality (catechinic acid, Figure I-9). Presumably, a similar arrangement occurs in the high molecular weight tannin fraction during alkali extraction.

In summary, the flavanoids found in western hemlock bark
are based on the standard flavanoid carbon skeleton. They range in size from the simple monomeric constituents such as catechin to the larger polymers of undetermined size contained in the phenolic acid fraction. Bark polyphenols can be divided into two basic types depending on the oxidation state of the C-ring in the monomeric unit. The phlobaphenes and "coloring matter" are more highly oxidized and present in smaller quantities than the less oxidized tannins and phenolic acids. Although data on the interflavanoid bonding in western hemlock polyflavanoids are limited, some inferences can be drawn from work done with bark.
extracts of other conifer species. Instead of a regular polymer containing exclusively C(4)-C(8) interflavanoid bonds, there is now indirect evidence for the presence of branching from the 6 position of the catechin and epicatechin moieties. This type of structure would give these polyflavanoids a globular conformation in solution.

I-B. Resins Made with Phenols and Formaldehyde

Phenols can react with formaldehyde under acidic or basic conditions to form rigid cross-linked polymers that have a variety of applications depending on the raw materials used, reaction conditions and the particular manufacturing process. One important use is in the formulation of phenol-formaldehyde (PF) thermosetting resins or phenol-resorcinol-formaldehyde (PRF) cold-setting resins used in the wood products industry. In 1979, approximately 37% (8.26 X 10^8 kg) of the thermosetting resin produced in the United States (including urea-formaldehyde types) was used in the manufacture of forest products (34). Some chemistry of the reactions between phenol and formaldehyde under basic conditions is presented below. Acidic resins are not normally used for wood applications because of problems with degradation of the wood, and will not be considered here. Catechol and other polyhydroxy phenols will react in a similar manner to phenol itself, but with faster reaction rates. The relative reactivity of some relevant
phenols with formaldehyde is given in Figure I-10.

Free formaldehyde is rather unstable in water solution, condensing readily into complex equilibrium mixtures of polymers primarily in the hemiformal form (36) (Figure I-11). Formaldehyde can be added to a resin in forms other than in a water solution. Paraformaldehyde, a solid, is a high molecular weight hemiformal polymer of formaldehyde. This material is available in various grades with different average molecular weights and particle sizes. Paraformaldehyde decomposes fairly rapidly in alkaline solution to release formaldehyde. The depolymerization process is accelerated by heat (36). Hexamethylenetetramine (also called hexa or hexamine) is most commonly used for the second stage of novolak phenol-formaldehyde resin curing, after the novalak has already been formed by reaction of phenol with formaldehyde solution.
Hexamine can also be used as a primary formaldehyde source under alkaline conditions. Its advantage in basic solutions is that it decomposes to formaldehyde only at high temperatures (approximately 150° C), allowing a longer pot-life for adhesives made with this formaldehyde source (37). The decomposition of hexamine in alkaline water solution is shown in Figure I-12. There have been conflicting reports on whether bonds made with bark adhesives containing hexamine, are more or less boil resistant than adhesives made with formalin or paraformaldehyde as the hardener (39,40). This is obviously an area requiring more study.

Commercial phenol-formaldehyde resol resins for wood adhesive applications are manufactured by mixing phenol with a basic catalyst in a reaction vessel. Sodium hydroxide is the most common base used, although barium hydroxide, calcium hydroxide, sodium carbonate, and organic amines can also be employed, depending on the specific application. Aqueous formaldehyde is added to give a formaldehyde/phenol molar ratio of 1.8 to 2.4. After initial heating, the temperature is controlled to allow the condensation reaction to proceed to the

Figure I-11. Self Condensation of Formaldehyde
Figure I-12. Decomposition of Hexamine in Alkaline Solution

\[ \text{N(CH}_3\text{)}_3 + 3 \text{H}_2\text{CO} + 3 \text{NH}_3 \]

desired extent. The resin is then cooled and and some water removed by evaporation (41).

The important reactions that occur in phenol-formaldehyde resin formulation are summarized in Figure I-13. Methylene and methylene ether bridges between phenolic moieties are probably both present, as well as unreacted methylol groups (42). A reaction mechanism with neutral quinone methide intermediates has also been proposed (Figure I-14) (42,43).

To make an adhesive mix for wood bonding purposes, some filler is usually added to the phenol-formaldehyde resin. Besides acting as a space filler and resin extender, the filler helps reduce the considerable shrinking that takes place during polymer crosslinking. Common filler materials are wood flour, ground corncobs and ground bark. The addition of more formaldehyde to the adhesive is not required for this type of
Figure I-13. Important Reactions in Phenol-Formaldehyde Resin Formulation
resin. Polymer cure occurs by a continuation of the condensation reaction to produce a highly crosslinked structure. Under basic conditions, any methylene-ether linkages present decompose to a simple methylene bridge with the release of a formaldehyde molecule that participates in formation of more methyol groups. The breakdown reaction is probably a reversal of equation III in Figure I-14 (43).
I-C. Western Hemlock Bark as a Source of Chemicals

Most of the work carried out on commercial chemical applications of western hemlock bark was done at ITT Rayonier, primarily by Herrick and Hergert, although neither of these authors is publishing in this field now. Several excellent reviews have been written on the chemistry and utilization of western hemlock bark extractives (17,29,44).

(1) Adhesive Applications

In the tannins derived from western hemlock bark, the A and B phenolic rings are quite different in reactivity. If a hemlock bark extract is simply mixed with formaldehyde under alkaline conditions, the phloroglucinol-derived A-ring reacts much faster than the catechol B-ring. Figure 1-9 gave the relative reactivities of these two phenolics; phloroglucinol is approximately 3 times more reactive to formaldehyde than catechol. A resin made in this way has a very short pot-life and low strength as a wood adhesive (39). The former is caused by the high reactivity of the A-ring, while the latter is probably due to the large size of the tannin molecule compared to the relatively short methylene bridges formed between molecules by the formaldehyde. Because of the steric problems, there would be relatively few covalent bonds between tannin molecules, forming a rather brittle three-dimensional structure.
A way around this problem is to have longer, more flexible bridging between polyphenol units. Low molecular weight phenol-formaldehyde resin can perform this function.

MacLean and Gardner (39) were the first investigators to show that strong, durable plywood bonds could be made with sulphited western hemlock bark extracts substituting for up to 75% of the phenol-formaldehyde adhesive. Bonds obtained using an adhesive not fortified with phenol-formaldehyde resins were not acceptable. Later, Herrick and Bock (40) tried a different approach by initially reacting an ammonia extract of hemlock bark with trimethylolphenol (Figure I-15) to form an adhesive. Besides forming a larger and more flexible bridging unit, the trimethylolphenol ties up the highly reactive position on the tannin A-ring, probably allowing the catechol B-ring to react as well. A resin made from 49% bark extract, 34%
polymetholphenol and 17% filler was fast-curing and produced acceptable exterior-grade plywood. At the time these adhesives were developed, commercial application was not feasible because of the high cost of the bark extract compared to the cost of phenol. Rayonier had a pilot plant producing the ammonia hemlock bark extract for about 10 cents per pound (in 1958) (40). Unfortunately, at that time phenol was even cheaper at about 9 cents per pound (45).

In order to avoid this cost problem, Herrick and Conca (46) developed a cold-setting resin using an ammonia extract of hemlock bark. As a cold-setting resin, the tannin adhesive would compete against the more expensive PRF adhesives. It was found that a maximum of 30% of a PRF resin or 60% of an RF resin could be substituted with the tannin extract and still meet pot-life and assembly-time requirements. Additional resin components such as polar organic solvents and pH-regulating buffers had to be added to the adhesive mix to accomplish this. The economics in this case were not much more favorable. Laminating PRF resin is normally 30%-40% phenol, so the extract is primarily substituting for phenol, which was less expensive than the extract anyway. In order to produce the bark extract for a reasonable price (12.5 cents/lb. in 1960), the extraction plant would have to be built on a fairly large scale (19 MM lbs./yr.) (46). To justify building such a plant, a market for the product would have to be developed first. This market never developed, probably because glulam lumber is such a high liability product. Manufacturers would not want to take any
chances on using an unproven adhesive.

The price of phenol decreased until the petroleum shortages of 1973-1974 (Figure I-16). Consequently, not much work was done in utilization of bark extracts in adhesives during this time. When it appeared that bark extracts might become cost-competitive with phenol, interest in this area was renewed.
Anderson et al. (47) described an application to particleboard. The particles were first sprayed with concentrated sulphite bark extract, then mixed with powdered paraformaldehyde (approximately 1 1/2% of furnish weight) and processed into a board. In the hot-press, formaldehyde is released which reacts with the phenolic bark extract to form a bond. The boards produced were comparable to conventional phenolic-resin bonded exterior-grade particleboard. In a particleboard application, the problems of short pot life and poor wet strength associated with western hemlock tannin adhesives are minimized. This may yet prove the best application for this type of adhesive.

On the basis of differential thermal analysis and plywood bonding tests, Steiner and Chow (48) concluded that an adhesive made from western hemlock extracts required higher than conventional temperatures to provide sufficient cross-linking for an acceptable bond. The adhesive was made from a water extract of fresh western hemlock bark and paraformaldehyde, and the pH adjusted to about 6.8 just prior to spreading. Exterior-grade bonds were obtained with a press-temperature of 180° C and a press time of 7.5 minutes. A typical press-temperature for PF resin-based adhesive used for plywood manufacture is 150° C. It was also observed that the age of the bark and extraction method had a great influence on the quality of the bond produced.

The variability of bark quality will probably prove to be the biggest obstacle to its large scale utilization. For plywood applications, the problem of the short pot life can be remedied by using slow releasing formaldehyde sources such as
paraformaldehyde or hexamine (39) or adding organic solvents to the adhesive mix (46), while reasonably good bonds can be obtained with high press temperatures (48) and/or limited fortification with conventional PF resin (40). However, it is much more difficult to get around the problem of log to log variation in bark quality due to inherent tree differences such as age, bark thickness and percentage outer bark, or differences in the treatment after harvesting, like water or dry sorting, length of time in storage and whether the log is debarked mechanically or hydraulically (48,44). The approach taken in this thesis of degrading the tannins to simpler components stabilizes the qualitative nature of the products obtained, although their yield will vary. This is advantageous in controlling the quality of the adhesives to be made in any commercial application.

(2) Non-Adhesive Applications

The non-chemical applications of conifer bark are quite extensive but will not be described here. Several good reviews on this topic are available (7,50,51).

Commercial chemical products have been produced from the barks of three North American conifers - redwood (*Sequoia sempervirens* (D. Don) Endl.), Douglas fir and western hemlock. Douglas fir has substantial amounts of wax associated with the bark phellem, that was at one time produced commercially by the
Bohemian Lumber Company in Oregon (49). Many products based on the polyphenolic portion of these three barks were produced and are summarized in Table I-3. Douglas fir is excluded from this table because Weyerhaeuser, the producing company, did not publish any information on availability or applications for their bark products. It is probable that these products were commercially available for only a short time. Hergert (17) infers that this may have been due to undesirable foaming in the Douglas fir bark extract in drilling mud and boiler water applications. This could have been caused by the high concentration of fatty acids in the species. Manufacture of the redwood bark products was discontinued in the late sixties. They were also available for only a short time (17).

The commitment of ITT Rayonier to their bark utilization program was really quite remarkable. The program started in 1948 and for some time had two commercial extraction plants operating. The Vancouver, B.C. plant was in operation from 1956 to 1976, producing between 5000 to 7600 tonnes/year, primarily of sulphited extract (44). Commercial production at the Grays Harbor plant extended from 1967 to 1972 (44). The bark utilization program at Rayonier was terminated in the late seventies.

"Rayflo", the drilling mud additive, was by far the most successful product marketed. When the sulphonated extract is added to the clay slurries used to lubricate the drill, the mud becomes less viscous and more slippery. Rayflo is a sulphonated polyflavanoid obtained by extracting ground bark with a water
<table>
<thead>
<tr>
<th>Species</th>
<th>Name</th>
<th>Type of Extract</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redwood (1)</td>
<td>Palcotan</td>
<td>Sulfite</td>
<td>Drilling mud additive</td>
</tr>
<tr>
<td></td>
<td>Palconate</td>
<td>NaOH</td>
<td>Boiler and cooling water treatment</td>
</tr>
<tr>
<td>Western (2)</td>
<td>Rayflo</td>
<td>Sulfite</td>
<td>Drilling mud additive</td>
</tr>
<tr>
<td></td>
<td>Rayflo-C</td>
<td>Sulfite</td>
<td>Boiler and cooling water treatment</td>
</tr>
<tr>
<td></td>
<td>Rayplex</td>
<td>Sulfite</td>
<td>Zn, Fe, Cu and Mn micronutrient deficiencies</td>
</tr>
<tr>
<td></td>
<td>HT-120</td>
<td>Ammonia</td>
<td>Resin intermediates</td>
</tr>
<tr>
<td></td>
<td>Terranier</td>
<td>Ammonia</td>
<td>Chemical grouting system</td>
</tr>
<tr>
<td></td>
<td>LB 451A</td>
<td>Sulfite</td>
<td>Penetrant iron spray for chloratic citrus trees</td>
</tr>
</tbody>
</table>

(1) Manufactured by Pacific Lumber Co., Scotia Calif.

(2) Manufactured by ITT Rayonier Inc., at Grays Harbor, Wash., and by Rayonier Canada Ltd. at Vancouver, B.C.

Table I-3. Commercial Polyflavanoid Products (17,40,44,46)

solution of 10-18% sodium sulphite and bisulphite at 150°C. The yield of this product from bark was about 35% (50).

The structure of sulphonated tannins was investigated by
Sears (52). By subjecting catechin to the same reaction conditions used to sulphonate and extract bark tannins, he found that the pyran C-ring of the model compound is opened and a sulphite group added to carbon-2 (Figure I-17). It is probable that the sulphonated bark tannins have similar ring-opened catechin or epicatechin moieties in its polymer structure.

Rayflo was shown to be superior to quebracho wood extract which was the standard additive at the time (9,53). This product eventually became uneconomical to manufacture, because of competition from cheaper additives derived from lignosulphonates.
"Rayplex" is a similar sulphited extract and was prepared as a complex of various metal salts in the form of a chelate. This material was used as a source of micronutrients in agricultural applications. It could be applied as a foliar spray or in the soil to field and orchard crops deficient in one or more mineral nutrients. Some of the metals that could be supplied in this way were zinc, iron, copper and manganese. Although this product had a low degree of phytotoxicity, it had to be applied early in the growing season in order to avoid fruit stain.

If hemlock bark is extracted with aqueous ammonia, a material with quite different properties results. The extract is obtained in 25-30% yield and has the ability to complex with iron, chromium and other metal ions to give gels. An extract of this type is also reactive with formaldehyde. Besides being developed for adhesive applications as described earlier, this extract could be used in chemical grouting systems for soil stabilization and control of water flow on heavy construction sites. Examples would be deep excavations, driving tunnels or streets, and earth-fill dams. The bark extract is dissolved in water, mixed with a solution containing formaldehyde and metal salt, then pumped through an injection pipe into the soil to be grouted. After settling, the stabilized soil has increased load bearing capacity, reduced water permeability and more stability to minimize deformation and subsidence. Although this system worked well and was competitive with other products, soil
grouting never became a common procedure in North America, and so a market never developed. However, chemical grouting has developed into a fairly routine procedure in Europe (44).

As an additive for boiler and cooling water, the sulphited extracts worked well as dispersants for scale-forming minerals and corrosion inhibitors (44). However, this is quite a limited market and could not support an extraction plant if there were no other important markets for bark chemicals.

It is apparent that although there have been some successful commercial chemical applications of western hemlock bark and its extracts, they have all been of low volume, and probably realized only a small profit. Potentially, the best application is still as a wood adhesive. This would certainly be a large scale use, and in a large forest-products company a bark based adhesive could be produced and utilized internally, reducing the company's dependence on external supply sources.

I-D. Bark Polyflavanoids of Other Species; Chemistry and Utilization in Adhesives

The bark polyflavanoids from a number of tree species besides western hemlock have been investigated for their suitability in adhesive formulation. These include the southern pines, Pinus radiata D. Don, Acacia (wattle) species, mangrove species (55,56), Finnish spruce (Picea obovata var. fennica (Regel) Henry) (57), Pinus brutia Ten. (58), and Larix
leptolepsis Gord. (59). The bulk of the literature is concerned with the first three species or species groups.

Figure I-18 shows the substitution patterns found in the polyflavanoids of the important bark extracts. The important differences are that wattle tannin has resorcinolic A-rings along with B-rings derived from pyrogallol or catechol, while the conifers (including western hemlock) have phloroglucinol-derived A-rings and catechol B-rings. The substitution patterns of the phenolic rings have a large effect on the reactivity of the tannins to formaldehyde and hence to its suitability as an adhesive. Another important factor is the average molecular weight. Some data have been published on the molecular weight ranges of bark polyphenols (see Table I-4), but these figures need careful interpretation. There is evidence that in aqueous solution, tannin molecules tend to associate to give apparent molecular weights much greater than the actual molecular weight (60). However, since it is these aggregates that give tannin solutions their viscosity and reactivity characteristics, the reported figures may be more useful from a practical point of view.

Some adhesive applications of the southern pines, Pinus radiata, and wattle bark extracts are given below.
(X substituents refer to possible continuations of the polymer chain)

<table>
<thead>
<tr>
<th>Extract</th>
<th>Substituent</th>
<th>Relative Amount</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wattle</td>
<td>H</td>
<td>OH, OH</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>OH, H</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>OH, H</td>
<td>5%</td>
</tr>
<tr>
<td>Southern Pine</td>
<td>OH</td>
<td>OH, H</td>
<td></td>
</tr>
<tr>
<td>Pinus radiata</td>
<td>OH</td>
<td>OH, H</td>
<td>major</td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>OH, OH</td>
<td>minor</td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>H, H</td>
<td>Trace</td>
</tr>
<tr>
<td>Mangrove</td>
<td>OH</td>
<td>OH, OH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OH</td>
<td>OH, H</td>
<td></td>
</tr>
</tbody>
</table>

Figure I-18. Substitution Patterns in the Basic Units of Bark Polyflavanoids
<table>
<thead>
<tr>
<th>Source of Bark Tannin</th>
<th>Molecular Weight</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wattle</td>
<td>Mn = 1250&lt;br&gt;Range=500(n=2) to 3000&lt;br&gt;(n=10 to 11)</td>
<td>68</td>
</tr>
<tr>
<td>Shortleaf and Loblolly pine (southern pines)</td>
<td>&lt;3500 - 50-60%&lt;br&gt;3500-8000 - 20%&lt;br&gt;12,000 - 10-20%</td>
<td>63,69</td>
</tr>
<tr>
<td>Radiata pine</td>
<td>Mn = 8400&lt;br&gt;&lt;10^3 = 31.4%&lt;br&gt;10^3-10^4 = 11.5%&lt;br&gt;10^4-10^5 = 29.2%&lt;br&gt;10^5-10^6 = 5.5%&lt;br&gt;10^6 = 22.4%</td>
<td>70,71</td>
</tr>
</tbody>
</table>

Table I-4. Molecular Weights and Distributions of Bark Polyphenols

(1) Wattle

In 1982 it was reported that approximately 300,000 tons of condensed tannins were produced commercially world-wide, of which only 15,000 tons were used for adhesive purposes (65). Most, or all, of the tannin used for adhesives was from the bark of the black wattle tree (*Acacia mearnsii* De Wild.). The development of plywood, particleboard, glulam, cardboard and finger-jointing adhesives from the polyflavanoids of this species has mainly been in South Africa. There are a number of reasons why these applications have been successful.

