HEMICELLULOSES AND PECTIC MATERIAL OBTAINED
FROM THE WOOD OF THE CATCLAW, ACACIA GREGGII, AFTER
CHLORINATION

by

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Major Professor  Date
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INTRODUCTION

A. Hemicellulose

In 1891, E. Schulze (1) proposed the name, hemicellulose, for a group of substances which entered into the cell membrane composition and which were, in some respects, similar to cellulose. He defined the term as being those carbohydrates occurring in the plant cell which are soluble in dilute alkalis and hydrolyzed rather readily by dilute mineral acids to give pentoses and hexoses. This was an arbitrary attempt to differentiate between true cellulose, an intermediate group of carbohydrates, and reserve foodstuffs in the plant, such as starch and some of the gums. However, Hawley and Wise (2) state that there is fallacy in seeking a chemical differentiation based on physical differences, since it is conceivable to change cellulose by mechanical methods so that it would satisfy Schulze's hemicellulose definition. Schorger (3) places a limitation by adding that the hemicellulose should be insoluble in boiling water in its natural condition, since otherwise starch would be included. According to Pringsheim (4), all other complex natural substances consisting solely of sugar remainders should be included. O'Dwyer (5) was the first to suggest that hemicellulose is not a true carbohydrate, such as pentosan and hexosan, since an acid group is often present either as glucuronic or galacturonic acid. Hence, these
compounds containing the acid group are more closely allied
to the pectic substances than to cellulose as Schulze thought.

Hemicelluloses are much more susceptible to dilute acid
hydrolysis than cellulose, but they were believed to be re-
lated in some manner, perhaps as intermediates in cellulose
formation; hence, the name. However, it is known now that
this relationship is not nearly as intimate, and so an at-
ttempt has been made to obtain a more suitable nomenclature.
Several workers (1) have suggested that the term "polyuronide"
be used to describe substances which have uronic acids present
in conjugation with sugars, especially pentoses. This would
include pectin and the structural hemicelluloses. If such a
class name were used the term "hemicellulose" could be reserved
for plant products which do not yield uronic acids on hydroly-
sis. Although the general nomenclature is confused at present,
it is impossible to adopt a more suitable system until further
knowledge of the structure, origin, and function of these ma-
terials is obtained (4). Specific hemicelluloses are named
according to the sugar contained in the molecule (3). If
xylose is obtained on hydrolysis, xylan is the name applied.
A galactoaraban will give galactose and arabinose when hydro-
lyzed.

Substances similar to cellulose, but which yield sugars
other than glucose on hydrolysis, have been known for a long
time to occur in the cell-walls of plants (7). According to
Norman (6), these hemicelluloses are not found free in the
natural state but are either associated or combined with some other cell-wall constituent. This is probably lignin, which gives rise to a lignin-hemicellulose. Support is given this view by Hagglund and Kurschner (3). These workers found that the lignin, which was isolated from pine wood by means of strong hydrochloric acid, contained four to six per cent pentosan. They assumed the lignin to be chemically combined with the hemicellulose, but Heuser (3) stated that the pentosan present was accidental and was due to incomplete hydrolysis and not to chemical combination.

The type of hemicellulose varies with the plant. F. H. Storer (3) found mannan in white pine, pitch pine, Norway spruce, Japanese larch, hemlock, red cedar, and white cedar. He concluded that the content of mannan was usually higher in the sapwood than in the heartwood. It seems that mannan is especially characteristic of the gymnosperms, while it occurs only sparingly in some hardwoods and is absent in others. Schorger (8) examined twenty-two different species of gymnosperms and six angiosperms and found appreciable quantities of mannan in all the conifers but none in the hardwoods. He also found that the mannan content is larger in the sapwood than in the heartwood and that the amount decreases from the base of the tree upward, but that the amount is uniform in a radial direction in the heartwood. In general the hardwoods contain more hemicellulose than the softwoods. Xylan is characteristic of the hardwoods, while galactan and mannan are characteristic
of the softwoods (3). It is found in considerable quantities in the hardwoods, particularly in beech and birch, where it amounts to 20 to 26 per cent of the total. The xylan content in conifers is low, usually amounting to four to six per cent (9). Schorger and Smith (10) found galactan to be characteristic of the common conifers, and Schorger (3) states that it has been found in hardwoods such as aspen, white oak, apple wood, and California live oak. Besides occurring in woods, hemicelluloses are found in stems, leaves, and many other plant parts. There are substances in the gums such as mesquite gum (11) and gum tragacanth (6) which are closely related in character to the hemicelluloses.