(1) Wattle tannins have primarily a resorcinolic A-ring, which has a relatively low reactivity with formaldehyde, and allows a much greater degree of control over the tannin-
formaldehyde condensation reaction (as compared to the conifer tannins).

(2) They have a comparatively low average molecular weight.

(3) A well established tannin industry exists in South Africa already producing bark extract for other applications.

(4) Conventional phenolic resins or their raw materials have to be imported into South Africa resulting in a high local cost. The economics of producing a tannin-based adhesive become more attractive for this reason.

Unmodified tannin resins are often unsuitable for particleboard applications because of the high viscosity when concentrated to 50 or 60% solids. This problem has been corrected by sulphonation (68), heating with base (72) or treatment with a series of acid and alkaline steps that hydrolyse the viscosity-influencing carbohydrate gums to simple sugars (73). Modified tannin extract has also been combined with urea-formaldehyde prepolymer to give a stronger but less waterproof particleboard, primarily used for flooring (74).

Particleboard manufacture has been the largest consumer of wattle tannin adhesives (65). In some cases, it has been reported that the product is superior to conventional PF bonded particleboard in resistance to weathering and dimensional stability (72).

Tannin adhesives used for exterior-grade plywood manufacture are usually fortified with 10% to 25% of conventional adhesives. PRF (75), PF (76) and UF (77) resins have all been shown to act as good crosslinking agents for
wattle tannin-based adhesives, and formulations using these resins have been in use industrially (65). Unfortified formulations have also been developed for plywood applications (72,78). One particularly interesting resin incorporates zinc acetate that acts as an accelerator of the reaction between tannin and formaldehyde, and as a B-ring activator to provide additional cross-linking (79). A typical formulation would have 15 to 16% paraformaldehyde as hardener and 10 to 25% coconut shell flour (or similar product) as a filler, based on the weight of tannin solids (65).

A number of cold-setting wattle tannin-based adhesives for glulam applications have been described. A system based on the reaction of resorcinol with methylol-tannin (formed by reaction of wattle tannin with formaldehyde in 50% methanol solution) has been described (72). It was later found that if the tannin extract is first reacted with sulphite in order to open the pyran C-ring, the amount of added resorcinol can be reduced to 10% of the total liquid adhesive (80). This concentration of resorcinol is substantially lower than what is required in conventional PRF resins.

PRF is also the resin normally used for finger-jointing. However, its use is not practical where large presses are not available, as the setting time is quite lengthy at ambient temperatures. Very fast setting adhesives using wattle tannins have been developed that are variations of the "honeymoon" resins described by Kriebich (81). These are two component systems where one part is a conventional slow-setting PRF or
tannin/formaldehyde resin with an excess of hardener, and the other part is a very reactive phenolic or tannin resin at a high pH with no hardener (82,83,84). These adhesives allow handling of the glued lumber within one hour of manufacture.

Some successful minor applications of wattle tannins are in adhesives for damp-resistant cardboard (85), phenolic foams (86) and in tannin based polyurethane adhesives (87).

(2) Southern Pines

Although quite a bit of work has been done on elucidation of the structure of polyflavanoids in southern pine barks (25,26,27,28), there has been relatively little information published on formulating adhesives with bark extracts from these species. Chen reports replacing 20% (88) and 40% (89) of the phenol in a commercial PF resin with alkaline extracts of southern pines. In both cases, bond strengths comparable to commercial PF resin could be achieved. At the higher substitution level, the mixed resin gave superior results at shorter press times. The use of alkaline extracts from various agricultural residues as phenolic extenders was also reported in the same papers.

Tannin interflavanoid bonds are prone to cleavage under acidic conditions (68,90). If a strong nucleophile, such as a thiol or phloroglucinol, is present, that compound can add to the 4 position of the cleaved flavanoid unit (91). Using
resorcinol as the nucleophile, Hemingway (92) reports formulating a cold-setting glulam adhesive with condensed pine bark tannin resorcinol adducts (Figure I-19) replacing more than 60% of the resorcinol in a conventional PRF resin. The main benefit of this treatment is a reduction in viscosity due to the cleavage of some interflavanoid bonds.

![Epicatechin-Resorcinol Adduct](image_url)

**Figure I-19. Epicatechin-Resorcinol Adduct**

A recent economic feasibility study has shown that the use of alkali or sulphite extracts of pine bark as a partial replacement for PF resins in particleboard manufacture can be economically justified. However, further research into adhesive formulations for this application that will meet industrial standards still needs to be done (93).
(3) Radiata Pine

The problems found with using Pinus radiata bark extracts in wood adhesives are the same as those associated with other conifers, namely:

1. Short pot life.
2. High viscosity of the adhesive mix at conventional solids content and conditions.
3. Relatively low strength and water resistance.

Attempts to circumvent these problems go back to 1952 when Dalton (94) used sulphonation to reduce the viscosity and reactivity of radiata pine extracts. This author reported strong water-resistant gluelines in plywood made with the modified extract, paraformaldehyde and a filler. Particleboard adhesives made with similar extracts have also been described (95).

Ultrafiltration has been evaluated as a means of purifying radiata bark polyphenols. This process removes the higher molecular weight flavanoid fractions that contribute to the viscosity and reactivity problems (71,96). The treatment does appear to improve the characteristics of the extract, although there have been doubts expressed as to how feasible such a treatment could be on a large scale industrial basis (97).

Recently Pizzi (97) has suggested that a diphenyldiisocyanate (MDI) (see Figure I-20) fortified tannin adhesive could be considered as a "universal" tannin adhesive for particleboard manufacture. The author reports that
excellent results are obtained with either a resorcinolic or phloroglucinolic type of tannin. The unmodified tannin along with some paraformaldehyde is sprayed as a dilute solution onto the wafers or chips first, followed by the separate application of MDI preferably in a following in-line blender (65). In this type of system it is thought the sugars and gums normally found in pine extracts would contribute to the strength of the glueline by reacting with the MDI to form urethanes, thus increasing the amount of crosslinking present. The problem with the use of MDI as a fortifier is its high cost. It is approximately 50% more expensive than a PF resin (60).

New Zealand Forest Products Limited (NZFP) has recently started producing plywood and particleboard bonded with adhesives based on radiata pine bark extracts (98). The large scale extraction plant completed in 1981 (22 tonnes/day) was the result of an adhesive development program started in 1955. Their commercial plywood adhesive is a fortified one, with a ratio of tannin to PF of approximately 2.5 to 1. The particleboard adhesive is unfortified but the extract has been
modified in order to control the viscosity (98). These adhesives have not yet been accepted outside of New Zealand, although mill trials will soon be underway in Australia (99).

I-B. Summary

Compared to other British Columbia native tree species, the bark of western hemlock is a very rich source of natural polyphenolics. For this reason, western hemlock has been one of the group of tree species that has been studied world-wide as a possible source of tree bark tannins that could substitute for the petroleum derived adhesives widely used in the manufacture of forest products. In some situations, particularly in South Africa with wattle tannins, there have been successful applications. The problems found with using tannin from western hemlock as adhesives, and from conifers in general, are:

1. Low bond strength due to the high average molecular weight of the bark tannins and relatively few crosslinking sites.

2. Overly high reactivity with formaldehyde, making the polymerization reactions rather difficult to control and resulting in a short adhesive pot-life.

3. Variable bark quality, resulting in variable bond strength for a conifer-tannin based adhesive. For western hemlock in particular, the bond quality seems to depend on the age and past history of the bark.
Some approaches that have been used to try and solve these problems are:

1. Ultrafiltration to isolate the optimum molecular weight range of the tannins.
2. Breakdown of the tannin polymer into sulphonated lower molecular weight oligomers and monomers.
3. Special tannin isolation and adhesive formulation techniques such as slow formaldehyde-release agents, specialized extraction techniques and high press-temperatures.

These techniques have not been entirely successful. At the present time, the only commercial application of conifer bark tannins as wood adhesives is in New Zealand using radiata pine.
CHAPTER II. THE TIME-TEMPERATURE DEPENDENCE OF THE YIELD OF CATECHOL FROM CATECHIN AND WESTERN HEMLOCK BARK POLYPHENOLS

In this thesis, the approach taken to the problem of how to use conifer tannins as adhesives was to break down the tannin into simpler phenolic molecules that would be easier to utilize as an adhesive base. A reaction was investigated that cleaves catechin (a model compound for conifer tannins) into catechol and other relatively simple compounds, the nature of which depends on the source of the tannin. The adhesive properties of catechol and some of the other simple phenolics that can be formed, are well known as monomers in PF-type resins. If these products were isolated from the product mixture in sufficient quantities they could be used to make excellent adhesives.

Catechol can be produced from simple flavanoids such as catechin, or from bark polyphenols by a cleavage reaction using a 30% solution of \((\text{NH}_4)_2\text{SO}_4\) in concentrated \(\text{NH}_4\text{OH}\) (this reagent and solvent is abbreviated to \((\text{NH}_4)_2\text{SO}_4/\text{NH}_3\)). Investigating the mechanism of this reaction and evaluating the feasibility of using the product mixture as an adhesive is the basis of this thesis. In order to obtain information on the mechanism of this reaction and also to develop reaction conditions that would give the maximum yield of catechol, it was necessary to first find or develop an assay method for this compound.

Two different assay procedures have been described in the literature. The traditional colorimetric method is to react the catechol with ethyl amine molybdate in methanol, then read the spectrophotometric absorbance of the resulting complex at 435 mu
(100). The reading can then be related to catechol concentration by a standard curve. Another method uses a gas chromatograph to obtain areas for the peaks corresponding to catechol and an internal standard. A known amount of the standard is added to the catechol solution, the mixture is then acetylated, and the resulting acetates analysed using an appropriately equipped gas chromatograph (101). The ratio of the catechol and standard peak areas can be related to a weight ratio obtained from a calibration curve.

Initially, the latter method was used in this study. However, it was soon apparent that the procedure was not suitable for rapid analysis of large numbers of samples, and a second GC-based assay procedure was developed. A description of the development of these procedures and the results obtained with them is given below.

(1) GC Assay Using Acetylated Catechol

The internal standard used by other workers was o-cresol (2-methylphenol). It was decided not to use this compound, mainly because it is quite volatile and hard to purify. Its low melting point (30°C) makes it difficult to recrystallize, and purification by distillation gives a colored product even under vacuum due to the high temperature required. Instead, phloroglucinol was chosen. This phenol is readily recrystallized from methanol. The triacetate is easily formed
using acetic anhydride/pyridine and has a retention time close to catechol diacetate. One disadvantage to using phloroglucinol as a standard is that it is unstable in solution. After about one week, the solution turns yellow, and a fresh solution has to be made.

The basic assay procedure was that after addition of the internal standard to the reaction product solution, the catechol and phloroglucinol were isolated by solvent extraction, acetylated and then analysed by gas chromatography. In the resulting chromatogram, the peaks had reasonable retention times (catechol diacetate - 250 s, phloroglucinol triacetate - 400 s) with a flat baseline (at a column temperature of 225°C). A final step to 290°C was required to clean compounds of low volatility from the column (Figure II-1).

The ratio of the catechol diacetate and phloroglucinol triacetate peak areas were then compared to a calibration curve of known weight ratios (catechol/phloroglucinol), versus the same area ratios obtained from integration of the chromatograph. The relationship between the area and weight ratios were determined by mixing appropriate volumes of catechol diacetate and phloroglucinol triacetate standard solutions, and then calculating the peak area ratios obtained from GC analysis. The ratio of the peak areas were then plotted against the known weight ratio (Figure II-2).

It was soon apparent that this procedure was too complicated and time-consuming. For ten, one-milliliter samples, the workup and analysis took about ten hours. Another
Figure II-1. Typical Gas Chromatogram of Acetylated Catechol and Phloroglucinol
Figure II-2. Calibration Graph for Acetylated Catechol

Problem was that the phenols isolated by solvent extraction of the reaction solution had to be reasonably dry before they could be acetylated. This meant drying under vacuum over a drying agent. Catechol sublimes readily, and if the residue from the evaporation of the extraction solvent is left under vacuum too long, a significant amount of catechol may be lost. For these
reasons, this assay procedure using acetylated catechol was used only until a better assay could be developed.

(2) Assay System for Underivatized Catechol

Ideally, it would have been desirable to analyse for catechol without isolating or derivatizing it. A colorimetric method would not be possible because the reaction mixture itself is highly colored. Normally, phenols are too reactive to be used directly in a GC analysis. Severe peak tailing makes reliable integration impossible. CSP-633 packing (Chromatographic Specialties) is completely deactivated and specifically designed for analysis of free phenols, although the manufacturers had never used polyhydroxy phenols such as catechol on this liquid phase (102). Using this packing, the catechol did not need to be isolated from the reaction mixture and derivatized, considerably shortening the assay procedure.

The first thing tried upon obtaining the CSP-633, was to pack a 48 in. stainless steel column with it. When a solution of catechol was injected, the resulting peak showed severe tailing. This was attributed to interaction between the catechol and the metal column. There were no analytical glass columns available for the gas chromatograph being used, so another column using 1/8 in. O.D. teflon tubing was made. In order to avoid the metal in the injection port, this column was designed for on-column injection with the sample injected
directly into the column (Figure II-3). This system gave chromatograms with symmetrical, sharp peaks and consistent retention times when using catechol/water solutions. However, when samples of the reaction mixture were analysed, the column seemed to degrade after only a few injections. The non-volatile components of the reaction mixture (including \((NH_4)_2SO_3\)) that are deposited on the walls of the column after sample injection were blocking the gas flow within the small I.D. tubing (1/16 in.). The solution to this problem was to go to a 1/4 in. O.D. glass preparative column 24 in. long, also designed for on-column injection. Occasional cleaning can be done by removing the column and wiping out the interior of the first couple of inches of the tubing using a damp tissue.

Initially, this system worked well and a standardization curve was determined using water solutions containing known amounts of catechol and o-cresol (Figure II-4). Phloroglucinol was not used as the standard because of its relatively high reactivity. It may interact with some components of the reaction mixture and lower its effective concentration. In this case it was felt that the more stable o-cresol was a better standard. However, a different problem developed after extended use of the column for analysis of the reaction mixtures. After 10 to 15 injections, the catechol peak started to broaden and its retention time got longer. Initially, it was thought that this was due to water being adsorbed to the column, as repeated injections of catechol/dry ether solutions did not produce this effect. Extensive heating (200° C for 12 hrs.) and repeated
Figure II-3. GC Injection Systems
injections of dry ether and ethanol did not restore affected columns, indicating that water adsorption was not the problem.

It had been noticed previously that water injected on a used CSP-633 column produces a large peak. This was unexpected as the flame ionization detector of this chromatograph is normally insensitive to water. It appeared that the water was washing out material that had been adsorbed to the packing which gave the apparent water peak. It was thought that whatever was being washed out could also be the cause of the peak tailing observed. Injection of water into an affected column until the

Figure II-4. Calibration Graph for Underivatized Catechol

\[ Y = (0.788)X + 0.0257 \]

\[ r = 0.99 \]
resulting peak was very small and not decreasing in size, followed by injection of a reaction mixture sample, showed that the packing had been restored to its original effectiveness. The adsorbed material was the cause of the peak broadening.

The basic assay procedure for underivatized catechol was to add an internal standard to the crude reaction mixture, inject a sample into the GC, and then analyse the resulting GC trace using a calibration graph. There is no need to isolate or derivatize the catechol, saving about eight hours of work per ten samples. A representative chromatogram is shown in Figure II-5.

(3) Optimization of Catechol Yield from Catechin

The yield of catechol from catechin using \((\text{NH}_4)_2\text{SO}_3/\text{NH}_3\), was investigated at a number of temperatures for various reaction times. Figure II-6 summarizes the results. Each point is the average of two results obtained from separate solutions, subjected to the same conditions of reaction temperature and time. The best molar yield of 30% was obtained at 175° C with a reaction time of 2 hours. Actual isolation of the catechol from a larger scale reaction (using the same conditions) gave a yield of 35% of pure recrystallized catechol. The higher yield in this case may have been due to a higher concentration of catechin in the reaction solution - 1 g catechin per 10 ml reaction solution versus 0.25 g catechin per 10 ml in the small
Figure II-5. Typical Gas Chromatogram of Catechol and o-Cresol
Figure II-6. Molar Yields of Catechol from Catechin at 125° C, 150° C, 175° C and 200° C for Various Reaction Times Using (NH₄)₂SO₃/NH₃.

The yield of catechol from solutions of catechin in 30% (NH₄)₂SO₃/H₂O was also done at reaction temperatures of 150° C and 175° C (Figure II-7). The maximum yields in these experiments were less than when concentrated NH₄OH was used as the reaction solvent, and also occurred at longer reaction times. TLC analysis of the solutions showed fewer reaction products in
the ammoniacal solvent, so the standard conditions chosen for investigating the nature of the catechin reaction used a concentrated ammonia solution containing 30% (NH₄)₂SO₃ at 175°C for 2 hours.

(4) Optimization of Catechol Yield from Bark Extract

A 95% ethanol extract of freshly collected bark was used in this section. The yield of extract was 26.5% (dry weights, w/w). Catechol was produced at different temperatures for various reaction times as for the experiments with catechin. The yields of catechol from bark extract at reaction temperatures of 150°C, 175°C and 200°C are shown in Figure II-8. The yields in this case are percentages of the weight of
Figure II-8. Weight Percent Yields of Catechol From Western Hemlock Bark-Ethanol Extract at 150° C, 175° C and 200° C for Various Reaction Times Using (NH₄)₂SO₃/NH₃
catechol produced from a known weight of extract. A maximum yield of about 3% was observed at 175° C, when a reaction time of 4 hours was used, and at 150° C with 10 hours reaction time. The higher temperature conditions were used where possible in further work with bark polyphenols because of the shorter, more convenient reaction time.

(5) Catechol Yield from Sequential Bark Extracts

Two sequential solvent extractions of western hemlock bark were done. The first followed the procedure of Fraser and Swan (32), with sequential extractions of benzene/ethanol, ethanol, water and 1% NaOH. A portion of each extract was reacted with (NH₄)₂SO₃/NH₃ at 175° C for 4 hours, and the amount of catechol produced was measured. A graph of yield of extract, and how much catechol is obtained from a particular extract versus extract type, is shown in Figure II-9.

The maximum catechol yield of 3.3% was obtained from the ethanol extraction. This result would be expected from analysis of the components of each extract type. The benzene/ethanol extract contained fats, waxes and non-flavanoid phenolics that could not decompose to catechol but which contribute to the weight of the extract. Simple flavanoids and biflavanoids are extracted with ethanol and would be expected to react to give catechol in the best yield, since accessibility and stereochemistry problems would be minimized. Higher molecular
weight polyflavanoids and some carbohydrates would be contained in the water extract and a lower catechol yield would be expected. To find out whether the phenolic acids of the base extract would react to form catechol, catechinic acid (Figure I-9) was prepared and tested. It was found that this compound does not decompose to catechol when treated with $\text{(NH}_4\text{)}_2\text{SO}_3/\text{NH}_3$ at 175°C. This probably explains why such a low yield of catechol was obtained from the base extract.

A second series of extractions used a sequence of solvents
in order of increasing dipole moment. The solvents used were petroleum ether, benzene, diethyl ether, acetone, ethanol, and water. It was hoped that the increased fractionation would result in an extract with a higher yield of catechol than the ethanol extract of the previous series. The components of this fraction could then be characterized to give better information on the nature of the bark compounds that will react to form catechol. In fact, the opposite occurred. Figure II-10 shows the extract and reaction yields of this experiment. A lower maximum yield of 2% was observed for the acetone extract. This
was probably due to the fact that bark extracts, and in particular those of western hemlock, are prone to aging (32). There was a gap of about 6 weeks between the two experiments, and although the bark was stored at 0° C, some polymerization of the low molecular weight polyflavanoids may have occurred. These are the components that are probably decomposing to form catechol in the degradation reaction, so a small change in the average molecular weight of this fraction may have a large effect on their reactivity to (NH₄)₂SO₃.

Another factor that may be important is the extraction time. In the first series, the benzene/ethanol extraction lasted for over 24 hours, while the ethanol extraction in this case proceeded for 12 hours. During this time the extract was being heated at the reflux temperature of the solvent, possibly resulting in an increased rate of polymerization. This could account for the rather low yield of catechol from the benzene/ethanol extract.

In order to determine the maximum possible yield of catechol from western hemlock bark using the reaction, ten grams of freshly collected, finely ground whole bark was tested with 30% (NH₄)₂SO₃/NH₃ at 175° C for 4 hours. The aqueous ammonia solution itself acted as the extraction medium, thus avoiding any degradation that might occur during the process of solvent extraction. Analysis of the products showed that 98 mg of catechol had been formed. On a whole bark basis, this is a yield of 1.1%. If it is assumed that 25% of the bark is composed of flavanoids and polyflavanoids accessible to this
reaction, the weight yield was 4.5% and the molar yield was about 13% (based on this portion being all catechol-containing monoflavanoids).

(6) Summary

A quick GC-based assay procedure for catechol was developed using a deactivated packing and an internal standard. With this method, the optimum reagent and conditions for production of catechol from catechin were determined to be a solution of 30% ammonium sulphite in concentrated ammonium hydroxide heated to 175° C for 2 hours. Under these conditions, a 35% molar yield of catechol was obtained.

Determining the yield of catechol from sequential extracts of western hemlock bark showed that the best results could be obtained from an extract containing the highest concentration of low molecular weight polyphenols. The extraction time and freshness of the bark were also found to be important factors in optimizing the catechol yield. On a whole bark basis, the best yield of catechol was 1.1%. This corresponds to a weight yield of 4.5% and a molar yield of 13%, if it is assumed that 25% of the bark is composed of flavanoids and polyflavanoids accessible to this reaction.
CHAPTER III. INVESTIGATION OF THE REACTION BETWEEN CATECHIN AND 
(NH₄)₂SO₃ IN CONCENTRATED NH₄OH

The determination of the mechanism of the reaction that 
cleaves catechin into catechol and some other products is 
fundamental to the research problem investigated in this thesis. 
The steps used to accomplish this aim were:

(1) Characterization of an intermediate compound in the 
cleavage reaction and identification of the "other 
products" that could have been derived from the A- and C-
ring portions of the catechin molecule. Identification of 
the former would provide a definite intermediate structure 
upon which further proposed modifications could be based. 
A knowledge of the co-products would show how the rest of 
the catechin molecule has been altered by the cleavage of 
the B-ring and should provide valuable clues to the 
mechanism.

(2) Observation of how compounds related to catechin react 
under the same conditions as the catechin reaction, by 
identification of their reaction products. Simple 
compounds related to the individual catechin rings or the 
etire molecule were investigated to see what changes to 
the A- and C- rings should be expected during the catechin 
cleavage.

(3) Development of a mechanism consistent with the observed 
products and testing the proposed reaction mechanism by 
using it to predict the results of a test reaction. This 
test should show how valid the proposed mechanism is.
A discussion of the results of these experiments is presented in this chapter.

III-A. Identification of an Intermediate and Some Products

\[ 1-(3,4\text{-Dihydroxyphenyl})-2\text{-hydroxy}-3-(1,3,5\text{-trihydroxyphenyl})\text{propanesulphonic Acid} \]

Sodium bisulphite will react with catechin in water solution to form the title compound. A probable mechanism for this reaction is shown in Figure III-1. Reaction conditions that have been described in the literature are refluxing solvent for 6 hours \(71\) or in a sealed container at \(170^\circ\text{C}\) for 0.5 hours \(52\). It seemed probable that this same compound would be an intermediate in the reaction of catechin with ammonium sulphite.

Catechin was reacted with ammonium sulphite in ammonia solution for 24 hours at \(60^\circ\text{C}\). The main product was isolated and characterized by its IR and NMR spectra. The presence of a sulphonic acid group was clearly indicated in the infrared spectrum by strong absorbances at 1145 and 1030 cm\(^{-1}\) and a weaker absorbance at 650 cm\(^{-1}\). Aromatic rings were also probable due to the carbon-carbon double bond absorbances around 1615 cm\(^{-1}\). Comparison of the NMR spectra of catechin and its sulphonated reaction product clearly shows that the pyran C-ring has been opened in the latter (see Figure III-2). The hydrogens
of the phloroglucinol A-ring become equivalent when the ether linkage is broken and form a singlet due to two protons. This singlet is shifted downfield to be superimposed over the signals from the catechol B-ring at a chemical shift of about 6.9 ppm.¹ The hydrogens at C-4 (g and f) also become essentially equivalent when the pyran ring is opened. The observed result

¹All chemical shifts given in this thesis are in delta units.
is a doublet with a relative intensity of 2, positioned at about the same chemical shift as the ddd (doublet - doublet - doublet) signal from the C-4 non-equivalent protons in catechin. The position of the doublet due to the hydrogen attached to C-2 (d in Figure III-2), is shifted upfield from 4.7 to 3.8 upon substitution of the aromatic ether substituent with the sulphonate group. This is expected because of the smaller deshielding effect of the sulphonate.

In order to determine whether the sulphonated catechin described above could be an intermediate in the high temperature degradation reaction to catechol, a sample of this compound was tested under the appropriate reaction conditions. When the sulphonated catechin was heated to 175°C for two hours in a sealed reaction tube, the same products were observed that were found in the direct reaction of catechin with ammonium sulphite. These experiments indicate that 1-(3,4-dihydroxyphenyl)-2-hydroxy-3-(1,3,5-trihydroxyphenyl)propanesulphonic acid is an intermediate in the cleavage reaction of catechin to catechol.

(2) 3-Amino-5-hydroxy-7-quinolinesulphonic Acid

The compound discussed here was isolated from the mixture of products produced along with catechol by the high temperature cleavage of catechin. The intensity of the spot on a TLC plate due to this compound indicated it is probably the catechol co-product formed in the highest concentration. This makes the
Figure III-2. Comparison of the NMR Spectra of Catechin and its Sulphonated Reaction Product
correct identification of this compound very important to the determination of the reaction mechanism, as it is probably derived from at least a portion of the A- and C-rings remaining after catechol is cleaved from catechin. The way in which this portion of the catechin molecule is structurally modified during the cleavage process would give valuable clues as to how the reaction must have occurred.

This compound can be isolated by passing the mixture of products from the high temperature reaction of catechin with ammonium sulphite through a column of strongly acidic ion exchange resin. Non-sulphonated compounds such as catechol pass through freely, while materials with a positively charged functional group are retained on the column. Alpha-amino acids would be strongly held to the column while species with a delocalized positive charge would only be weakly adsorbed to the resin and eventually pass through. 3-Amino-5-hydroxy-7-quinolinesulphonic acid (AHQSA) is of the latter form, and is the only compound of this type present in the product mixture in significant quantities. By simply passing the reaction mixture through an ion-exchange column and discarding the initial highly colored fractions, fairly large quantities of AHQSA can be isolated. Yellow-brown crystals can be obtained from recrystallization in water.

AHQSA was analysed exhaustively, using standard spectroscopic techniques. The IR spectra showed the strong characteristic absorbances of a sulphonic acid at 1190, 1040 and 640 cm$^{-1}$. The aromatic region shows several absorbances ranging
in position from 1570 to 1640 cm$^{-1}$, indicating the presence of a number of different bonds of these types. A weak doublet at 3450 and 3540 cm$^{-1}$ is consistent with the presence of a primary aromatic amine (103).

The NMR spectrum is quite simple and is shown diagrammatically in Figure III-3 along with the signal assignments. The only deuterated solvent in which AHQSA is soluble enough to give good spectra is DMSO-d$_6$. Most labile hydrogens will exchange in this solvent and result in a single broad signal for these protons at a chemical shift of about 5. The four signals between 7 and 9 are due to the carbon-bonded hydrogens on the aromatic ring system and can be divided into

![Chemical shift diagram](image-url)

**Figure III-3.** The NMR Spectrum of 3-Amino-5-hydroxy-7-quinolinesulphonic Acid
two pairs. The downfield signals are both split into doublets with a small coupling constant of about 1.3 Hz, while the other two have a negligible coupling constant. Examination of the spectral data for quinoline shown in Figure III-4 shows that the 2,4 coupling constant is substantially larger than the 6,8 2,4 — 1.7 Hz.
6,8 — 1.0 Hz.

Figure III-4. NMR Spectral Data of Quinoline (104,105)
at least in the solid form, is a zwitterion with the heterocyclic nitrogen being protonated. This hydrogen seems to be the source of the 11.23 signal. The signal is quite broad, due to interaction with the electric quadrupole moment of the nitrogen. In trifluoroacetic acid solutions of quinoline, a signal due to the protonated nitrogen can also be observed with a chemical shift of 14 ppm (106). The $^{13}$C-NMR spectra of AHQSA in DMSO clearly showed the nine carbon signals expected, with shifts ranging from 113 to 162 ppm. No attempt was made to assign these signals.

A UV spectra of this compound showed absorption maxima at 223 and 263 mu. Addition of acid or base caused a shift in the absorbance to higher wavelengths (bathochromic shift). This observation is consistent with both acidic and basic groups being attached to the UV chromophore.

Mass spectral analysis of AHQSA was not very successful due to the low volatility of sulphonic acids. The largest fragment observed had m/e of 149 with a base peak at 66. It has been reported that tetramethylammonium salts of sulphonic acids decompose upon heating in the mass spectrometer to methyl sulphonates, which can give good spectra (107, Figure III-5). The tetramethylammonium salt of AHQSA was made by neutralizing a solution of the acid with tetramethylammonium hydroxide and then evaporating the solvent. Although trimethylamine was observed in the mass spectrum of this derivative (m/e=59), the largest fragment observed was still at 149. An attempt to make the methyl sulphonate directly, by reaction of methyl iodide with
The silver salt of the acid according to the method of Gierer (108, 109), was also unsuccessful.

The acetylated derivative was isolated and characterized by IR and NMR analysis. Two different carbonyl absorbances were noted at 1763 and 1670 cm⁻¹. These correspond to acetate ester and amide groups respectively. The presence of the two acetate derivatives was also obvious from the NMR spectrum, which showed two 3-hydrogen singlets at shifts of 2.18 and 2.46 ppm. These signals were due to the acetylated amine and alcohol, respectively.

The titration curve of AHQSA with sodium hydroxide solution (Figure III-6) shows two pKa's at pH's of 3.3 and 7.5. The first would be due to the neutralization of the sulphonic acid group, and the second to the aromatic hydroxyl at C-5. A molecular weight of 247 g/mole was calculated by noting the equivalents of base required to neutralize the known weight of acid to the first end point. This value is reasonably close to the true molecular weight of 258 g/mole.

Conclusive evidence for the identification of this catechol
co-product as 3-amino-5-hydroxy-7-quinolinesulphonic acid came from an analysis by x-ray crystallography. Crystals of sufficient size and quality proved to be difficult to grow. A number of different solvent systems and evaporation regimes were tried before suitable crystals were obtained. A freshly isolated sample of the compound was first recrystallized twice from methanol/water. The purified material was then dissolved in the minimum amount of boiling water, and then an equal volume of cold water added. The concentration of the compound in the water at room temperature was below the saturation point, so there would have to be some loss of solvent before precipitation
and crystallization could occur. Very slow evaporation was induced by placing the Erlenmeyer flask containing the solution in a tunnel of aluminum foil that had one end sealed around a nitrogen gas inlet tube. In this way, the crystallizing solution was protected from light, oxygen and dust, while a slow rate of evaporation was induced by the flow of gas over the solution. Seed crystals, selected using a microscope from previous crystallization attempts, were added periodically until enough solvent had been evaporated to make the solution saturated, at which point the seed crystals would not dissolve. About two weeks after this point, the resulting crystals were isolated and proved to be adequate for analysis.

The structure of AHQSA along with the numbering system and a three dimensional representation is shown in Figure III-7. The compound exists as the zwitterion shown in the solid form with one water of crystallization. Bond lengths and angles are shown in Tables III-1, 2, 3 and 4. Estimated standard deviations are in parentheses.

(3) 3,5-Diamino-7-quinolinesulphonic Acid

The title compound can be isolated from the product mixture formed from the reaction of catechin with ammonium sulphite in concentrated ammonia. It was chosen for isolation based on the intensity of its spot on a TLC plate, it was the compound
Figure III-7. Structural and Three-Dimensional Representations of 3-Amino-5-hydroxy-7-quinolinesulphonic Acid

present in the third largest concentration after catechol and AHQSA. This compound was isolated as the ammonium salt by preparative TLC, because it would not pass through an ion exchange column. Adsorption to the column would be expected if it had a localized positive charge similar to the protonated amino group of a 2-amino carboxylic acid in the zwitterion form.

Proton-NMR analysis showed that this compound must have a similar carbon framework and substitution pattern to AHQSA.
Again, four isolated non-labile signals were observed with small or no splitting, and with chemical shifts similar to AHQSA. The IR spectrum showed the characteristic strong absorbances of a sulphonic acid group and a rather broad doublet in the 3300 to
<table>
<thead>
<tr>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(4)-H(O4)</td>
<td>0.78(4)</td>
<td>N(2)-H(N2b)</td>
<td>0.91(5)</td>
</tr>
<tr>
<td>O(5)-H(O5a)</td>
<td>1.00(5)</td>
<td>C(2)-H(2)</td>
<td>0.99(3)</td>
</tr>
<tr>
<td>O(5)-H(O5b)</td>
<td>0.85(4)</td>
<td>C(4)-H(4)</td>
<td>0.87(3)</td>
</tr>
<tr>
<td>N(1)-H(N1)</td>
<td>0.84(4)</td>
<td>C(6)-H(6)</td>
<td>0.89(3)</td>
</tr>
<tr>
<td>N(2)-H(N2a)</td>
<td>0.95(4)</td>
<td>C(8)-H(8)</td>
<td>0.88(3)</td>
</tr>
</tbody>
</table>

Table III-3. AHQSA Bond Lengths Involving Hydrogen Atoms (in Angstroms)

<table>
<thead>
<tr>
<th>Bonds</th>
<th>Angle (deg)</th>
<th>Bonds</th>
<th>Angle (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(5)-O(4)-H(O4)</td>
<td>111(3)</td>
<td>C(3)-C(2)-H(2)</td>
<td>121(2)</td>
</tr>
<tr>
<td>H(O5a)-O(5)-H(O5b)</td>
<td>105(4)</td>
<td>C(3)-C(4)-H(4)</td>
<td>120(2)</td>
</tr>
<tr>
<td>C(2)-N(1)-H(N1)</td>
<td>119(3)</td>
<td>C(4a)-C(4)-H(4)</td>
<td>118(2)</td>
</tr>
<tr>
<td>C(8a)-N(1')-H(n1)</td>
<td>117(3)</td>
<td>C(5)-C(6)-H(6)</td>
<td>118(2)</td>
</tr>
<tr>
<td>C(3)-N(2)-H(N2a)</td>
<td>118(2)</td>
<td>C(7)-C(6)-H(6)</td>
<td>121(2)</td>
</tr>
<tr>
<td>C(3)-N(2)-H(N2b)</td>
<td>113(3)</td>
<td>C(7)-C(8)-H(8)</td>
<td>120(2)</td>
</tr>
<tr>
<td>H(N2a)-N(2)-H(N2b)</td>
<td>127(4)</td>
<td>C(8a)-C(8)-H(8)</td>
<td>122(2)</td>
</tr>
<tr>
<td>N(1)-C(2)-H(2)</td>
<td>118(2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table III-4. AHQSA Bond Angles Involving Hydrogen Atoms (Degrees)

3500 cm\(^{-1}\) region that is typical of amines. One obvious difference between the IR spectra of this compound and AHQSA, was that the strong C-O aromatic phenol absorbance at 1400 cm\(^{-1}\) present in the latter was not observed in the former.