The true physiological significance of hemicellulose in the plant is for the most part somewhat obscure. However, it is agreed by most workers that there seem to be two main functions of hemicelluloses. One type serves as structural or skeletal material, while the other serves as a reserve substance. Doree (9) states that the reserve materials occur in kernels and seeds, that the products of hydrolysis are chiefly hexoses, and that the structural substances which are found in seed and fruit husks yield pentoses on hydrolysis. However, Norris and Freece (1) do not feel that the preponderance of pentose residues, which consist chiefly of xylose and arabinose, is a universal property of substances in the latter group. Pentosans have been found to be low in reserve materials, and Pringsheim (4) says that, since both animal and vegetable organisms are usually
more adapted to utilizing hexoses, the content of pentosan in such materials is naturally small and the hexoses large. He finds further that pentoses are not deposited in wood or straw until the later stages of growth are reached, and, hence, the young plants must contain less pentosan than the mature ones. This might be interpreted to indicate that during the growth of the plant the hemicelluloses, which consist of hexoses and are reserve materials, are used and converted to pentoses, which constitute the structural forms. This is somewhat substantiated by the work of Buston and Houghton (13) who found some evidence for the conversion of hexosan to pentosan in their studies on the hemicellulose constituents of the pods of runner beans. During the final stages of ripening the pods show a marked decrease in hexosans and an increase in the pentosan content.

Although most workers agree that pentosans do not function as reserve material, there are those who believe that they are utilized when the more readily used carbohydrates are exhausted. However, it is generally conceded that they are structural and not reserve materials in wood and that they serve to cement the fibers together. No special function in nature of general applicability has been assigned to them at present (3). Castoro (3), in 1906, noticed that during the growth of seeds some of the hemicelluloses were consumed, those in the shells being excepted. Confirmation to the belief that hemicelluloses do serve as food stores was given by Schulze (7), who estimated
them during the germination of seeds. He found that during this germinating period the content of hemicellulose in seeds diminished. According to Norman (6) the seeds of plants should be studied because of the possibility of the hemicelluloses having a role in germination. He believes that these will be the ordinary type of polyuronide hemicellulose. By using acetylation methods, O'Dwyer (13) found that 80 per cent of hemicellulose fraction "A" from beechwood, which was 80 years old, could be acetylated readily, while the same substance from an older beech was only acetylated 20 per cent. She concludes that the nature of the hemicellulose depends on the age of the wood. Perhaps these facts may at some time give an indication as to the functional part played by the hemicellulose, but no definite interpretation in this light can be given until further knowledge of this substance is gained.

Little had been done toward classification of hemicelluloses until Norman differentiated them according to Table 1 (6). Preece (14) in his studies of boxwood called the hemicelluloses soluble in cold sodium hydroxide "free hemicellulose" and those soluble in hot sodium hydroxide "combined hemicellulose." These terms were used relatively, not literally, to emphasize the difference in the treatment necessary to remove them. "Lignosaccharide" was the hemicellulose extracted with alcoholic sodium hydroxide, because this solvent readily extracts some of the lignin from woody tissues. Classification of specific hemicelluloses is usually based on the
TABLE I

HEMICELLULOSES
Extracted by dilute alkalis
Hydrolyzed by hot dilute acids
Giving hexoses and pentoses and often uronic acid

not associated with cellulosic fraction

not containing uronic acid

POLYSES
hexosans, pentosans, hexo-pentosans

reserves?

mannot, araban, xylan

containing uronic acid

associated with lignin?

POLYRONIDES
cyturonides

encrusting substance

not containing uronic acid

CELLULOSANS
cellulosic framework substance

xylan, mannan, glucosan?

not associated with cellulosic fraction

associated with natural cellulose

pentose uronic acid

hexose uronic acid

pentose hexose uronic acid
type of sugar produced on hydrolysis (4).

When Schulze defined the hemicelluloses they were thought to be made up solely of anhydrohexoses and anhydropentoses, which were called mannans, galactans, xylans, and arabans, depending on the hydrolysis product. Combinations of pentoses and hexoses were also found which were substances like mannogalactans, galactomannans, xylomannans, and so on. Workers, such as Pringsheim (4), Hawley and Wise (2), and Schorger (3), found that it was practically impossible to decide whether an isolated polysaccharide fraction was a mixture of two or more distinct substances or whether it represented one chemical individual. If a hemicellulose gave galactose and arabinose on hydrolysis, it was not possible to say whether the "galacto-araban" was a chemical entity or an intimate mixture of galactan and araban.

There are hemicelluloses, however, which contain not only sugar units, but also methoxy uronic acids. Such compounds are called encrusting hemicelluloses by Norman (6). He further states that the plant hemicelluloses contain sugar units which belong to two configurational groups, one being the glucose series, and the other the galactose series, and that it is very unusual to find constituents from both groups present. Tottingham, Roberts, and Lepkovsky (15) found a little galactose, with large amounts of xylose and glucose in the hemicellulose from the fruiting branches of apple wood. Sugars which have been found commonly are xylose,
arabinose, mannose, and galactose. Schorger (3) states that no adequate proof exists for the occurrence of glucose. Recently, however, glucose has been found in small amounts in the hemicelluloses of lemon wood (16), sapwood of black locust (17), and English oak sapwood (19). The composition of many of the hemicelluloses which have been isolated is given in Table II.