On the basis of the above information it was concluded that this co-product must either have two sulphonic acid groups and one amino group, or one sulphonic acid and two amines as substituents on a quinoline ring system. The pattern of substitution would have to be 3, 5 and 7 again in order to give
the observed NMR spectrum. Two sulphonic acid groups were unlikely, because the relative intensity of the sulphonic acid IR absorbances to the aromatic absorbances were about the same as these parameters measured in the AHQSA spectrum. The 3,5 diamino, 7-sulphonic acid arrangement was decided on, because this is very similar in structure to AHQSA. The replacement of the hydroxyl group by an amine is very reasonable and would even be expected under the reaction conditions (see section III-C(1)). The structure of 3,5 diamino, 7-quinolinesulphonic acid as isolated, and after acidification is shown in Figure III-8.

![Figure III-8. Ammonium Salt and Acid Forms of 3,5-Diamino-7-quinolinesulphonate](image)

III-B. The Reaction of Some Model Compounds with (NH₄)₂SO₃ in Concentrated NH₄OH

Just as catechin was used a model of conifer bark polyflavanoids, it was found necessary to use models and
anallogues of catechin, in order to further understand the nature of the reaction with \((\text{NH}_4)_2\text{SO}_3\). For example, the behaviour of phloroglucinol under the cleavage reaction conditions would give information on how the catechin A-ring is modified. It was found that an understanding of a replacement reaction named after Bucherer was initially necessary. A discussion of this reaction is presented first.

(1) The Bucherer Reaction on Resorcinol and Phloroglucinol

In the presence of sulphite or bisulphite ions and heat in ammonia solution, certain hydroxylated aromatic compounds can be converted to amines. The reverse reaction also occurs; i.e., aniline derivatives can be converted to phenols in alkaline sulphite solution. These interconversions proceed by the Bucherer reaction. Compounds that will undergo this process are limited to derivatives of naphthalene (110), quinoline (111,112), resorcinol (113,114) and related compounds. Commercial applications of the Bucherer reaction have been restricted to the synthesis of naphthalene derivatives used in the manufacture of dyes (110). Accordingly, much of the more modern work on the mechanism of this reaction has been done using naphthalene and naphthylamines rather than monocyclic phenols.

The conditions required for the conversion of a suitable
phenol to an aniline derivative - a hot ammonia solution containing sulphite ion - are the same that are needed for the cleavage of tannins or catechin into catechol and other products. A study of the latter reaction is the main subject of this thesis. In fact, the cleavage reaction was first observed during an experiment to determine whether the phloroglucinolic A-ring of catechin could be aminated by this reaction. It is obvious that an understanding of the Bucherer reaction could be helpful in determining the mechanism of the cleavage reaction studied here.

Originally, it was thought that the Bucherer reaction proceeded by formation of the bisulphite addition product of the keto form of a phenol, which then decomposed to the amine (Figure III-9). This mechanism was originally proposed by Fuchs (115, 116). All the reaction steps are equilibria so the reverse transformation, aniline to phenol derivative, would go by the same mechanism, only in reverse. The most important evidence that supported this mechanism was the isolation of ketone-derived bisulphite addition products from phloroglucinol (117), resorcinol (113) and other compounds. In the case of resorcinol, action of the bisulphite leads to the introduction of a sulphonate residue in addition to the ones at the carbonyl carbons (Figure III-10).

The mechanism described above was later questioned by Rieche and Seeboth, at least for the 2-naphthols (118,119). They showed that the bisulphite adduct that can be isolated from naphthols are not 1,1-hydroxy sulphonate structures, but are in
Figure III-9. The Mechanism of the Bucherer Reaction According to Fuchs (116)

Figure III-10. Structure of the Bisulphite Addition Products of Phloroglucinol and Resorcinol

fact β-keto sulphonates. A summary of the mechanism according to these workers is shown in Figure III-11.

The evidence used to support this mechanism does not exclude the possibility of an intermediate like the sulphite
addition product shown in Figure III-9.

There has been no direct evidence for the gem-amino, hydroxy intermediate (Structure 3, Figure III-11) postulated by Seeboth. The compound that was isolated is a tetralone-sulphonic acid (structure 2, Figure III-11). In the reaction solution, this type of compound probably exists as the bisulphite adduct which decomposes to the keto form during the isolation procedure. One step in the workup of these compounds is treating with HCl gas to drive off SO$_2$ (119). Since bisulphite addition products of ketones decompose in even mild acid (120), this is probably the point at which the formation of the free tetralonesulphonic acids occurs. The exact nature of the intermediate in which the oxygen group is replaced by a nitrogen group, has not yet been determined.
The rate at which the Bucherer reaction proceeds, and in fact whether it proceeds at all, will depend on the rate of the step in which the aromaticity of the substituted ring is lost. Table III-5 shows the resonance energy (r.e.) of some aromatic organic compounds. This number provides a measure of the degree of aromatic stabilization of a given compound. These values are determined by taking the difference between the calculated and observed heats of combustion. Although there are problems with the assumptions required in calculating the theoretical heat of combustion for an aromatic compound, the stabilization energy can be used to obtain a good qualitative idea of the degree of electron delocalization (121).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Resonance Energy (Kcal/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>36.0</td>
</tr>
<tr>
<td>n-Propyl Benzene</td>
<td>35.4</td>
</tr>
<tr>
<td>Styrene</td>
<td>38.1</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>61.0</td>
</tr>
<tr>
<td>Pyridine</td>
<td>23.0</td>
</tr>
<tr>
<td>Quinoline</td>
<td>47.3</td>
</tr>
<tr>
<td>Phenol</td>
<td>36.0</td>
</tr>
<tr>
<td>Aniline</td>
<td>38.0</td>
</tr>
<tr>
<td>2-Naphthol</td>
<td>61.0</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>36.0</td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td>36.0</td>
</tr>
</tbody>
</table>

Table III-5. Resonance Energies of Some Aromatic Compounds (122)

Kinetic studies have shown that the rate of the Bucherer reaction for naphthols depends on the addition of bisulphite to a C=C and not a C-O linkage (124,125). This evidence supports
Seeboth's mechanism, where bisulphite adds first to the substituted ring and destroys its aromaticity. The resulting enol tautomerizes to a ketone (Figure III-12). The addition of the second bisulphite to the carbonyl carbon would help stabilize the compound and push the equilibria to the non-aromatic side. The change in resonance energy in this reaction series would be about the same as the difference in r.e.'s for 2-naphthol and n-propylbenzene: 61-35.4 = 25.6 Kcal/mole. This is still a substantial energy barrier, but there is another factor that has to be taken into account. Summation of bond energies shows that the keto form of a tautomer pair, >CH-C=0, is 10 to 16 kcal/mole more stable than the enol, >C=C-OH, depending on the method of calculation (123,126). If we assume an average energy difference between keto and enol forms of 13 Kcal/mole, the energy change between 2-naphthol and the tetrалonesulphonic acid would be 61-35.4-13 = 12.6 Kcal/mole.

Of course, all these numbers are approximations and can only be used in a semiquantitative manner. However this does indicate why a mononaphthol will react but phenol will not. In the latter case the difference in energy between the aromatic enol and non-aromatic ketone forms would be 36-13 = 23 Kcal/mole, a value almost twice as large as calculated for 1-naphthol.

The greater stability of the keto form explains the relative reactivities of phenol, resorcinol and phloroglucinol under Bucherer reaction conditions. Phenol is unreactive, resorcinol is relatively reactive and phloroglucinol is very reactive. For a reaction time of 2 hours at 175° C, about 50%
of the resorcinol present is converted into 3-amino phenol and about 5% into the diamino derivative. Under the same conditions phloroglucinol is converted to 1,3,5-triaminobenzene in about 80% yield. The energy difference between the enol and keto forms of resorcinol is 36-13-13=10 Kcal/mole. This value is comparable to naphthol, and the reactivity of these two compounds is similar. A 95% yield of 2-naphthylamine is obtained after reaction at 150° C for 8 hours (110). The bisulphite adduct of resorcinol (Figure III-10) also had addition of bisulphite to the ring in the 5 position which is similar to the isolated adduct of 1-naphthol. Whether the bisulphite added first to the ring or to a carbonyl of the resorcinol, is not known.

Phloroglucinol has three enols so the energy difference in this case will be negative, 36-13-13-13=-3 Kcal/mole. The negative value indicates that at room temperature a large
proportion of the phloroglucinol molecules should be in the keto form. This is not observed in the solid or in solution although many reactions of phloroglucinol require that it first tautomerize to the keto form (126). The semiquantitative nature of this type of calculation is shown here. Other factors such as the extent and strength of solvent-phenol hydrogen-bonding undoubtedly have an effect but are not taken into account.

From this analysis, there appear to be two factors that can make an aromatic compound amenable to amination via the Bucherer reaction. First, if the aromatic ring to which the hydroxyl is bonded has sufficiently low aromatic character, bisulphite can add to the ring, destroying its aromaticity and allowing the enol to tautomerize to its keto form. The ketone can then react to form the imine, and, after loss of the bisulphite, an aniline derivative. This situation occurs with mononaphthols. For phloroglucinol and related compounds, the loss of aromaticity is caused by the addition of sulphite to a carbonyl carbon of the keto form of the phenol. This intermediate goes on to form the aminated product. Whether or not amination at the carbonyls proceeds by an amino-sulphonic acid intermediate is not known. However, it has been observed that phloroglucinol can be aminated in the absence of sulphite when kept at room temperature in ammonia solution for several days (123). The presence of sulphite does speed the reaction up. However, it may do this simply by destroying the aromaticity of the ring and not forming the actual reactive intermediate. For this type of phenol amination to occur, the keto form of the phenol must be
relatively stable. If neither of these conditions are met—that is, the hydroxyl is a substituent on a ring with a high resonance energy and it has a weak ketone character—then amination doesn't occur. Phenol itself is an example of this type of compound. Of course these same arguments would be true for conversion of aromatic amines to phenols.

The question of what is the actual mechanism for the substitution of a hydroxyl by an amino function is still left unresolved. There is no firm evidence for either route (summarized in Figure III-13). However, mechanism A seems to

```
OH

\[ \text{Mechanism A} \]
```

have been based solely on the fact that gem-hydroxy, sulphite addition products of phloroglucinol and resorcinol can be isolated. The observation that a phenol with strong ketone

```
OH

\[ \text{Mechanism B} \]
```
character like phloroglucinol can be aminated in the absence of sulphite suggests that the sulphite may act as a catalyst by destroying the ring aromaticity but not participate in the actual amination. The addition of ammonia to aldehydes to form adducts and the reaction of amines with ketones to form imines are well known transformations (Figure III-14). This evidence supports mechanism B as the method by which aromatic hydroxyls are replaced by an amino function.

\[
\begin{align*}
R CO + NH_3 &\rightleftharpoons R C\equiv NH_3 &\rightleftharpoons R C\equiv NH_2 \\
R' C=O + NH_2-R'' &\rightleftharpoons R' OH &\rightleftharpoons R' C=NR'' &\rightleftharpoons R' CNR''
\end{align*}
\]

Figure III-14. The Reaction of Ammonia and Amines with Carbonyl Compounds (120)

(2) Catechol

Based on the structural similarities between catechol and resorcinol, it would be reasonable to assume that these two
compounds will behave in a similar manner under Bucherer reaction conditions. In fact, the extent of amination was found to be quite different. While resorcinol is approximately 50% aminated when reacted for 0.5 hours at 175° C, catechol does not react at all. This difference cannot be explained in terms of resonance versus ketone stabilization energy, as both compounds have the same resonance stabilization and two hydroxyls capable of enolizing to ketones. The difference appears to lie in the inherent stabilities of the keto forms of the two phenols (Figure III-15).

Figure III-15. Keto-enol Equilibria of Catechol and Resorcinol

This difference can be seen in a comparison of 1,3 and 1,2 cyclohexadiones (Table III-6). In aqueous solution, the former is almost entirely enolic while the latter is mainly in the keto form. The same trend can be seen in the alicyclic compounds
<table>
<thead>
<tr>
<th>Compound</th>
<th>Percent Enol in Dilute Water Solution</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketone</td>
<td>Enol</td>
<td></td>
</tr>
<tr>
<td>[Chemical Structure 1]</td>
<td>[Chemical Structure 2]</td>
<td>0</td>
</tr>
<tr>
<td>[Chemical Structure 3]</td>
<td>[Chemical Structure 4]</td>
<td>20</td>
</tr>
<tr>
<td>[Chemical Structure 5]</td>
<td>[Chemical Structure 6]</td>
<td>40</td>
</tr>
<tr>
<td>[Chemical Structure 7]</td>
<td>[Chemical Structure 8]</td>
<td>-100</td>
</tr>
<tr>
<td>[Chemical Structure 9]</td>
<td>[Chemical Structure 10]</td>
<td>-100</td>
</tr>
</tbody>
</table>

Table III-6. Concentration of the Enol Tautomers of Some Diketones in Aqueous Solution
acetylacetone and biacetyl. It seems probable that the same situation occurs in the monoketo tautomer of catechol and resorcinol. Structure V would be more stable than II.

In the analysis done in the previous section for resorcinol, it was assumed that the most stable non-aromatic form would be the diketone. Since it seems that the monoketo form of 1,3 diketones is more stable, the energy difference between the aromatic and non-aromatic tautomers must be even less than the 10 Kcal/mole calculated above for resorcinol. This shows the relative crudeness of these calculations. The factor that has been ignored is the resonance stabilization in the non-aromatic monoketo isomer. For resorcinol, the resonance stabilization energy of the mono-enol must be greater than the approximately 13 Kcal/mole that would be gained by tautomerizing to the diketo isomer. In comparing resorcinol and catechol for the saturated diketones, the resonance stabilization energy of the mono-enol forms should be about the same. Since it is observed that this is not true, there must be another factor involved here.

One possible explanation for the different stabilities of the monoketo forms of the two dihydroxy phenols involves the electrostatic repulsion of the dipole moments of the 1,2 carbonyl or alcohol groups when they are coplanar. For biacetyl, this repulsion seems to be greater than the stabilization of the mono-enol form with hydrogen bonding between the oxygen groups (Figure III-16), and explains why this compound has no enolic character at all.
Figure III-16. Hydrogen-Bonding in the Mono-Enol Tautomers of Acyclic Diketones

In 1,2-cyclohexanedione the carbonyls are forced to be approximately coplanar. To reduce the electrostatic repulsion, one of the carbonyls can tautomerize to the enol form, in which the alchohol group would have a smaller dipole moment than $>\text{C} = \text{O}$. The hydrogen-bonding possible in this tautomer will also help stabilize it. The result is that, in solution, about 40% of this compound is in the mono-enol form. For 1,2-cyclopentadione, where the ring is very close to being planar, the repulsion between the carbonyls will be even greater and a larger proportion of the compound will be forced into the enol form. As noted before (Table III-6), 1,2-cyclopentadione is entirely in the mono-enol form in solution.

From this reasoning, it follows that catechol is less reactive than resorcinol to amination via the Bucherer reaction because the most stable non-aromatic catechol tautomer is not as

\[ \text{For example, the dipole moments of formaldehyde and methanol in the gas phase are 2.33 and 1.70 debyes, respectively (131).} \]
stable as the corresponding resorcinol isomer. The greater stability of the latter means that the energy barrier to the non-aromatic sulphonated intermediate is small. Thus, resorcinol is more reactive under these condition.

(3) Wattle Bark Extract

As shown in Figure I-18, wattle bark polyflavanoids have a different hydroxyl substitution pattern from catechin or western hemlock bark flavanoids. Instead of the phloroglucinolic A-ring, this part of the monomeric unit of the wattle tannin is derived from resorcinol. Reaction of wattle bark extract with \((\text{NH}_4)_2\text{SO}_3/\text{NH}_3\) at 175°C for two hours not only produced catechol as expected, but also resorcinol and some hydroxy aniline derivatives. Analysis of the volatile aromatics produced in this reaction was done by GC-MS. The computerized spectra search routine used to identify the products could not distinguish between 3-aminophenol and 2-aminophenol or the corresponding diaminated products. In order to determine whether these aniline derivatives were ortho or meta substituted, the GC retention times were compared to the products obtained from the amination of resorcinol. The retention times were approximately the same, indicating that the hydroxy-aniline and diaminobenzene isolated from the wattle extract reaction were meta substituted and so derived from resorcinol. This conclusion was verified by co-injection of the
acetylated products from both reactions. At the appropriate retention times only one peak was observed. The ratio of the peak areas due to resorcinol, 3-amino-phenol and 1,3-diaminophenol were approximately the same in both reactions.

The conclusion that can be drawn from these data is that, in wattle tannin polyflavanoids, both the A- and B-rings can be cleaved off. This observation provided valuable clues in the determination of the mechanism of the cleavage reaction.

III-C. A Mechanism for the Cleavage of Catechin into Catechol and Quinoline Derivatives

The products that have been isolated from the cleavage of catechin are shown in Figure III-17. Compound I can be isolated in larger quantities than II and is assumed to be the major co-product of catechol. It is obvious that the catechol must be derived from the B-ring and the two quinoline products from the A- and C-rings of catechin. In light of the discussion on the Bucherer reaction, the relationship between I and II is quite clear. Amination of the phloroglucinolic A-ring has simply proceeded to a greater extent in II. The latter compound cannot be directly produced from the 5-hydroxy derivative (see Figure III-18 for numbering system), as it was observed that heating pure I with sulphite in ammonia does not produce II. In retrospect, this result should have been expected, since the benzene ring of the quinoline could enolize only at the 5-
position so its keto tautomer would be relatively unstable. The position meta to the hydroxyl (C-7), where the sulphite would add in a naphthalene-type mechanism, is already occupied by a sulphonic acid group. A second sulphur group adding to this position would be very unlikely due to steric hindrance. It is evident that the 7-amination must occur at some intermediate stage in the cleavage reaction. The probable point in the reaction sequence where this amination would occur is mentioned below.

In the conversion of catechin to I and catechol, there are some substitutions and other reactions that must occur:

2. Substitution of the C9 hydroxyl by an amino group.
Figure III-18. Standard Numbering System For Quinoline Derivatives

(5) Substitution of the C7 hydroxyl by a sulphonic acid group.
(6) Dehydrogenation to form a double bond between C3 and C4.
(7) Substitution of the C3 hydroxyl by an amino group.

These steps are not necessarily presented in the order in which they would occur in the complete reaction.