Little is known as to the composition of the hemicelluloses in situ. Anderson (20) has suggested two possibilities based on the fact that alkali dissolves these materials out of the plant. They may either contain an acid and be linked as an ester to some constituent, possibly cellulose, or they may contain no acid group and be held by a glucosidic union. Both unions are probably present and the former would give rise to an acid hemicellulose and the latter to a polysaccharide type. O'Dwyer (21) has found definite differences between the heartwood and sapwood of oak. The heartwood itself is more resistant to heat than the sapwood, and the hemicelluloses from these differ in that that from heartwood on hydrolysis gives a methoxy aldobionic residue while that from sapwood gives a uronic acid residue. She concludes that the building units are the same but that there is a transition from sapwood to heartwood, marked by definite constitutional changes, which are probably different structural arrangements in the hemicellulose molecules.
<table>
<thead>
<tr>
<th>SOURCE</th>
<th>INVESTIGATOR</th>
<th>DATE</th>
<th>HYDROLYSIS PRODUCTS</th>
<th>Uronic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian clearing nut</td>
<td>Baker and Pope (22)</td>
<td>1900</td>
<td>galactose, mannose</td>
<td>none</td>
</tr>
<tr>
<td>Ivory nut</td>
<td>Baker and Pope (22)</td>
<td>1900</td>
<td>laevulose, mannose</td>
<td>none</td>
</tr>
<tr>
<td>Tagua palm seed</td>
<td>Patterson (23)</td>
<td>1923</td>
<td>mannose</td>
<td>none</td>
</tr>
<tr>
<td>American White Oak</td>
<td>O'Dwyer (24)</td>
<td>1923</td>
<td>xylose, arabinose, mannose, galactose</td>
<td>none</td>
</tr>
<tr>
<td>Beechwood</td>
<td>O'Dwyer (5)</td>
<td>1926</td>
<td>xylose, arabinose</td>
<td>glucuronic (?)</td>
</tr>
<tr>
<td>Fraction A</td>
<td></td>
<td></td>
<td></td>
<td>galacturonic (?)</td>
</tr>
<tr>
<td>Fraction B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat Bran</td>
<td>Norris and Preece (1)</td>
<td>1930</td>
<td>xylose, arabinose, xylose</td>
<td>none</td>
</tr>
<tr>
<td>Fraction A</td>
<td></td>
<td></td>
<td></td>
<td>glucuronic (?)</td>
</tr>
<tr>
<td>Fraction B₁</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction B₂</td>
<td></td>
<td></td>
<td>glucose, a pentose, arabinose</td>
<td>none</td>
</tr>
<tr>
<td>Fraction C₂</td>
<td></td>
<td></td>
<td></td>
<td>glucuronic (?)</td>
</tr>
<tr>
<td>Maize Cob</td>
<td>Preece (25)</td>
<td>1930</td>
<td>xylose, xylose, methylpentose, arabinose</td>
<td>present</td>
</tr>
<tr>
<td>Fraction A</td>
<td></td>
<td></td>
<td></td>
<td>present</td>
</tr>
<tr>
<td>Fraction B₁, C₁</td>
<td></td>
<td></td>
<td></td>
<td>present</td>
</tr>
<tr>
<td>Fraction C₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOURCE</td>
<td>INVESTIGATOR</td>
<td>DATE</td>
<td>HYDROLYSIS PRODUCTS</td>
<td>Uronic acid</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------------------</td>
<td>-------</td>
<td>----------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Western Larch</td>
<td>Schorger and Smith (10)</td>
<td>1916</td>
<td>galactose</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>Wise and Peterson (26)</td>
<td>1930</td>
<td>arabinose, galactose</td>
<td>none</td>
</tr>
<tr>
<td>Boxwood</td>
<td>Preece (14)</td>
<td>1931</td>
<td>xylose</td>
<td>present</td>
</tr>
<tr>
<td>Mesquite Wood before $Cl_2$</td>
<td>Sands and Gary (27)</td>
<td>1933</td>
<td>xylose</td>
<td>methoxy uronic acid</td>
</tr>
<tr>
<td></td>
<td>Sands and Nutter (18)</td>
<td>1935</td>
<td>xylose, glucose (?)</td>
<td>methoxy-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>uronic acid</td>
</tr>
<tr>
<td>Lemon Wood</td>
<td>Fruin (16)</td>
<td>1933</td>
<td>xylose, glucose</td>
<td>methoxy-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>uronic acid</td>
</tr>
<tr>
<td>English Oak</td>
<td>O'Dwyer (19)</td>
<td>1940</td>
<td>xylose, glucose</td>
<td>methoxy-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>uronic acid</td>
</tr>
<tr>
<td>Oat Hulls</td>
<td>Krznarich (28)</td>
<td>1935</td>
<td>galactose, xylose,</td>
<td>glucuronic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>arabinose</td>
<td></td>
</tr>
<tr>
<td>Cottonseed Hulls</td>
<td>Kinsman (29)</td>
<td>1932</td>
<td>xylose</td>
<td>glucuronic</td>
</tr>
</tbody>
</table>
B. Pectic Substances