The breaking of the catechin ether linkage probably occurs first. This reaction has been investigated by other workers and probably proceeds by the mechanism shown in Figure III-1 to form a C2 sulphonated derivative. As mentioned earlier, it was demonstrated that this compound is an intermediate in the cleavage reaction to catechol. It could be isolated when catechin was reacted with sulphite in ammonia solution at low temperature. If a portion of the solution containing the ring-opened compound was then reheated at 175° C, catechol and the quinoline compounds are obtained in yields comparable to the direct reaction of catechin with \((\text{NH}_4)_2\text{SO}_3/\text{NH}_3\).
(1) Amination on the Phloroglucinolic A-Ring

The amination at C9 could only occur after the pyran B-ring had been opened. At this point the A-ring would take on many of the characteristics of phloroglucinol including its strong ketone character. The initial addition of sulphite would probably occur at the least sterically hindered position - para to the alkyl group. Amination could then proceed at the other two sites (see Figure III-19). This is the point at which the C5 amination may occur and determines whether the final quinoline product is 3-amino-5-hydroxy-7-quinolinesulphonic acid (I) or 3,5 diamino, 7-quinolinesulphonic acid (II).

(2) Amination at C3

Looking backwards from the final products, the most likely point for the amination at C3 to occur would be at the end of the sequence after the aromatic quinoline structure has been formed. The Bucherer reaction has been reported to occur with quinoline derivatives, although only compounds with the hydroxyl on the benzene ring have been investigated (111, 112). In this case, the hydroxyl is on the pyridine ring. Calculation of the energy difference between the aromatic and keto forms of a $\beta$-hydroxy quinoline using values from Table III-5 gives a value of -1.1 Kcal/mole:
Figure III-19. Introduction of the C5 and C9 Amino Groups
47.3 (r.e. of quinoline) - 35.4 (r.e. of Benzene) - 13 (Ketone stabilization energy) = -1.1 Kcal/mole

The small negative number indicates that the keto form would be relatively stable. In fact, it has been reported that bisulphite easily adds to pyridine itself at the 4 position (132). With this information, the amination can be envisaged to occur as shown in Figure III-20.

Figure III-20. A Mechanism For the Amination at C3
When the structure of the quinoline product is compared to the catechin starting material, it is evident that a dehydrogenation must occur at some point in its formation. One of the double bonds in the heterocyclic ring could result from the elimination of the B-ring, but the other could only be formed in some oxidation step, since there is no other change in the substitution pattern in the A- and C- ring residues. There are at least three ways in which this dehydrogenation could occur:

(a) Oxidation by an inorganic constituent of the reaction solution.

(b) Oxidation by molecular oxygen.

(c) Disproportionation.

In strongly basic solution, the sulphite ion has a strong reducing action (133). Of course, sulphate can act as an oxidizing agent by the reverse of this half-reaction. However, sulphate is present only as a very minor impurity in the \((\text{NH}_4)_2\text{SO}_3\) used as the reagent and could only act with a relatively strong reducing agent. The possibility of an inorganic oxidation can be ruled out.

Atmospheric oxygen has been known to act as an oxidizing agent of alkenes (134). This possibility was tested using different reaction conditions. In one case, the solution
containing catechin, sulphite and ammonia was saturated with nitrogen by slowly bubbling the gas through the reaction solution. An N\textsubscript{2} atmosphere was kept in the tube while it was flame-sealed. In this way the extent of the reaction in the absence of oxygen could be tested. Another tube was prepared in the same way but using oxygen gas. In this case the reaction would proceed in the presence of a large quantity of oxygen. A third control tube was also prepared in the usual manner with no gas bubbling done at all. There was no significant difference in the amount of catechol produced from the three experimental solutions after reaction at 175° C. The obvious conclusion is that oxygen does not participate in the cleavage of catechin to catechol and quinoline derivatives.

Disproportionation is an auto-oxidation reaction whereby one molecule of a compound oxidizes another identical or closely related molecule. The result is two products, one with a higher and the other with a lower oxidation state than the starting material. Some examples of disproportionation are given in Figure III-21. In the third example the quinone starting material oxidizes its addition product to form the substituted naphthoquinone. The potential of the substituted quinone product is much less than that of the starting material, so the reaction is essentially quantitative; two moles of naphthoquinone are required to produce one mole of the substituted product. Quinones can act as very efficient dehydrogenating agents.

The intermediate formed in the sulphonation of catechin
Figure III-21. Examples of Disproportionation
(see Figure III-1) is called a quinone methide. This type of compound undergoes many of the reactions of quinones and could also be expected to act in a similar manner in self-oxidation reactions. Disproportionation seems to be the most likely mechanism for the oxidation step in the cleavage process. Likely points at which this step could occur are presented later.

(4) Cleavage of the C2-C1' Bond

This is the most important step in the cleavage mechanism, since it is the point at which the catechol molecule is formed. Methods for the breaking of carbon-carbon bonds are relatively few in number and usually occur only with a good leaving group. At first glance, the catechol B-ring of catechin appears to be a poor candidate for this function. However, at the high temperature and basic conditions required for this cleavage reaction, the catechol ring will tautomerize rapidly. One of the tautomeric forms (III, Figure III-22) would be a relatively good electron-acceptor and leaving-group.

One possible mechanism for breaking the C2-C1' bond is a simple β-elimination as shown in Figure III-23. The dehydrogenation at C3-C4 would have to occur first, so the driving force for the elimination would be the formation of the stable quinoline aromatic structure. However, this mechanism is unlikely because it will not work for elimination of the A-ring
as was observed in the wattle bark tannin. It is not possible
to end up with an aromatic product from A-ring elimination since the catechol B-ring does not have a hydroxyl group ortho to the C2-C1' bond that can be aminated to eventually form the nitrogen in the quinoline heterocyclic ring. Another mechanism would have to be proposed for A-ring cleavage that would also explain why phloroglucinol (or 1,3,5-triaminobenzene) is not formed from catechin. Also, β-elimination does not suggest how the rather anomalous substitutions of the hydroxyl at C7 with sulphite occurs. The breaking of the C2-C1' bond and the substitution reaction are both unexpected and likely to be linked in some way. This makes an elimination mechanism like this unlikely.

The observation that the cleavage can go either way in wattle tannin to form resorcinol and catechol is a very important one. If the complication of two different mechanisms for A- and B-ring cleavages is to be avoided, then the cleavage will have to occur at a rather symmetrical intermediate. The 1,3-diarene-propane structure of sulphonated catechin fulfills this requirement. The equivalent sulphonated wattle monomer (fisetinidol) is even more symmetrical since the two aromatic rings are more similar in reactivity and structure (Figure III-24).

As was mentioned in the previous section, the quinone-methide intermediate in the sulphonation of catechin could act as the oxidizing agent in a disproportionation reaction. If the self-oxidation was to happen at this point, two products would be formed, one of which would have an extended conjugated structure (Figure III-25). Sulphonation of this compound is
most likely to occur at the carbon alpha to the catechol ring, because of the steric hindrance of the hydroxyl groups ortho to the carbon chain on the phloroglucinol ring. With a resorcinolic A-ring, there is only one ortho hydroxyl, so sulphonation at the A-ring alpha carbon would proceed more readily in wattle tannins.

An extended type of elimination can be envisaged to occur in the dehydrogenated, C2-sulphonated catechin (Route A, Figure III-26). This cleavage mechanism is closely related to heterolytic fragmentation as described by Grob (138,139,140). In this class of reactions, a molecule symbolized by $a-b-c-d-x$ cleaves into three fragments: $a-b$, $c=d$ and $x$. The letters $a$, $b$, $c$ and $d$ represent a sequence of atoms such as C, O, N, S, P or B. Basically, the mechanism involves electron-donation from one end of the molecule ($a-b$) to a leaving group ($x$) with the formation of an unsaturated intermediate fragment ($c=d$).

$$a-b-c-d-x \rightarrow a-b + c=d + x$$

Some examples of the types of compounds and the fragments
they form during heterolytic fragmentation are shown in Table III-7. In the case of the modified catechin reaction, the phloroglucinol ring acts as the electron-donating group and the catechol ring as the leaving group. The middle group does not physically separate from the A-ring; there is just a shift in the double bonds. For catechin, the cleavage at the A-ring (Route B, Figure III-26) is less likely because of:

1. Steric hindrance to sulphonation at C4.
2. A higher energy barrier in Route B. The aromaticity of the catechol ring is lost which requires a larger input of
Figure III-26. Formation of Catechol and Phloroglucinol by an Extended Elimination from Sulphited, Dehydrogenated Catechin

energy as compared to route A where the aromaticity of the phloroglucinol ring is lost (see sections III-B.(1) and (2)).

(3) The inability of the non-aromatic product of Route B to stabilize by rearrangement to an aromatic product (see below).

The first two reasons listed do not apply with a resorcinolic A-ring, and so explain why more of the Route B type
Table III-7. Examples of Some Common Electron-Donating, Middle and Leaving Groups Found in Compounds Undergoing Heterolytic Fragmentation (138,139)
reaction to cleave off the A-ring occurs in the cleavage of wattle tannin.

(5) Rearrangement of the Non-Aromatic Catechin Cleavage Product

As mentioned previously, ketones form an addition product very readily in concentrated sulphite solution. This is also likely to happen with the quinone methide cleavage product. A mechanism for the addition and subsequent rearrangement of the aminated non-aromatic cleavage product to a sulphonated quinoline is presented in Figure III-27. At C7, the hydroxyl is lost, rather than the sulphite, because the latter is already negatively charged. There would be a strong electrostatic repulsion to the addition of another electron pair to the sulphite group. The driving force for the final elimination of bisulphite (step 3) is the subsequent formation of the aromatic quinoline system. A related reaction has been described by Lauer and Langkammerer (141). These authors demonstrated the formation of 3-phenolsulphonic acid from the action of sodium bisulphite on resorcinol (see Figure III-28).

A summary showing the key intermediates in the proposed mechanism for the cleavage of catechin to catechol and 3-amino-5-hydroxy-7-quinolinesulphonic acid is presented in Figure III-29.
In an attempt to test the cleavage mechanism described above for catechin, a related compound, dihydroquercetin (DHQ), was subjected to the same reaction conditions, and the nature and yield of the products examined. The C-ring of DHQ is in a higher oxidation state than the comparable part of the catechin molecule since there is a ketone at C4. It was thought that in this case the C4 ketone would tautomerize to an enol, making disproportionation unnecessary to get to a cleavable intermediate. If this did occur, the yield of catechol should double, since it would not be necessary for half of the starting material to act as an oxidizing agent. The reduced product of the disproportionation shown in Figure III-29 cannot proceed by the proposed mechanism to form catechol. It was expected that
DHQ would react as shown in Figure III-30.

In fact, no catechol at all was observed in the product mixture. The only solvent-extractable product observed was 1,3,5-triaminobenzene, the Bucherer reaction product of phloroglucinol. What appears to have happened is that the ketone formed a sulphite addition product first that prevented any possible enolization. Even in its underivatized form, the ketone probably has very little enolic character due to the hydrogen bonding possibilities with a hydroxyl on the A-ring. In the adduct form, a double bond cannot form between C3 and C4, so the mechanism for catechol formation described above is not possible. However A-ring cleavage is possible and does appear to occur. A mechanism for phloroglucinol formation from DHQ is
Figure III-29. Key Intermediates in the Proposed Mechanism for the Cleavage of Catechin to Catechol and 3-Amino-5-hydroxy-7-quinolinesulphonic Acid
Figure III-30. The Expected Reaction Sequence of Dihydroquercetin with $(\text{NH}_4)_2\text{SO}_3/\text{NH}_3$
Figure III-31. A Mechanism for the Production of Phloroglucinol from Dihydroquercetin
(7) Cleavage of Western Hemlock Bark Tannin

The reaction mechanism described above for the cleavage of catechin should also apply to the cleavage of catechin-based tannins due to their structural similarities. However, the polymeric nature of the latter material with their globular, tightly packed conformation could cause accessibility problems. Sulphite has to be able to reach C2 and C7 and the disproportionation step would have steric requirements as well. These factors would contribute to the observed lower yield of catechol from western hemlock tannins.

It is well known that conifer tannins rapidly degrade after tree harvesting and drying of the bark or after extraction by organic solvents. The degradation appears to be due to polymerization reactions that raise the average tannin molecular weight. This was the cause of the reduced catechol yields from aged bark. The best results were obtained with fresh material. Isolation by solvent extraction also seems to affect the polymer structure as the highest catechol yield (see Chapter II) was obtained using fresh whole bark rather than an ethanol extract.

GC-MS analysis of the solvent-extractable phenolics formed from the cleavage of western hemlock bark tannins, showed the major products to be catechol and phenol. Minor products observed were methyl phenol, methoxyphenol and dimethoxyphenol.
Para-hydroxybenzoic acid has been reported to be an alkali fusion product of western hemlock bark tannin (17) which implies that a certain proportion of the B-rings in the tannin polymer are mono-hydroxylated. Phenol would be produced from these groups during the cleavage reaction rather than catechol. This explains the phenol observed in the reaction product mixture. The other phenolics, as well as a certain proportion of the catechol and phenol, are probably produced from the decarboxylation of the corresponding para-hydroxy carboxylic acids, most of which have been reported to be a component of western hemlock bark (see Figure 1-3). Under the cleavage reaction conditions, protocatechuic acid decarboxylates to form catechol in high yield.

III-D. Summary

A mechanism for the cleavage reaction of catechin was proposed partially based on the structures of an intermediate and two catechol co-products. A low temperature reaction of catechin with ammonium sulphite formed 1-(3,4-dihydroxyphenyl)-2-hydroxy-3-(1,3,5-trihydroxyphenyl)propanesulphonic acid. If heated to 175° C with the ammonium sulphite reagent, this compound reacts further to give catechol, showing that it is an intermediate in the cleavage of catechin. The two co-products isolated along with catechol were 3-amino-5-hydroxy-7-quinolinesulphonic acid and 3,5-diamino-7-quinolinesulphonic
acid. Other information on the reaction mechanism of catechin came from the identification of reaction products obtained under the same conditions from related compounds such as phloroglucinol, dihydroquercetin and wattle tannin.

The reaction requirements of high temperature, high concentration of ammonia and the presence of sulphite ion are the same as those needed for the Bucherer reaction, a classic substitution reaction by which certain hydroxylated aromatic compounds can be converted to aniline derivatives and vice versa. An understanding of this reaction proved to be critical in understanding the cleavage of catechin.

The reaction mechanism envisaged for the cleavage of catechin into catechol is summarized below (See Figure III-29).

1. Opening of the catechin C-ring to give the isolated 2-sulphonic acid intermediate.
2. Disproportionation of the quinone-methide in equilibrium with the sulphonic acid to give two products with the same carbon backbone but in different oxidation states.
3. Amination of the phloroglucinolic A-ring of the higher oxidation state product via the Bucherer reaction.
4. Cleavage to form catechol and a 9-carbon quinone methide by a mechanism related to heterolytic fragmentation.
5. Cyclization to form a partially aromatic bicyclic compound.
6. Loss of bisulphite to give the fully aromatic quinoline structure.
CHAPTER IV. FORMULATION OF ADHESIVES AND BOND EVALUATION

The underlying purpose of the research presented in this thesis was to develop a commercially feasible adhesive from western hemlock bark. This aim was accomplished in that catechol, produced here from the cleavage of western hemlock tannin, has been used successfully in adhesive applications by other workers (101, 142). Simply isolating catechol from the reaction mixture and testing it as an adhesive base would not have been useful as this kind of work has already been done. Instead, the adhesive properties of the entire complex product mixture was evaluated. It was hoped that the catechol and other simple phenols produced would act as crosslinking agents. The most successful tannin-based adhesives use a low-molecular weight PF resin as a fortifier; methylolated catechol could fulfill a similar function.

The approach to adhesive development used in this work was to first develop the preparation conditions and formulation components using a fast qualitative method of bond evaluation. When good results were obtained on a consistent basis, then "fine-tuning" was done using quantitative bond-testing. In order to minimize the number of variables, the same batch of bark was used in all the bonding work. After collection, the bark was ground as quickly as possible and stored at $0^\circ$C to maintain the quality of the bark.
(1) Qualitative Adhesive Development

The basic technique used here was to bond two 10cm X 30cm strips of veneer together at right angles so that there was 100 cm² of glueline. After cooling, the assembly was manually twisted apart and the quality of the bond evaluated based on the extent of wood failure. A 175°C press temperature and 2 minute press-time were chosen. These rather extreme press conditions should allow formation of a good bond if it is at all possible, thus reducing the number of variables involved. If the results were encouraging, then milder conditions could be investigated.

The crude, alkali-soluble reaction mixture obtained from the filtration of the reacted bark slurry was not used 'as is' because of the low solids content and high concentration of inorganic ions ((NH₄)₂SO₃). As well as removing water, evaporation of the solution also resulted in the loss of NH₃ and neutralization to about pH 7.5. An alkali-soluble precipitate formed at this pH was filtered out. This material was designated as the high molecular weight fraction (HMWF). To remove the excess (NH₄)₂SO₃ from the filtrate, either ethanol or a hot aqueous solution of Ba(OH)₂ was added and the precipitate filtered out. The alcohol reduced the solubility of the (NH₄)₂SO₃ in solution while the Ba(OH)₂ formed BaSO₃ which is only slightly soluble in cold water (0.02g/100ml at 20°C, 143). The filtrate was evaporated to give the catechol-containing organic fraction called the low molecular weight fraction (LMWF). In adhesive formulation, the two fractions were used in the same ratio in which they were produced.
The glue formulation described by Steiner and Chow (48) was used as a starting point for the adhesive development:

Bark Extractives 70g (35% Solids)
Modal 8g
Wheat Flour 6g
Water 10g
Paraformaldehyde 6g
pH - 6.5-7.0

Modal was not available, so CoCob was used as a filler instead. Using a formulation similar to the one above, the effect of pH on bond quality was investigated. It was found that the best bond was obtained with a pH between 10 and 11. However, under these basic conditions it was found that paraformaldehyde could not be used as the formaldehyde source, because it caused rapid gellation of the adhesive. The less reactive formaldehyde source, hexamine, had to be used instead. Varying the assembly time showed that the best results were obtained with an open assembly time of 20 to 25 minutes. Closed assembly times were not investigated. The last important formulation parameter investigated in this phase was the effect of a pre-reaction with formaldehyde before bonding. It was hoped that a pre-reaction of the low molecular weight fraction would produce methylolated catechol and low molecular weight catechol polymers that would act as crosslinking agents between the large tannin and tannin residue molecules. The LMWF was heated at 100°C for various times up to one hour with about 20%
of the total formaldehyde to be added (as paraformaldehyde) at pH 10.5. The resulting methylolated phenolic solution was then combined with the HMWF, filler and hexamine, and then tested. A pre-reaction time of 1 hour gave the highest bond strength. The best adhesive preparation procedure and formulation determined qualitatively was as follows:

2 ml LMWF + 0.1 g NaOH + 0.14 g Paraformaldehyde (heated to 100°C for 1 hour)
10 g HMWF
1 g H2O
1.2 g Hexamine
2 g Cocob
0.4 g Wheat Flour
pH = 10.5, solids content-45%, Assembly Time - 25 min

Two-ply samples glued with this adhesive gave very good dry strength (about 95% wood failure) and wet strength with some wood failure.