The jellying principle of fruits, pectin, was found by Braconnet in 1831. Pectic compounds are generally described as gelatinous substances of carbohydrate nature which are widely distributed in plant tissue in varying amounts. They are especially characteristic of fruits and vegetables and are also found as constituents of gums and mucilages (30). Ehrlich (7) in his work on beet residues discovered that the basal unit of pectic substances was pectic acid, a complex of galactose, galacturonic acid, and arabinose. It is insoluble in water and forms salts with the alkali metals which are soluble in water and which are precipitated by alcohol as thick, gelatinous precipitates. When solutions of these salts are acidified, pectic acid separates out, also as a gelatinous precipitate. The salts of pectic acid with the alkaline earth metals are insoluble.

According to Onslow (7) the term pectin is used to include the derivatives of pectic acid in which some or all of the carboxyl groups are esterified by methyl alcohol. These pectins are water soluble and are precipitated from solution by alcohol in gelatinous forms. When free carboxyl groups are present, water soluble salts are formed with alkali and alkaline earth metals, which are precipitated by alcohol.

Hawley and Wise (2) state that there is dubious significance
in using pectin to apply to the components of the middle lamellae of the cells in the woody tissues. They felt that there was no convincing experimental evidence up until 1926 for the presence of a substance or group of substances in the cell walls of wood analogous in chemical behavior to the gel-forming pectins in fruit. They feel that if pectin is present in wood at all, it is there only in very small amounts. However, since that time pectic substances have been definitely isolated from wood by many workers.

The nomenclature of the pectic materials is in a state of confusion. Workers until recently have not followed any definite scheme of naming the various substances and hence a wide variety of names have been given to one compound, and often the same name is used by separate investigators to indicate entirely different substances. Table III, compiled by Ahmann and Hooker (31), gives some indication of the lack of uniformity in the naming of pectic compounds.

Branfoot (30) has worked out a nomenclature which is now most generally accepted:

Pectose — the insoluble modification present in association with cellulose in the cell-walls of tissues, which is the precursor of the soluble form.

Pectin — the soluble form, now recognized to be a methoxylated form of pectic acid.

Pectinic acids — the primary hydrolysis products of pectin with varying methoxy content.

Pectic acid — completely de-methoxylated pectin.

Metapectic acid — the final stable product of hydrolytic decomposition of pectin.
TABLE III — NAMES GIVEN BY VARIOUS AUTHORS TO PECTIC SUBSTANCES
extracted from Ahmann and Hooker

<table>
<thead>
<tr>
<th>INVESTIGATOR</th>
<th>Pectic acid slightly sol. in H₂O</th>
<th>Ca, Hg. salts insol. in H₂O</th>
<th>Pectin H₂O sol. Esters</th>
<th>Protopectin esters insol. in H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braconnot</td>
<td>Gallertsaure</td>
<td>Pectic acid salts</td>
<td>Pectin</td>
<td>Pectin</td>
</tr>
<tr>
<td>Fremy</td>
<td>Pectic acid</td>
<td>Pectose</td>
<td>Pectin an isomer of pectic acid, Pectosic acid, Parapectin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pectin</td>
<td>Pectose</td>
<td>Pectin</td>
<td>Pectin</td>
</tr>
<tr>
<td>Mangin</td>
<td>Tetra-galacturonic acid</td>
<td>Pectose</td>
<td>Pectin</td>
<td>Pectin</td>
</tr>
<tr>
<td>Ehrlich</td>
<td>Pectin</td>
<td>Pectin</td>
<td>Pectin</td>
<td>Pectin</td>
</tr>
<tr>
<td>Tromp de Haas, and Tollens</td>
<td>Pectin</td>
<td>Pectin</td>
<td>Pectin</td>
<td>Pectin</td>
</tr>
<tr>
<td>Onslow (7)</td>
<td>Pectin</td>
<td>Pectin</td>
<td>Pectininogen</td>
<td>Pectocellulose</td>
</tr>
<tr>
<td>Fellenberg</td>
<td>Pectic acid</td>
<td>Pectin</td>
<td>Pectin</td>
<td>Protopectin</td>
</tr>
<tr>
<td>Sucharipa (32)</td>
<td>Pectic acid</td>
<td>Protopectin</td>
<td></td>
<td>Pectose</td>
</tr>
<tr>
<td>Carre and Haynes</td>
<td>Pectic acid</td>
<td>Ca pectate</td>
<td>Pectin</td>
<td>Protopectin</td>
</tr>
</tbody>
</table>
Dore (33) suggests another classification and nomenclature which is also used:

Protopectin -- formerly called pectose, is the insoluble form which occurs in unripe fruits and in most other parts of plants and is the original pectic substance from which the soluble forms are derived.

Pectin -- is a term used to designate the soluble pectic substances occurring naturally in the juice of ripe fruits or obtained artificially by treating protopectin with mild hydrolytic agents.

Pectic acid -- is obtained by subjecting pectin to hydrolysis and occurs naturally in over-ripe fruits and vegetables.

It is evident that different investigators have failed to agree on nomenclature and classification and have caused the literature to become tangled and obscure. However, now the trend is to use the nomenclature of Branfoot and, in some cases, that of Dore.

Pectic materials are widely distributed in plant tissues, either in the free state or in combination with other substances. Mangin (30), in 1889, recognized the invariable presence of pectic substances which he thought to be associated with cellulose in the cell-walls and suggested that they were of some possible importance in the constitution and development of the cell membranes. He found pectin and cellulose in the middle lamella, which he decided was a cement of insoluble pectates (34). This middle lamella is generally taken to be calcium pectate in older tissues, while
the primary cell-walls laid down in mitosis and the youngest and thinnest cambium walls in the young, green plant tissues are, at least in part, pectic in nature (6).

Protopectin is supposedly the insoluble forerunner of pectin. It was found that unripe fruit did not yield as much pectin as ripe fruit when extracted with water, so it was assumed that the pectin was in an insoluble form and that making it soluble was a feature of the ripening process. However, protopectin was never isolated, so Tutin (35) thought perhaps it was a lack of complete mechanical disintegration which made the pectin unavailable to the solvent. He worked on unripe apple tissue and disintegrated it completely by mechanical means. He obtained a large amount of water soluble pectin and concluded that protopectin does not exist, that all the pectin is in the soluble form, and that the problem is to attain complete disintegration.

Succulent tissues, such as currants, pears, strawberries, oranges, and rhubarb, seem to contain pectic materials to the greatest extent (7). Norris and Schryver (36) found pectinogin in turnips, onions, and pea pods. In his studies on lemon peel, Suchariya (32) found that free pectin occurred in the fruit and in many other soft places of the plant, both in the juice and in the cell-wall. It was located in the cell-wall just on the periphery, where the two cells meet. The "protopectin" occurred only in the cell-wall closest to the cellulose layer near the inner part of the wall under
the free pectin film. Nanji and Norman (37) investigated a
great variety of plant materials and found pectic substances
in the leaves of the sycamore, alder, potato, sumach, lilac,
laurel, and ivy; in cereal grains such as barley, malt, wheat,
rye, maize, oat, and rice; and in the fruits of apple, orange,
and lemon.

Anderson, et. al., (38) isolated pectic materials from
four different trees, both soft and hard woods, and found that
the mature softwoods contained smaller amounts than the mature
hardwoods. The woods examined were lemon wood, mesquite wood,
white pine, and black locust. Pectic material was not isolated
from the white spruce which was studied, but its presence was
indicated in small amounts. From beechwood O'Dwyer (39) iso­
lated a pectic substance, some of which came from wood where
there was no evidence of a tertiary layer, which indicates that
pectic substances are not confined to this particular layer in
the cell-wall. She also states (5) that the pectins occur in
unlignified tissue to a much greater extent than do the hemi­
celluloses, and that the hemicelluloses are found in greater
amounts in lignified substances than are the pectic materials.

Norman (40) says that pectin seems to disappear almost
completely during the lignification process, and in wood where
it has gone to an extreme only traces, if any, of the pectic
substances are found. Even the middle lamella undergoes modi­
fication, and the calcium pectate in maturity gives place to
lignin in senescence. When there is large percentage of lignin
in the middle lamella it must be of considerable importance in
giving rigidity and mechanical strength to the tissue. Buston
(41) does not consider that pectin constitutes the source of
lignin during primary lignification, but that lignin probably
arises from some carbohydrate related to the glucosan-xylan
series, with members of which series it is usually associated
in the plant. Anderson, et. al., (38) agree with this in that
they state that the pectic materials are apparently laid down
early in the growth of the tissue, remain unchanged as the wood
ages, and do not seem to be converted to lignin or hemicellu-
lose in the wood. Mature woods have been thought to be lacking
in pectin, but the failure to isolate pectic substances from
wood, according to Anderson (42), is due to the presence of
other substances which have been gradually deposited on the
pectin and protect it from the action of solvents.