(2) Quantitative Adhesive Development

With the qualitative development of the adhesive to a reasonable level, the next step was to evaluate its strength and develop it further quantitatively. Work on this phase began about one month after the end of the development described in the previous section (approximately two months after collection.
of the bark).

A 12 X 18 inch three ply panel was prepared using a larger scale preparation of the adhesive formulation above, and tested for dry and wet shear strength. The results are summarized in Table IV-I. These were not the findings expected. The dry strength was very low and the shear samples fell apart while soaking in water. Qualitative testing of this formulation also gave poor results.

<table>
<thead>
<tr>
<th>Dry Strength</th>
<th>Wet Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shear Strength(psi)</td>
<td>Wood Failure(%)</td>
</tr>
<tr>
<td>125</td>
<td>10</td>
</tr>
<tr>
<td>105</td>
<td>10</td>
</tr>
<tr>
<td>80</td>
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<td>20</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>80</td>
<td>0</td>
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Table IV-1. Results of Shear Tests On a Three-Ply Panel

At first it was thought that an error had been made either in the reaction and preparation conditions or in the formulation. Another batch of adhesive was prepared but again gave the same poor results. The best explanation was that the bark had degraded in storage even though it was kept in a sealed
bag at 0° C. This conclusion was confirmed by testing a small sample for catechol yield. Only about one quarter of the yield from fresh bark was observed. It was also found that paraformaldehyde could be used instead of hexamine in the adhesive formulation. This was another indication of the lower reactivity of the products obtained from the degraded, older bark.

More quantitative bond evaluation was done using the degraded bark in order to gain more information about the system. In all cases no wet strength was observed. The figures reported are for dry strengths only. The results, with a statistical analysis are given in the Appendix. First, reaction product isolations were done using different amounts of Ba(OH)$_2$ (30, 40 and 50 grams) to precipitate out the sulphite. Uniformly poor results were obtained with very little wood failure. Next, the quality of bonds produced using the entire reaction product mixture, including unreacted ammonium sulphite, were tested. Again, poor results were obtained, but it was interesting that the largest amount of ammonium sulphite in the reaction mixture also gave the best bond strength. It seems that the additional strength resulting from the higher concentration of low molecular weight products obtained from the cleavage reaction with the higher $(\text{NH}_4)_2\text{SO}_3$ concentration overcame the disadvantage of the higher inorganic salt concentration. Finally, glueline loadings and paraformaldehyde contents were evaluated using a smaller number of shear samples. Lower adhesive loading and smaller paraformaldehyde
concentrations than had been used previously were found to give significantly better results. In the best situation, with a glueline loading of 1.94 g/100cm$^2$ and 0.2 g paraformaldehyde per 5.9 g of adhesive, an average dry shear strength of 220 psi with 60% wood failure was observed, but no wet strength was evident.

(3) Summary

Qualitative and quantitative testing of adhesive formulations based on the mixture of organic compounds produced from the cleavage of western hemlock bark tannins were done using standard techniques. Although early results from the qualitative testing were promising, they could not be duplicated later quantitatively. It was found that the quality of the bark used and its corresponding ability to undergo the cleavage reaction, degraded rapidly during storage. Adhesives made with bark stored for more than two months did not have any wet strength at all. Assembly time, methylation of low molecular weight phenolics, paraformaldehyde content and adhesive loading were found to be factors important in determining the strength of a bond made with this type of bark-based adhesive.
CHAPTER V. SUMMARY

Over the past 35 years a great deal of research has been done world-wide on the problem of using tree bark tannins as substitutes for the petroleum derived adhesives widely used in the manufacture of forest products. In some situations, particularly in South Africa with wattle tannins, there have been successful applications. In British Columbia, the tannins from western hemlock bark have attracted the most attention. The problems found with using tannin from this species as adhesives, and from conifers in general, are:

(1) Low bond strength due to the high average molecular weight of the bark tannins and relatively few crosslinking sites.
(2) Overly high reactivity with formaldehyde making the polymerization reactions rather difficult to control and resulting in a short adhesive pot-life.
(3) Variable bark quality, resulting in variable bond strengths for a conifer-tannin based adhesive. For western hemlock in particular, the bond quality seems to depend on the age and past history of the bark from which it is extracted.

Some approaches taken by other workers to solve these problems have been:

(1) Ultrafiltration to isolate the optimum molecular weight range of the tannins.
(2) Breakdown of the tannin polymer into sulphited lower molecular weight oligomers and monomers.
(3) Using special tannin isolation and adhesive formulation
techniques such as slow formaldehyde-release agents, specialized extraction techniques and high press-temperatures.

The approach to the problem taken here was to break down the tannin into simpler phenolic molecules that would be easier to utilize as an adhesive base. A reaction was investigated that cleaves catechin (a model compound for conifer tannins) into catechol and quinoline derivatives. This reaction can also be applied to western hemlock bark extracts, in which case some phenol is produced as well. The properties of catechol and phenol in adhesive mixtures with formaldehyde are well known. If these products were isolated from the product mixture they could be used to make excellent adhesives.

After development of a quick GC-based assay procedure for catechol, the optimum reagent and conditions for production of catechol from catechin were determined to be a solution of 30% ammonium sulphite in concentrated ammonium hydroxide heated to 175°C for 2 hours. Under these conditions a 35% molar yield of catechol was obtained.

A mechanism for the cleavage reaction of catechin was proposed partially based on the structures of an intermediate and two catechol co-products. The compound 1-(3,4-dihydroxyphenyl)-2-hydroxy-3-(1,3,5-trihydroxyphenyl)propanesulphonic acid was formed from a low temperature reaction of catechin with ammonium sulphite. If heated to 175°C with the ammonium sulphite reagent, this compound reacts further to give catechol. The two co-products
3-amino-5-hydroxy-7-quinolinesulphonic acid and 3,5-diamino-7-quinolinesulphonic acid. Other information on the reaction mechanism of catechin came from the identification of reaction products from related compounds such as phloroglucinol, dihydroquercetin and wattle tannin, obtained under the same conditions.

The reaction requirements of high temperature, high concentration of ammonia and the presence of sulphite ion are the same as those needed for the Bucherer reaction, a classic substitution reaction by which certain hydroxylated aromatic compounds can be converted to aniline derivatives and vice versa. An understanding of this reaction proved to be critical in understanding the cleavage of catechin.

The reaction mechanism envisaged for the cleavage of catechin into catechol is summarized below (See Figure III-29).

1. Opening of the catechin C-ring to give the isolated 2-sulphonic acid intermediate.
2. Disproportionation of the quinone-methide in equilibrium with the sulphonic acid to give two products with the same carbon backbone but in different oxidation states.
3. Amination of the phloroglucinolic A-ring of the higher oxidation state product via the Bucherer reaction.
4. Cleavage to form catechol and a 9-carbon quinone methide by a mechanism related to heterolytic fragmentation.
5. Cyclization to form a partially aromatic bicyclic compound.
6. Loss of bisulphite to give the fully aromatic quinoline structure.
Adhesive formulations based on the mixture of organic compounds produced from the cleavage of western hemlock bark tannins were qualitatively and quantitatively tested for strength using standard techniques. Although early results from the qualitative testing were promising, they could not be duplicated later quantitatively. This was the result of the quality of the bark used and its ability to undergo the cleavage reaction, degrading fairly rapidly during storage. Adhesives made with bark stored for more than two months did not have any wet strength at all. Some factors found to be important in determining the strength of a bond made with this type of bark-based adhesive were assembly time, methylation of low molecular weight phenolics, paraformaldehyde content and adhesive loading.

The results of the bonding experiments were not as good as they might have been. In retrospect, these poor bond strengths could have been foreseen. It is likely that the cleavage reaction in tannins proceeds by the same mechanism as with catechin. If this is true, there should be sulphonation of the remaining tannin aromatic rings after the catechol B-ring has been split off. The sulphonic acid group is a strong electrophilic substituent, and on an aromatic ring would have a deactivating effect on electrophilic aromatic substitution. This means the substituted ring would be less reactive to formaldehyde than might be expected. If the aromatic ring is also aminated and in the zwitterion form as was observed with AHQSA, the material will be even less reactive, as the \(-\text{NH}_3^+\)
substituent has a very strong deactivating effect. Probably the bond strength that was observed was only due to the free catechol, phenol and unreacted tannin molecules. It is unlikely that good bond strengths can be achieved using the whole mixture of organic compounds produced from the cleavage of western hemlock bark tannins.

The possibility still remains of using only the catechol and phenol produced by the reaction as an adhesive base. These compounds can be isolated very easily from the reaction mixture by solvent extraction. However, at the present state of development, the reaction probably does not have a high enough yield to make it commercially feasible.

Based on the mechanism of the cleavage reaction proposed, there appears to be two important factors limiting the phenolic yield from bark tannin. First, by the nature of the products formed from catechin, it is necessary that there be an oxidation step in the reaction sequence leading up to the elimination of catechol. The most likely oxidizing agent is a quinone methide as discussed in section III-C(3). After the methide has been reduced it could not rearrange further and eliminate catechol by the type of mechanism described. This could account for the yield from catechin being less than 50%; half of the potential catechol-producing material is not reactive after being reduced. It is possible, that if a reagent capable of performing this oxidation step was added to the reaction mixture, the yield of catechol from catechin (or tannin) could be doubled.

The second problem concerns the nature of the bark tannin
molecule. As discussed earlier, the tannin polymer probably has a randomly-oriented, globular configuration in solution, with strong intramolecular hydrogen-bonding and perhaps some covalent crosslinking as well. Not only will a conformation of this type limit accessibility of the reagents to the interior of the molecule, but the rotational freedom of some bonds would be severely limited and may not allow reactive centers to approach close enough to permit the cleavage reaction to occur. If the tannin polymer could be broken down into smaller units, the reaction would probably go more readily and to a greater extent.

Recently, Foo et al. (144) reported that Pinus taeda L. bark tannin was cleaved at the interflavanoid bonds by NaHSO$_3$ when refluxed for 24 hours in water. A 20% yield of monomeric sulphited tannin was reported. If this type of reaction also occurs with western hemlock bark tannins, pre-reaction of the tannin with NaHSO$_3$, before attempting the cleavage reaction may improve the yield of simple phenols.

The combination of an added oxidizing agent and pre-cleavage of some interflavanoid bonds may bring the yield of simple phenols derived from softwood bark by this reaction to commercially acceptable levels. Experiments investigating these approaches were not done here. They are a logical 'second phase' to the preliminary investigations discussed in this thesis.
CHAPTER VI. EXPERIMENTAL

VI-A. General Information

(1) Spectroscopy and Chromatography

Infrared (IR) spectra were obtained using a Perkin Elmer 681 Infrared Spectrophotometer. Samples were prepared either as a thin solid film on an AgCl plate or as a KBr pellet. The proton nuclear magnetic resonance (P-NMR) spectra were taken on Varian Associates spectrometric models XL-100 or HA-100 (100 MHz) and a Bruker WP-80 (80 MHz) instruments. DMSO-d6 (Sigma, 100% deuterated) and acetone-d6 (Sigma, 95% deuterated) were the deuterated solvents normally used, with tetramethylsilane (TMS) as an internal standard. Signal positions are given in parts per million (ppm) downfield from the internal standard signal (delta). 13C-NMR spectra were determined on a Varian CFT-20 spectrometer. Ultraviolet (UV) absorption spectra were obtained on a Unicam SP8000 UV Recording Spectrophotometer. Low resolution mass spectrograms were carried out on a Varian/MAT CH4B mass spectrometer while GC-MS work was done on a Hewlett Packard 5985B system. The capillary column used in GC-MS analysis contained a SE-54 liquid phase. The components of product mixtures analysed by this technique were identified by use of a computerized mass spectrum library search routine and conventional analysis of the individual mass spectrum. Spectra are listed as m/e (relative abundance).

Analytical gas liquid chromatography was done on a Hewlett Packard 7620A Research Chromatograph equipped with a 2 ft. long, 0.25 inch O.D. glass column packed with CSP-633
(Chromatographic Specialties). Integration of peaks was performed by an attached Hewlett Packard 3370B Integrator. An on-column injection system was used in this case. This system was used for analysis of mixtures containing polar compounds such as phenols. For non-polar compounds, a 6 ft. X 0.125 inch stainless steel column containing 5% OV-17 on Chromosorb W was used.

Analytical thin-layer chromatography (TLC) was carried out on Whatman K5F pre-coated silica gel plates containing a fluorescent indicator. Visualization was done by staining with I$_2$ vapor or visualization with short-wavelength ultraviolet light. The 20 cm X 20 cm plates were normally cut into 4 cm X 10 cm pieces for analytical work. Preparative TLC was done on full-size Whatman PLK5F plates. Unless otherwise noted, the developing solvent system was ethyl acetate/isopropanol/water in proportions of 50:25:11. Solvent ratios are volume to volume. All solvents for chromatographic or reaction medium applications were distilled before use. Elemental analyses were done by Canadian Microanalytical Services.

(2) General Methods

Small plywood panels were made with an Elmes Engineering Works 15 inch X 15 inch hot press. The panels were cut into standard 1 inch X 3 inch shear test samples with a 1 in$^2$ test area. The shear samples were cut such that in half of the test
pieces, the lathe checks were pulled closed upon testing while in the other half the lathe checks were pulled open.

Moisture contents (M.C.) are reported on a net basis, e.g. MC=weight of H₂O/weight of H₂O + oven-dry weight of bark. Evaporation of solvents was done using a rotary evaporator with a bath temperature of about 43° C

Melting points were determined using a Fisher-Johns Melting Point Apparatus and are uncorrected.

(3) Materials

Western hemlock bark was collected on two occasions. On May 5, 1982, approximately 20 kg of bark was collected from two logs about 60 cm in diameter that had been felled 10 days previously at the UBC Research Forest, Haney, B.C.. After stripping from the logs, the bark was coarsely chopped into pieces not exceeding 25 cm² in area and let air-dry for 72 hrs. No attempt was made to separate inner and outer bark layers. The dried bark was then passed through a #2 Wiley Mill using a screen with 5 mm openings. After further air-drying overnight, the bark was ground in a Pallman grinder to about 200 mesh. At this point, the moisture content was 10.87%. The ground western hemlock bark was stored in a freezer at -10° C. This material was used in the work described in Chapters II and III.

The second batch of western hemlock bark was collected on March 13, 1983 from a 45 cm DBH tree that had been blown down.
two days previously at the UBC Research Forest. The 50 kg of bark was dried and ground in the same manner as described above. After processing, the moisture content of the ground bark was 17.5%. The bonding experiments in Chapter IV were done using this material.

Veneer (0.1 inches thick) used in the bonding experiments had been peeled at Forintek Canada Corp. from Douglas fir logs. The veneer had a moisture content of 6% when used.

Catechin used in this work was obtained from Sigma and contained two and one half moles of water of crystallization per mole of catechin.

VI-B. Chapter II
(1) Acetylated Catechol Assay Procedure

One gram of reaction mixture containing catechol was added to 10 ml of distilled water along with 1.00 ml of phloroglucinol standard solution (3.50 mg/ml in water). The solution was then extracted with three 15 ml portions of diethyl ether. After drying with MgSO₄, the combined ether layers were filtered and then evaporated in an air stream. The residue of catechol and phloroglucinol was redissolved in 3 ml of 1:1 pyridine/acetic anhydride and heated to 60°C for 1 hour in a water bath. After cooling, 10 ml of distilled water was added and the resulting solution extracted with 40 ml of diethyl ether. The ether layer was washed by shaking with concentrated aqueous NaHCO₃, followed
by two washings with distilled water. This step removed any acetic acid present, and most of the pyridine, from the ether layer. After drying with MgSO₄, the ether was evaporated to about 2 ml, a portion of which, (2 ul), was then analysed on a gas chromatograph using an OV-17 column. The temperature program used was:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time at That Temp.</th>
<th>Heating Rate to Next Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>120° C</td>
<td>4 min.</td>
<td>30° C/min.</td>
</tr>
<tr>
<td>225° C</td>
<td>10 mins.</td>
<td>30° C/min.</td>
</tr>
<tr>
<td>290° C</td>
<td>10 mins.</td>
<td>recover to 120° C</td>
</tr>
</tbody>
</table>

The peaks in the resulting chromatogram were integrated and the ratio of the catechol acetate to phloroglucinol acetate peak areas determined. This ratio was then related to a concentration of catechol in the original reaction solution by using a calibration equation.

(2) Underivatized Catechol Assay Procedure

o-Cresol standard solution (0.500 ml, 10 mg/ml) was added to 1.00 g of reaction solution (containing up to 5 mg of catechol). About 2 ul of the mixture was injected into a gas chromatograph equipped with a CSP-633 glass preparative column using on-column injection.

Initial Temperature - 100° C
Final Temperature - 200° C
Heating Rate - 20° C/min.
Time At Final Temp. - 10 min.

After each sample, water was injected until the resulting peak was small and of consistent size. When necessary, the column was manually cleaned by wiping out the non-volatile deposits contained in the first few centimeters of column tubing. The peaks in the resulting chromatogram were integrated and the ratio of the catechol to o-cresol peak areas related to a weight of catechol in the one gram of sample by using a calibration equation.

(3) Typical Procedure for Investigating the Time-Temperature-Yield Relationship of Catechol Produced from Catechin

Catechin (25.0 mg) was put in each of ten pyrex ignition tubes (ID-6mm, OD-10mm, length-70mm, source-Fisher). 1.00 grams of 30% (NH₄)₂SO₃ in concentrated ammonia solution was then added to each test-tube. The tubes were sealed by heating one end to red heat in a oxygen/methane flame then crimping it together using pre-warmed forceps. The sealed tubes were then annealed by holding the melted end in a methane flame until a thick coating of carbon was formed on the glass. After air-cooling, the tubes were heated in hot tap water and then shaken until all the catechin had dissolved. The heating to reaction
temperature was done in a modified GC oven. This allowed accurate control of the temperature, while the forced-air circulation resulted in rapid heating of the reaction tubes. For specific reaction times at the oven temperature, pairs of tubes were removed from the oven at appropriate intervals (usually 1 hour, 2 hours, etc.), labelled and then cooled to -5°C in a freezer. Normally, the reaction tubes were stored overnight in the freezer before being assayed for catechol content the next morning. The contents of the reaction mixtures were also examined by TLC.