Workers have, in recent years, been attempting to show the
relationships between lignin, hemicellulose, and pectic materials
in the plant tissues. Linggood (43) decarboxilated pectin with
hot water under pressure to obtain an insoluble product which,
because of its general behavior, indicated a hemicellulose and
which on hydrolysis gave sugars and uronic anhydride equal to
6.2 per cent. Norman and Norris (44) oxidized pectin with
Fenton's reagent, which is barium acetate and ferrous sulfate,
at a temperature controlled between 30 and 35 degrees. The
products resembled in appearance and general properties the
structural hemicelluloses. These results give support to the
view that the hemicelluloses may be formed in nature by the protracted mild oxidation of pectin. Onslow (7) has tenta-
tively suggested a scheme of interrelationship between the plant substances. This is reproduced in Table IV. The limita-
tions of such a system should be fully realized and accepted only as an unproven suggestion.

In 1914, von Fellenberg (6) showed the presence of arabinose and galactose in pectin, and, in 1917, Ehrlich (36) showed the molecule to contain galacturonic acid, together with arabinose and galactose. He said that crude pectin was the calcium salt of pectic acid. Norman (45) found that pectin was a mixed calcium-magnesium salt of an acid, since the ash of pectin contained calcium and magnesium. Nanji, Paton, and Ling (46) found that when the supposed calcium-magnesium salt was decomposed by the addition of oxalic, acetic, and formic acids, the salt could not be reformed by the addition of calcium and magnesium chlorides, acetates, or bicarbonates. Since they found the ash of pectin ran as high as 80 per cent iron, ferric chloride was used in an attempt to reprecipitate the insoluble salt. This method was successful, and so they concluded that the salt was probably an iron-calcium salt, rather than a calcium-magnesium salt.

These same workers suggested a molecule which contained a six-membered ring consisting of four molecules of galacturonic acid, one of galactose, and one of arabinose. This structure has been the source of considerable controversy. Norris (46) thought that he had confirmed this ring structure by the results
TABLE IV
Interrelationships of various substances (from Onslow)

Glucose (cellulose) →
  ↓
  by oxidation

Glucuronic acid (oxycellulose) →
  ↓
  by decarboxylation

Xylose (Hemicellulose)

Glucose →
  ↓
  by inversion
  ↓
  Galactose →
  ↓
  by oxidation
  ↓
  Galacturonic acid (Hemicellulose) →
  ↓
  by decarboxylation
  ↓
  Arabinose (Hemicellulose)

Galactose →
  ↓
  by inversion

[1 galactose
4 galacturonic acid
1 arabinose
methyl alcohol
(?) Ca, (?) Fe,
(?) Mg]

Mannose (Hemicellulose)

Pectin →
  ↓
  by saponification

Pectic acid →
  ↓
  by decarboxylation

Hemicellulose
he obtained from furfurals and uronic acid contents which he ran on pectin. However, Morrell, Baur, and Link (47) in methylating pectin, found that a portion which was very resistant to this treatment, contained eight to ten molecules in an ordinary saccharide linkage. They concluded that the structural unit must contain a minimum of eight to ten units. Norris and Resch (48) also found that the central nucleus of galacturonic acid, arabinose, and galactose were united in a chain formation. At the present time the ring structure has been discarded, and the chain structure suggested above has been accepted.

From orange albedo an arabino-galacturonic acid was isolated by Bowman and McKinnis (49). It yielded two moles of furfural and one mole of carbon dioxide. This substance, or a polymer of this, is probably the nuclear unit of orange albedo pectin. McKinnis (50) states that no pentoses, free or combined, occur in the apple pectin. A pentose was isolated, but he states that it comes from the decarboxylation of galacturonic acid in a weakly acid solution. From pectin in the flax plant Henderson (51) isolated galactose and a tetragalacturonic acid. The beech wood examined by O'Dwyer (39) contained a pectic substance which was 43 per cent pentose and which was very similar to the pectic acid obtained by other workers. From the cambium layer and from the sapwood of black locust pectic substances were isolated by Anderson (42). The composition of some of the pectic substances closely
approximated the pectinic acids, while others approximated the polygalacturonic acid obtained from commercial citrus pectin. The presence of d-galacturonic acid in the calcium pectate precipitated from these was established. The sugars were not identified, but apparently methylpentose sugars were absent.