(4) Catechol Yield of Products from the Extraction Procedure of Fraser and Swan (32)

Ground western hemlock bark (41.95 g, M.C.=12.9%, dry weight = 36.5 g) was sequentially extracted with 200 ml each of benzene/ethanol (2:1), ethanol and water in a Soxhlet. Individual extract solutions were evaporated and dried under vacuum with P₂O₅ overnight. After air-drying, the extracted bark was stirred with 250 ml of 1% NaOH solution for 24 hours. The suspension was then filtered in a Buchner funnel to give 200 ml of dark brown solution. The bark residue was washed with water and the dried in an oven (102°C) to a constant weight of 23.51 g. The pH of the alkali extract solution was adjusted to 3 and the suspension filtered to give a brown precipitate. This material was washed three times by suspending in water,
sedimenting the suspension using a table-top centrifuge and pouring off the wash water. The residue was then dried overnight under vacuum. The extraction times and yields are given in Table VI-1 while the major IR absorbances (KBr pellet) are shown in Table VI-2. The total yield of extracts was 35.4%. Each extract (30 mg) was tested for catechol yield using the same procedure as described above.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extraction Time</th>
<th>Weight Yield</th>
<th>Percent Yield</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene/Ethanol (2:1)</td>
<td>24 hrs.</td>
<td>7.051</td>
<td>19.3</td>
<td>Purple Solid</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12 hrs.</td>
<td>2.743</td>
<td>7.5</td>
<td>Purple Solid</td>
</tr>
<tr>
<td>Water</td>
<td>72 hrs.</td>
<td>1.572</td>
<td>4.3</td>
<td>Purple Solid</td>
</tr>
<tr>
<td>1%NaOH</td>
<td>24 hrs.</td>
<td>1.630</td>
<td>4.3</td>
<td>Brown Solid</td>
</tr>
</tbody>
</table>

Table VI-1. Conditions And Yield Of Products From The Extraction Procedure According To Fraser & Swan (32)

(5) Extraction with Neutral Solvents and Catechol Yield

Granulated bark (49.38 g, MC=12.9%, dry Weight=43.01 g), as described in section VI-B. Chapter II(3) was sequentially extracted in a Soxhlet with 200 ml each of petroleum ether,
Table VI-2. Major IR Absorbances of Products From the Extraction Procedure According To Fraser And Swan (32)

<table>
<thead>
<tr>
<th>Extract</th>
<th>Major Absorbances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene/Ethanol</td>
<td>3400,2900(weak),1720(weak),1610,1510,1450</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3400,1610,1515,1440</td>
</tr>
<tr>
<td>Water</td>
<td>3400,1610,1515,1440</td>
</tr>
<tr>
<td>1% NaOH</td>
<td>3400,1700(weak),1610,1510</td>
</tr>
</tbody>
</table>

benzene, diethyl ether, acetone, ethanol and water. The dry weight of the bark after extraction was 31.02 g, total yield of extract was 34.0%. The extract solutions were evaporated then put under vacuum over P₂O₅ overnight. The extraction times, yields and a description of the solid extract are given in Table VI-3. IR spectra (KBr pellet) were taken of each extract and are summarized in Table VI-4. Each extract (30 mg) and 150 mg of the extracted bark were reacted with (NH₄)₂SO₃/NH₃ for 4 hours at 175°C in sealed glass tubes as described above. The yield of catechol was then determined using o-cresol as a standard.

(6) Preparation of Catechinic Acid and Reaction with 30% (NH₄)₂SO₃/NH₃

NaOH (0.25 g) was dissolved in 50 ml of water and the solution refluxed under a continuous N₂ flush. Catechin (0.5 g) was added and the solution refluxed for 45 minutes. The reaction mixture was cooled and the pH adjusted to 4 with dilute
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extraction Time (hrs.)</th>
<th>Weight Yield (g)</th>
<th>Percent Yield</th>
<th>Description of Dry Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet. Ether</td>
<td>4</td>
<td>1.0572</td>
<td>2.46</td>
<td>Yellow wax, m.p. - 25°C</td>
</tr>
<tr>
<td>Benzene</td>
<td>6</td>
<td>0.1556</td>
<td>0.36</td>
<td>Orange Gum</td>
</tr>
<tr>
<td>Diethyl Ether</td>
<td>14</td>
<td>0.7482</td>
<td>1.74</td>
<td>Purple solid</td>
</tr>
<tr>
<td>Acetone</td>
<td>6</td>
<td>6.3262</td>
<td>14.71</td>
<td>Purple solid</td>
</tr>
<tr>
<td>Ethanol</td>
<td>20</td>
<td>4.3968</td>
<td>10.71</td>
<td>Brown-Purple Solid</td>
</tr>
<tr>
<td>Water</td>
<td>20</td>
<td>1.7157</td>
<td>3.99</td>
<td>Brown-Purple Solid</td>
</tr>
</tbody>
</table>

Table VI-3. Conditions and Yields of Products From the Neutral Solvent Extraction Series
<table>
<thead>
<tr>
<th>Extract</th>
<th>Major Absorbances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet. Ether</td>
<td>2920, 2850, 1740, 1510, 1460, 1370, 1160</td>
</tr>
<tr>
<td>Diethyl Ether</td>
<td>3400, 1700 (weak), 1620, 1510, 1460, 1360, 1240, 1140</td>
</tr>
<tr>
<td>Acetone</td>
<td>3400, 1700 (weak), 1610, 1515, 1440, 1360</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3400, 1610, 1515, 1440, 1280</td>
</tr>
<tr>
<td>Water</td>
<td>3400, 1610, 1515, 1440, 1280</td>
</tr>
</tbody>
</table>

Table VI-4. Major IR Absorbances Of Products From The Neutral Solvent Extraction Series

HCl. Water was removed by evaporation and the brownish residue dried under vacuum overnight. The dry solid was then triturated with dry acetone and filtered. TLC (40% methanol in CHCl₃) showed essentially one compound was present. IR (KBr pellet) - 3400(S), 1700(S), 1580(S), 1500(S). NMR (D₂O) - 2.1(d, 1, 1), 2.7(ddd, 1), 3.1(dd, 1), 3.2(m, 2), 4.5(m, 1), 6.8(m, 2).

The prepared catechinic acid (30 mg) was sealed in a glass tube with 1 g of 30% (NH₄)₂SO₃/NH₃ and heated for 2 hours at 175° C. No catechol was observed among the reaction products examined by TLC.

(7) Maximum Yield of Catechol from Western Hemlock Bark

Ten grams of freshly collected, finely ground, western hemlock bark (the second batch described in VI-A(3), M.C. = 11.92%, Dry Weight = 8.81 g) was heated with 9 g of (NH₄)₂SO₃ and 21 ml of concentrated NH₄OH in a stainless steel reaction
vessel at 175°C for 4 hours. The resulting suspension was filtered through Buchner funnel and the bark residue washed with three 25 ml portions of water to give 4.68 g of oven-dried extracted bark. The aqueous solutions were combined, and extracted three times with 100 ml of diethyl ether. After drying over MgSO₄, the combined ether layers were evaporated, then redissolved in water to a volume of 10.00 ml. An assay of this solution for catechol (underivatized procedure) showed that 98 mg of catechol had been produced in this reaction.

VI-C. Chapter III

(1) Isolation of 1-(3,4-Dihydroxyphenyl)-2-hydroxy-3-(1,3,5-trihydroxyphenyl)propanesulphonic Acid

Catechin (125 mg) was dissolved in 7 ml of water along with 3 g of (NH₄)₂SO₃. The solution was put into a screwtop vial with a teflon seal in the cap, and heated to 60°C in a water bath. After 24 hours, TLC showed one main product formed, and no catechin present. Methanol (10 ml) was added with stirring to the aqueous solution, then the precipitated (NH₄)₂SO₃ filtered out. The filtrate was evaporated, and triturated with methanol. After filtration and evaporation of the filtrate, 100 mg of a white amorphous solid was obtained. The main component (60 mg) was isolated by preparative TLC (4 plates, solvent system - 75:25:7 ethanol/CHCl₃/H₂O). IR (KBr pellet) - 3250(s),
1615(m), 1520(m), 1450(m), 1145(s), 1030(s), 650(m). NMR (D$_2$O) - 6.9(m,3), 6.85(s,2), 4.5(m,1), 3.9(d,1), 2.6(d,2).

This compound was identical by IR and TLC analysis to the product produced by the reaction of catechin with NaHSO$_3$, prepared and isolated by the methods described above.

(2) The Reaction of 1-(3,4-Dihydroxyphenyl)-2-hydroxy-(1,3,5-trihydroxyphenyl)propanesulphonic Acid with (NH$_4$)$_2$SO$_3$ in Ammonia Solution at High Temperatures

A solution of catechin and (NH$_4$)$_2$SO$_3$ in concentrated NH$_4$OH was prepared as described in the previous section. After the reaction at 60° C, a portion of the solution was resealed in a pyrex ignition tube and heated to 175° C for two hours. Analytical TLC showed the presence of the same products (including catechol) that were observed from the direct reaction of catechin under these high temperature conditions. No assay for catechol yield was done.

(3) Isolation of Catechol and 3-Amino-5-hydroxy-7-quinolinesulphonic Acid

Catechin (4 g) was dissolved in 28 g of concentrated NH$_4$OH along with 12 g of (NH$_4$)$_2$SO$_3$. The solution was sealed in a stainless steel reaction vessel equipped with a pressure gauge
and valve. The reaction vessel was heated by placing in a thermostatically controlled glycerin bath set at 175°C for 2 hours. After cooling, the clear, orange-colored solution was extracted with three 100 ml portions of diethyl ether. The organic layers were combined, dried with MgSO₄, then evaporated to give 600 mg of a residue showing essentially one spot on a TLC plate. This material was recrystallized from benzene and dried under vacuum to give 454 mg of catechol (35% molar yield). NMR (CDCl₃) - 6.47(s,4), 4.62(s,broad,2). IR (KBr pellet) - 3450(s), 3330(s), 1615(m), 1600(m), 1510(s), 1470(m), 1360(s), 1250(s), 1190(s), 1095(s), 740(s). M.P. - 103°C. The identity was confirmed by measuring the mixed melting point with authentic recrystallized catechol. There was no melting point depression.

Methanol (100 ml) was stirred into the aqueous layer. The precipitated ammonium sulphite was filtered out, and the solution evaporated to a small volume. The methanol addition, filtration and evaporation was repeated twice more. TLC analysis of the product (50:25:11, EtOAc/EtOH/H₂O) showed at least 5 different compounds were present. Two of the spots were much more intense than the other (Rf's - 0.57, 0.35).

The organic material was dissolved in the minimum amount of water possible and divided into 4 portions. Each portion was individually applied to a column made of 100 ml of Amberlite IR-120 ion exchange resin (H⁺ form), and eluted with distilled water. Several fractions were collected and the similar fractions from the four different elutions combined. Compounds
with a TLC Rf greater than 0.57 (TLC solvent system defined above) passed through the column freely and composed the organic material in the first fractions collected. The major component at Rf=0.57 passed through the column slowly, and after the few initial highly colored fractions a clear, yellow eluant containing only this compound was obtained. Distilled water was continually flushed through the column until the resulting solution was very lightly colored. The volume of eluants containing this compound from the four eluant series was about 3 liters. The compounds with an Rf<0.57, including the major component at Rf=0.35, were retained on the ion-exchange resin, and could not be removed by elution with water. The three liters of solution containing the isolated compound was evaporated to give a yellow-orange amorphous solid. Recrystallization of the solid from methanol/water gave small needle-like crystals of the pure compound.

IR (KBr pellet) - 3440(m), 3350(s), 1640(m), 1615(m), 1585(s), 1570(s), 1400(s), 1295(s), 1260(m), 1190(s), 1040(s), 850(m), 640(s).

NMR (DMSO) - 11.23(s,1,broad,labile), 8.68(d,1,J=1.3 Hz), 8.14(d,1,J=1.3 Hz), 7.71(s,1), 7.30(s,1).

UV (H₂O) - absorbance maxima at 223 nm and 263 nm. There is a bathochromic shift of both absorbances upon addition of acid or base. MS - 149(1), 86(4.6), 85(2.9), 84(90), 68(4.7), 67(2.0), 66(100).

¹³C-NMR (DMSO-d6) - 162, 160, 152, 144, 140, 130, 126, 115, 113.

Elemental Analysis - found: C-43.11, H-3.87, N-10.87, O-30.91,

Titration with 0.0260 N NaOH showed a pKa at pH 3.3 and a pKb at pH 7.5. The molecular weight calculated from the titration data was 247 g/m.

Crystals for x-ray analysis of this compound were formed by dissolving 50 mg of the purified crystallized compound in 50 ml of water, and then seeding with small crystals previously selected for good form using a microscope. The solution was contained in a 100 ml Erlenmeyer flask, protected from the light by placing in an aluminum foil tunnel. A low-velocity nitrogen stream was introduced from one end to maintain gas circulation within the tunnel and induce a slow rate of evaporation. After two weeks the crystals were filtered out and washed with cold distilled water. X-Ray crystallographic analysis was done at the Department of Chemistry, UBC.

(4) Acetylation of 3-Amino-5-hydroxy-7-quinolinesulphonic Acid

The isolated sulphonic acid (200 mg) was acetylated by dissolving in 10 ml of 4:1 acetic anhydride/pyridine, and leaving overnight at room temperature. Methanol was added with cooling, until no more heat was generated. The solution was then stirred with additional methanol for an hour. After evaporation of the methyl acetate, the viscous residue was put
under vacuum overnight to remove the remaining pyridine. 190 mg of the resulting solid was purified by preparative TLC on four plates. The main component at Rf=0.51 was isolated.

IR - 3450(s), 1765(s), 1670(s), 1610(m), 1555(s), 1460(m), 1375(m), 1280(m), 1190(vs), 1100(s), 1050(s), 650(s).

NMR - 10.55(s,1), 9.05(d,1,J=1.3 Hz), 8.58(d,1,J=1.3 Hz), 8.13(s,1), 7.62(s,1), 2.46(5,3), 2.18(s,3).

(5) Isolation of 3,5-Diamino-7-quinolinesulphonic Acid

The organic products from the reaction of 1 g of catechin with 3 g of (NH₄)₂SO₃ in 7 ml of concentrated NH₄OH at 175°C, were prepared and isolated in the manner described in VI-C. Chapter III(2). The product mixture (160 mg) was separated on 4 preparative TLC plates. The bands containing the major component with an Rf=0.35 were collected.

NMR - 8.36(d,1,J=1.4 Hz), 7.36(s,1), 7.29(d,1,J=1.4 Hz), 6.94(s,1).

IR - 3415(s), 3360(s), 1630(s), 1580(m), 1460(m), 1400(m), 1190(s), 1050(s), 650(s).
(6) Reaction of Phloroglucinol with \((NH_4)_2SO_3/\text{NH}_3\)

Phloroglucinol (2 g, recrystallized from water) was dissolved with \((NH_4)_2SO_3\) (6 g) in 14 ml of concentrated ammonia. The solution was sealed in a stainless steel reaction vessel, and heated to 175° C in a glycerin bath for 2 hours. After cooling, methanol (100 ml) was added, and the precipitated salt filtered out. The filtrate was evaporated to dryness, then triturated with methanol. After filtering, the solution was evaporated, and the resulting residue dried under vacuum over \(P_2O_5\) for 5 hours to give 1.56 g of product. This material was acetylated by reaction at room temperature overnight with 40 ml of 1:1 acetic anhydride/pyridine. Unreacted acetic anhydride was destroyed by the addition of excess methanol, and the resulting solution was evaporated to a small volume. Water and diethyl ether (50 ml each) were added. The ether layer was separated, and washed with several portions of water. The organic layer was dried with MgSO\(_4\) and evaporated to give a white amorphous residue. The material (200 mg) was analyzed by separation on four preparative TLC plates (9:1, methanol/chloroform). The three main bands were collected and characterized.

1,3,5-Triaminobenzene triacetate (Rf=0.55, yield=54.3 mg).
IR (KBr pellet) - 3440(s), 3310(s), 3260(s), 1660(s), 1610(s), 1560(s), 1450(s), 1365(m), 1285(s), 860(m). NMR (DMSO)- 9.93(s,3), 7.62(s,3), 2.05(s,9). MS - 249, 207, 165, 123, 95, 59, 43.

1-Hydroxy-3,5-diaminobenzene triacetate (Rf=0.64,
yield = 11.2 mg). IR (KBr pellet) - 3450(m), 3260(m), 1755(m), 1655(s), 1610(s), 1560(s), 1460(m), 1420(s), 1365(m), 1275(s), 1210(s).

The last component isolated appeared to be incompletely acetylated, probably 1,3,5-triaminobenzene diacetate (Rf = 0.32, yield = 10.1 mg). IR (KBr pellet) - 3430(m), 3300(m), 1665(m), 1610(s), 1550(s), 1440(m), 1280(m), 1195(m), 840(m).

(7) Reaction of Catechol with (NH₄)₂SO₃/NH₃

The reaction products of catechol (1 g) with 3 g of (NH₄)₂SO₃ in 7 g of concentrated NH₄OH were prepared, acetylated and isolated by the procedure outlined in section VI-C. Chapter III(5). The single product obtained was found to be indistinguishable from authentic 1, 2-dihydroxybenzene diacetate when examined by analytical TLC, GC (SE-30 column)(separate and co-injection) and mixed melting point.

(8) Reaction of Resorcinol with (NH₄)₂SO₃/NH₃

The reaction products of resorcinol (1 g) with 3 g of (NH₄)₂SO₃ in 7 g of concentrated (NH₄)OH were prepared and acetylated by the procedure outlined in section VI-C. Chapter IV(5). The product mixture was then analysed by GC-MS. The
following compounds were identified by use of a computerized mass spectrum library search routine and examination of the individual mass spectra.

1,3-Dihydroxybenzene diacetate (major component) - MS - 194(4), 152(16), 110(100), 43(61).

1-Amino-3-hydroxybenzene diacetate (major component) - MS - 193(6), 151(28), 109(100), 81(6), 80(8), 43(61).

1,3-Diaminobenzene diacetate (minor component) - MS - 192(21), 150(30), 149(44), 108(100), 81(11), 80(17), 43(71).

(9) Reaction of Wattle Bark Extract with (NH₄)₂SO₃/NH₃

Three grams of spray-dried wattle bark extract (Tannins and Chemicals Inc.) was combined with (NH₄)₂SO₃ (6 g) and 14 g of concentrated NH₄OH in a stainless steel reaction vessel which was then heated to 175° C for 2 hours. The resulting solution was extracted three times with 100 ml portions of diethyl ether. The organic layers were combined, dried with MgSO₄ and evaporated. Acetylation was then performed with 50 ml of 1:1 acetic anhydride/pyridine for 4 hrs. at 50° C. Excess acetic anhydride was destroyed by adding methanol. Water and diethyl ether (50 ml each) were then added, shaken, and the organic layer isolated. After evaporation, the resulting residue was analysed by GC-MS. The positions of the GC peaks were compared to those of the compounds obtained from resorcinol to determine
substitution patterns. The important components identified are listed below.

1,2-dihydroxybenzene diacetate (major component) MS - 194(2), 152(18), 110(100), 81(4), 64(2), 52(6), 43(62).

1,3-dihydroxybenzene diacetate (major component) - MS - 194(4), 152(15), 110(100), 81(3), 80(3), 43(63).

2,6-dimethoxyphenol acetate (minor component) - MS - 196(5), 154(100), 139(28), 111(9), 110(9), 95(8), 93(11), 65(9), 43(36).

1-amino-3-hydroxybenzene diacetate (major component) - MS - 193(5), 151(25), 109(100), 81(6), 80(8), 43(64).

1,3-diaminobenzene diacetate (minor component) MS - 192(22), 150(30), 149(45), 108(100), 81(10), 80(16), 77(16), 57(13), 43(68).

(10) Reaction of Dihydroquercetin with (NH₄)₂SO₃/NH₃

The reaction products of dihydroquercetin (200 mg) with 1.2 g of (NH₄)₂SO₃ in concentrated NH₄OH (2.8 ml) were prepared, acetylated and isolated by the procedure outlined in section VI-C. Chapter III(8), except that chloroform was used for the initial extraction of organics from the reaction solution. Initial TLC analysis of the products (9:1, chloroform/methanol) indicated that there was no catechol diacetate present, although a spot that could have been due to 1, 3, 5-triaminobenzene triacetate was observed. GC analysis (Carbowax 20M on
Chromosorb W confirmed the absence of the catechol derivative. Co-injection of the reaction product with authentic triaminobenzene triacetate showed just one peak. The major isolatable product from dihydroquercetin formed by this reaction and workup was 1,3,5-triaminobenzene triacetate.

(11) Reaction of Protocatechuic Acid with (NH₄)₂SO₃/NH₃

Protocatechuic acid (20 mg) was sealed in an ignition tube with 300 mg of (NH₄)₂SO₃ and 0.7 ml of concentrated NH₄OH. The tube was heated in an oven at 175°C for two hours. TLC analysis of the solution indicated the presence of catechol. o-Cresol standard solution was added, and the amount of catechol present assayed by the procedure outlined in section VI-B. Chapter II(2). The formation of catechol was confirmed by co-injection of authentic catechol with the reaction products. The molar yield was 82%.

(12) The Formation of Catechol from Catechin in the Presence and Absence of O₂

Pyrex reaction tubes were made by flame-sealing one end of four pieces of 15 cm long, 7 mm O.D., 5 mm I.D. tubing. A solution made from catechin (25 mg), (NH₄)₂SO₃ (150 mg) and
concentrated NH₄OH (350 mg) was put in each tube. A disposable pipette drawn to a fine diameter tip was then used to bubble either O₂, N₂ or air into the solution in one of the tubes. The untreated solution and the one bubbled with air were the control samples. After bubbling the gas through the solution for 1 minute, the open end of the reaction tube was closed with a pipette bulb. Then the reaction tube was sealed by crimping the flame-heated tubing 2 cm from the rubber bulb. After heating in an oven at 150° C for 3 hours, the reaction solutions were assayed for catechol content following the procedure outlined in section VI-B. Chapter II(2). No significant differences in the yields of catechol from the different reaction conditions could be determined.

(13) Identification of Products from the Reaction of Western Hemlock Bark Polyphenols with \((\text{NH}_4)_2\text{SO}_3/\text{NH}_3\)

Freshly collected, coarsely ground bark (1466 g, MC=46.8%, Dry weight=779.9 g) was extracted with ethanol (4 liters) in a Soxhlet for 24 hrs. Evaporation of the ethanol solution gave 206.9 g of solid (26.5% yield).

Five grams of ethanol extract prepared above was heated to 175° C for 2 hrs. with \((\text{NH}_4)_2\text{SO}_3\) (5 g) and concentrated NH₄OH (45 g) in a sealed stainless steel reaction vessel. After cooling and bubbling with N₂ to remove most of the NH₃, the solution was extracted with four portions of diethyl ether (50
ml each). The organic layers were combined, dried with MgSO₄ and evaporated. The solids were analysed by GC-MS. Besides the aromatic compounds listed below, some fatty acids and hydrocarbons were identified.

Catechol (major component) - MS - 110(100), 92(11), 82(4), 81(10), 64(16), 63(14).

Phenol (major) - MS - 95(7), 94(100), 66(21), 65(21), 55(9), 50(5).

Methylphenol (minor, position of methyl group uncertain) - MS - 109 108(100), 107(86), 91(8), 90(23), 89(14), 79(31), 77(35), 53(15), 51(17).

Methoxyphenol (minor component, position of the methoxy group unclear) - MS - 125(5), 124(75), 109(100), 81(61), 65(7), 63(8), 53(24).

Dimethoxyphenol (minor component, position of methoxy groups uncertain) - MS - 155(8), 154(100), 139(47), 111(27), 96(34), 93(23), 79(14), 68(14), 65(24).

VI-D. Chapter IV

(1) Isolation of the Organic Products from the Reaction of Western Hemlock Bark with (NH₄)₂SO₃/NH₃ for Adhesive Formulation

Ground western hemlock bark (330 g, 17.5% MC, 247.5 g dry weight), 100 g of (NH₄)₂SO₃ and 1.2 liters of concentrated ammonia were placed in a 2 liter Parr hydrogenation apparatus
equipped with a glass liner and a mechanical stirrer. The mixture was stirred with an aluminum rod to obtain a slurry of even consistency. After sealing the reaction vessel, it was heated to 175° C using an external heating jacket and kept at that temperature, with stirring, for 2 hours. A pressure of about 400 psi was generated in the reaction vessel. After cooling in a large cold water bath, the apparatus was opened and the reaction mixture filtered through a large Buchner funnel. Most of the liquid was pressed out of the bark residue by using a rubber sheet sealed across the top of the funnel. The solid was washed with two 200 ml portions of concentrated ammonia solution and then 500 ml of distilled water. The bark residue was dried in an oven to a constant weight of 157 g. Assuming that no sulphite was remaining in the solid, the extraction/reaction yield was 36.5%.

The initial filtrate and washings from above were combined and evaporated to a solid. This amorphous, dark-brown material was suspended in 200 ml of distilled water (pH of the solution was 7.5) and vacuum filtered. The solid was washed several times with water and refiltered to give 88.6 g of product (45% MC, 44 g dry weight). Excess (NH₄)₂SO₃ was removed from the combined filtrate and washings by one of two methods:

1. After evaporation to about 25 ml, 200 ml of ethanol was added to the solution with stirring. The precipitated salts were filtered out and the solution evaporated to 15 ml. The ethanol addition, filtration and evaporation was repeated to give 15 g of a viscous, yellow-brown solution
(45% solids, 7 g dry weight).

(2) After evaporation to about 100 ml, the solution was heated to 50° C on a hot plate with magnetic stirring. A certain amount of Ba(OH)$_2$ (20 g to 60 g) dissolved in 100 ml of boiling water was then slowly added, and the resulting solution was allowed to cool to room temperature. The precipitated BaSO$_3$ was then filtered out and the filtrate was evaporated to a small volume.

(2) General Method of Adhesive Formulation

Usually, two fractions resulted from the isolation of organic material from the cleavage reaction on hemlock bark - organics soluble and insoluble in water at pH 7.5. The ratio of the fractions in the glue mix was the same as the ratio in which they were produced. The soluble fraction (2 g, 45% solids) was normally pre-methylolated by reacting with paraformaldehyde, (0.13 g) and 0.4 g of 50% NaOH solution at 100° C for 0.5 hrs. The pH of the solution was 10.5. After cooling, the solution was combined with 10 g of the neutral-insoluble material (55% solids), more 50% NaOH solution (1.6 g), hexamine (1.0 g) or paraformaldehyde (0.8 g), CoCob filler (2 g), wheat flour (0.4 g) and water (2 g). This mixture was manually stirred until smooth. If necessary, more water was added to reduce the viscosity to an easily spreadable level. The solids content of the final glue mix was 45 to 50%.
(3) Qualitative Technique for Adhesive Bond Evaluation

The adhesive mixture to be tested was applied with a spatula to a 10 cm by 10 cm area on the tight face of a piece of veneer (0.1 inches thick) with dimensions of 10 cm X 30 cm. The loading was approximately 3 g/100 cm². After a measured open assembly time (typically 20 min.), another similar piece of veneer was placed at right angles over the adhesive-spread area. The second piece of veneer had its loose surface (lathe-checked) facing downward and contacting the adhesive in the glueline. This assembly was then pressed under the following conditions:

Press Temperature - 175 degrees C  
Press Time - 2 min.  
Pressure - 200 psi

After letting cool for about 30 min., the quality of the glueline was evaluated by manually twisting the pieces of veneer apart at the glueline.

The qualitative wet strength of a bond was determined by submerging the 10 cm X 10 cm sample under water in a small desiccator. The air space was evacuated using an aspirator, and left at least 12 hours under suction. Bond quality was evaluated by manual breakage.
(4) Quantitative Evaluation of Bond Strength

Three-ply 8 X 12 inch plywood panels were made using the adhesive mixture to be tested with the longitudinal direction of the face and back veneers oriented parallel to the short axis of the panel. Typical assembly and press parameters were as follows:

- **Assembly Time** - 20 - 25 min.
- **Adhesive Loading** - 2.33 g/100 cm² (single glueline)
- **Press Time** - 4 min.
- **Press Temperature** - 175° C
- **Pressure** - 200 psi

The 8 X 12 inch size allowed twenty 1 X 3 inch shear samples to be cut from each panel. Grooving was done such that half of the samples from a given panel were tested with the lathe checks being pulled open and the other half with the lathe checks pulled closed (Figure VI-1). Ten samples (five of each lathe check orientation) were tested dry and the resulting shear strengths and wood failure determined. The other ten samples were submerged in water in a filtration flask with suction from an aspirator for 12 hours. These wet samples were also tested for shear strength.

Some testing was also done with 4 inch X 5 inch test panels. In this case only 4 shear samples could be cut from each board. Their testing and analysis were as described above for samples from the larger panels.
Figure VI-1. Shear Test Samples


eds., J. Wiley and Sons, New York, Ch.3.


(37) Steiner, P.R. Personal Communication


2,964,469 to Rayonier Inc.


APPENDIX.

Statistical Analysis of Quantitative Bond Evaluation
I. Varying Amounts of Added Barium Hydroxide

<table>
<thead>
<tr>
<th>Amount Added</th>
<th>Mean Shear Strength</th>
<th>Mean Wood Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A= 30g</td>
<td>125</td>
<td>5.5</td>
</tr>
<tr>
<td>B= 40g</td>
<td>142</td>
<td>18.0</td>
</tr>
<tr>
<td>C= 50g</td>
<td>149</td>
<td>17.0</td>
</tr>
</tbody>
</table>

A. Shear Strength

ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>Mean Square</th>
<th>Computed F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Means</td>
<td>3047</td>
<td>2</td>
<td>1523.5</td>
<td>1.25</td>
</tr>
<tr>
<td>Error</td>
<td>32850</td>
<td>27</td>
<td>1216.7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35897</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ F_{0.05(2,27)} = 3.35 > F(\text{calc.}) = 1.25 \]

Therefore, there is no significant difference in the shear strengths in adhesives made with varying amounts of barium hydroxide at the 0.05 level of significance.
B. Wood Failures

ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Means</td>
<td>965</td>
<td>2</td>
<td>482.5</td>
<td>3.11</td>
</tr>
<tr>
<td>Error</td>
<td>4192.5</td>
<td>27</td>
<td>155.28</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5157.5</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
F_{0.05(2,27)} = 3.35 > F_{\text{calc.}} = 3.11
\]

Therefore, there is no significant difference in the wood failure means for adhesives made with varying amounts of barium hydroxide at the 0.05 level of significance.
II. Adhesives made with Whole Soluble Reaction - Product Mixture

<table>
<thead>
<tr>
<th>Amount of Ammonium Sulfite Used</th>
<th>Mean Shear Strength</th>
<th>Mean Wood Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = 50g</td>
<td>137</td>
<td>12.5</td>
</tr>
<tr>
<td>B = 40g</td>
<td>76</td>
<td>3</td>
</tr>
<tr>
<td>C = 20g</td>
<td>91</td>
<td>5.5</td>
</tr>
</tbody>
</table>

A. Shear Strengths

ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Means</td>
<td>20207</td>
<td>2</td>
<td>10103</td>
<td>7.45</td>
</tr>
<tr>
<td>Error</td>
<td>36640</td>
<td>27</td>
<td>1357</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>56847</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ F_{0.05(2,27)} = 3.35 < F(\text{calc.}) = 7.45 \]

Therefore, at least one mean is different.
Duncan's Multiple Range Test

Means:

\[ \bar{X}_B \, \bar{X}_C \, \bar{X}_A \]

76  91  137

<table>
<thead>
<tr>
<th></th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rp</td>
<td>2.888</td>
<td>3.035</td>
</tr>
<tr>
<td>Rp</td>
<td>33.6</td>
<td>35.4</td>
</tr>
</tbody>
</table>

At alpha = 0.05

\[ \bar{X}_B \, \bar{X}_C \, \bar{X}_A \]

(Any subset of adjacent means joined by a line underneath are not significantly different)
### B. Wood Failures

#### ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Means</td>
<td>485</td>
<td>2</td>
<td>242.5</td>
<td>6.58</td>
</tr>
<tr>
<td>Error</td>
<td>995</td>
<td>27</td>
<td>36.85</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1480</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
\text{F } 0.05(2,27) = 3.35 < \text{F(calc.)} = 6.58
\]

Therefore, at least one mean is different.
Duncan's Multiple Range Test

Means:

\[ \bar{X}_B \quad \bar{X}_C \quad \bar{X}_A \]

\[ 3.0 \quad 5.5 \quad 12.5 \]

<table>
<thead>
<tr>
<th>P</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rp</td>
<td>2.888</td>
<td>3.035</td>
</tr>
<tr>
<td>Rp</td>
<td>5.54</td>
<td>5.83</td>
</tr>
</tbody>
</table>

at alpha = 0.05

\[ \bar{X}_C \quad \bar{X}_B \quad \bar{X}_A \]
III. Varying Glueline Loading

<table>
<thead>
<tr>
<th>Loading (g/100cm², single glueline)</th>
<th>S.S.</th>
<th>Mean</th>
<th>W.F.</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.71</td>
<td>200</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2.33</td>
<td>208</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.94</td>
<td>190</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.55</td>
<td>130</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

A. Shear Strength

ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Means</td>
<td>7485</td>
<td>3</td>
<td>2495</td>
<td>119</td>
</tr>
<tr>
<td>Error</td>
<td>63</td>
<td>3</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7547</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ F_{0.05(3,3)} = 9.28 < F_{\text{calc.}} = 119 \]

Therefore, at least one mean is different.
Duncan's Multiple Range Test

Means:

<table>
<thead>
<tr>
<th>XD</th>
<th>XC</th>
<th>XA</th>
<th>XB</th>
</tr>
</thead>
<tbody>
<tr>
<td>130</td>
<td>190</td>
<td>200</td>
<td>208</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rp</td>
<td>4.501</td>
<td>4.516</td>
<td>4.516</td>
</tr>
</tbody>
</table>

at alpha = 0.05

<table>
<thead>
<tr>
<th>XD</th>
<th>XC</th>
<th>XA</th>
<th>XB</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X</td>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>
B. Wood Failures

ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Means</td>
<td>2260</td>
<td>3</td>
<td>753</td>
<td>2.48</td>
</tr>
<tr>
<td>Error</td>
<td>913</td>
<td>3</td>
<td>304</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3172</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ F_{0.05(3,3)} = 9.28 > F_{\text{calc.}} = 2.48 \]

Therefore, there is no significant difference in the means.
IV. Varying Paraformaldehyde Content

<table>
<thead>
<tr>
<th>Grams of Paraformaldehyde in 5.9g of Adhesive</th>
<th>S.S. Means</th>
<th>W.F. Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 0.5</td>
<td>83</td>
<td>25</td>
</tr>
<tr>
<td>B 0.4</td>
<td>168</td>
<td>65</td>
</tr>
<tr>
<td>C 0.3</td>
<td>168</td>
<td>40</td>
</tr>
<tr>
<td>D 0.2</td>
<td>220</td>
<td>60</td>
</tr>
</tbody>
</table>

A. Shear Strength

ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Means</td>
<td>19435</td>
<td>3</td>
<td>6478</td>
<td>39.99</td>
</tr>
<tr>
<td>Error</td>
<td>487</td>
<td>3</td>
<td>162</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19922</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F 0.05(3,3) = 9.28 < F(Calc.) = 39.99

Therefore, at least one mean if different.
Duncan's Multiple Range Test

Means:

\[
\bar{X}_A \ \bar{X}_B \ \bar{X}_C \ \bar{X}_D
\]

83 168 168 220

\[
\begin{array}{|c|c|c|c|}
\hline
P & 2 & 3 & 4 \\
\hline
R_p & 4.501 & 4.516 & 4.516 \\
R_p & 40.51 & 40.64 & 40.64 \\
\hline
\end{array}
\]

\[
\bar{X}_A \ \bar{X}_B \ \bar{X}_C \ \bar{X}_D
\]

at alpha = 0.05
B. Wood Failure

ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Means</td>
<td>2050</td>
<td>3</td>
<td>683</td>
<td>6.83</td>
</tr>
<tr>
<td>Error</td>
<td>300</td>
<td>3</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2350</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$F_{0.05(3,3)} = 9.28 > F(\text{calc.}) = 6.83$

Therefore, there is no significant difference in the means.