Anderson, et. al., (38) have found that the pectins isolated from wood have the same general physical and chemical properties as those from other plant tissues. It would seem that pectic substances do not have a definite composition but vary with the treatment to which they are subjected. Norris and Resch (48) support this view and state that the action of extractants on the pectin may alter the composition in at least two ways. It may act on the cell-wall substances, dissolving out the central pectin nucleus together with varying quantities of araban and galactan, causing the pectin to have a lower uronic acid content owing to "dilution" with non-uronic constituents. Or the extractant may react not only on the cell-wall material but on the extracted pectin, removing the non-uronic constituents and causing the final product to contain a higher uronic acid content and a correspondingly lower non-uronic content. In conclusion, they state that pectic acid is not a chemical entity of definite constitution, but a mixture which under certain conditions of preparation approaches constant composition.
C. Statement of the Problem

Chlorination has the effect of rendering lignin soluble. Cross and Bevan (52) studied the effect of chlorine on lignin in plant tissue. They state that the chlorine evidently resolves the union of the lignin to the cellulose and that the lignone chloride formed is dissolved away by treatment with alcohol after washing with water to remove the hydrogen chloride formed. They found that the furfural yielding constituents pass over into the chlorinated derivative with a minimum of change, since the gross yield of furfural obtainable from the fiber is the same after chlorination as it was before. Evidence of the simple or limited character of the reaction with chlorine was found in that the amount of hydrochloric acid formed was approximately equal to that combining with the lignin, indicating that there were no secondary oxidations of any moment. Browne (53) obtained the same results from the chlorination of sugar-cane fiber. In studying redwood Dore (54) chlorinated the wood in an attempt to free the cellulose of lignin and hemicelluloses. He thought that this treatment removed the hemicelluloses by a hydrolytic action, since he found reducing sugars in the filtrates and washings incidental to the chlorination operations. However, he had previously treated the wood with 17.5 per cent sodium hydroxide which probably accounts for the absence of the hemicelluloses, which
is not due to the action of the chlorine as he thought. Heuser and Hang (3) found that the xylan in straw was only very slightly attacked by the chlorination procedure. They obtained a small amount of xylan chloride with an equivalent amount of hydrogen chloride being formed, indicating that the chlorine attacked the xylan by substitution.

From methylation data Harris, Sherrard, and Mitchell (55) concluded that the hydroxyls of lignin were not free for methylation until after hydrolysis and that the lignin must be attached to some of the carbohydrates, possibly the hemicellulose, in the wood cell. Such a chemical combination between lignin and carbohydrates is not difficult to conceive in the cell-wall lignin which is intimately mixed with a large proportion of carbohydrates. Norman (6) states that chlorination is not known to produce a specific effect on the hemicelluloses themselves. It seems to merely render them soluble, the most probable explanation being that the hemicellulose and lignin exist in some form of combination, and that the solution of the hemicellulose depends on the rupture of the linkage with lignin. He treated wood with sodium hydroxide and then chlorinated and extracted the hemicellulose with sodium hydroxide and found that such treatment brought most of the polyuronide hemicelluloses into solution (56).

Norman and Shrikhande (57) gave three possible explanations for the fact that hemicellulose is rendered soluble by
chlorination. The first is that the lignin and hemicellulose are not combined, but the chlorine has a specific effect on hemicellulose which renders it soluble. This has been discarded because non-reducing polysaccharides are not known to undergo any reaction upon brief treatment with halogen. Norman (6) also found that the general pattern of the hemicellulose fractions isolated after chlorination is the same as those prepared before. The second suggestion made was that the hemicellulose and lignin are not combined, but the presence of an insoluble layer of lignin, which is removed by the chlorination, mechanically and physically hinders the solution of the hemicellulose. This theory has been rejected because it is not in accordance with the present views on the structure of the cell-wall. The last possibility was that the lignin and hemicellulose are combined and that the hemicellulose dissolves when the linkage is ruptured by chlorine. This is the accepted explanation because the hemicellulose is only dissolved from the tissue by agents which dissolve lignin and rupture the linking, but after isolation the hemicelluloses are sometimes even soluble in hot water.

According to the work of Anderson (42) previously mentioned, the pectin is often not isolated due to a covering of other substances, the chief of which is probably lignin. Chlorination would remove the lignin and should make the pectin available. Pectin in highly lignified tissues, according to O'Dwyer (5) occurs only in very small amounts.
Nanji and Chinoy (58) found that chlorine water caused the breakdown of pectin. If only small amounts of pectin are present in lignified tissues and chlorine does cause the destruction of the pectin, then it would be highly possible that it could not be isolated after chlorination.

The present study was undertaken in order to isolate and identify the hemicellulose and pectin obtained from catclaw wood, and to see how well the results obtained correlate with the work which has already been done and mentioned above. When a study of these substances is made before the wood is chlorinated, a comparison of the two can be obtained.
PRELIMINARY TREATMENT OF CATCLAW WOOD

The material used as the source of the hemicellulose and pectic materials for this investigation was catclaw wood, Acacia greggii, which was cut January 1, 1959, and freed from sapwood. It was necessary to saw it up while still green, since the wood is so hard that when dry it is practically impossible to cut without injuring the saw. This wood was converted to sawdust and ground in a Wiley mill to a fineness that permitted it to pass through a one millimeter mesh screen. This fine dust from the wood was dark red, very highly pigmented and lignified. To remove such materials as fat, waxes, resins, proteins, soluble pentoses and hexoses, alcohols, and acids (59), the sawdust, in three lots of 500 grams each, was covered with acetone. This treatment was carried out repeatedly in the cold and then with the aid of heat under reflux until the filtrates after the extractions became tea-colored. Although the filtrates and the wood were still very dark, the extractions were discontinued at this point because successive treatments removed only negligible amounts of impurity. The extraction of the wood was continued with alcohol in the cold and on a boiling water bath under reflux until the filtrates were again tea-colored. Such treatment removed tannins, linoxyn, highly oxidized resins, and resinous substances related to
lignin (3). The sawdust was then extracted with water on a boiling water bath until the filtrates were tea-colored. These water filtrates gave no test for starch with iodine and did not reduce Fehling's solution. The wood was so highly pigmented and lignified that in every case a large number of extractions had to be carried out for extended lengths of time to remove as much of these materials as possible. Even with the large number of extractions which were made and the large amount of soluble material which was removed the wood remained very dark.

Four per cent sodium hydroxide was used to remove the hemicelluloses. To insure the removal of all those available to this extractant before chlorination the wood was treated four times with the sodium hydroxide. The last extraction yielded such a small amount of hemicellulose on acidification that it indicated a maximum amount of the hemicelluloses had been removed without the aid of chlorination. To free the insoluble calcium pectate of its calcium the wood was treated with twentieth-normal hydrochloric acid. This treatment liberates insoluble pectic acid which can then be extracted in later treatments with ammonium hydroxide. The hydrochloric acid also extracts a certain amount of soluble pectic substances. To remove the liberated pectic acid and other alkaline soluble pectic materials the wood was extracted four times with five per cent ammonium hydroxide. The last extract, on acidification, showed only traces of pectic substances,
indicating that the greatest part of the pectin available to this extractant, without the use of chlorination, had been removed from the wood. Schorger (3) states that ammonia also has a high solvent action on tannins, coloring matter, resins, and lignin. With the readily accessible hemicelluloses and pectic substances removed, the wood was now ready to chlorin-ate.
Whether or not hemicelluloses and pectic substances are combined mechanically or chemically with lignin in the cell-wall, the removal of the lignin from the wood has been shown definitely to liberate these materials and to allow them to be extracted by the usual procedure. The most effective method of removing lignin is by chlorination which renders it soluble to alcohol as lignone chloride. To carry out this removal of lignin the sawdust was covered with water and made slightly acid with dilute hydrochloric acid. Chlorine, which had first been washed by bubbling through water, was passed through the mixture while it was shaken vigorously by mechanical means. Any excess chlorine was destroyed by passing through strong sodium hydroxide. After a period of two and a half hours the wood was filtered immediately and washed thoroughly with distilled water to remove the hydrochloric acid which had been added and which was formed during the course of the reaction. To remove the lignin which had been made soluble during the chlorination, the sawdust was covered with alcohol and allowed to stand for 48 hours. The wood was somewhat lighter but the filtrate was so dark that the sawdust was again covered with alcohol and allowed to stand for 72 hours. The sawdust and alcohol mixture was then refluxed for two hours, filtered while still hot, and washed generously
with hot alcohol. The wood was now tan in color, indicating that much of the lignin had been removed. After the pectic materials and hemicellulose had been removed the chlorination procedure was repeated exactly as described except that the duration of the chlorine treatment was reduced to one and a half hours. After the wood had been washed with water and extracted with alcohol, it was a light straw color. A third chlorination was not deemed necessary. The quantities of hemicellulose and pectic material liberated by the second chlorination were exceedingly small and the appearance of the extracted residue was that of completely delignified cellulose.
Ammonium oxalate has long been considered the classical solvent for pectin and has been widely used as a means of removing pectic substances from plant materials. However, it was avoided in this investigation because it has been found to present certain difficulties, and because Anderson, et al., (38) have found that successive treatments with dilute hydrochloric acid and then ammonium hydroxide successfully isolate pectic materials from wood. There are several disadvantages in using this extractant. Siegle (60) used ammonium oxalate and found that it did not remove all the pectic materials. According to Preece (14), besides removing some of the pectins, it also removes some of the non-pectic constituents of the cell-wall. It has been found by Norris and Resch (48) to remove the non-urono constituents of the pectin in the wood, thus altering the composition. Farnell (61) states that this reagent is strongly absorbed by the pectins. It has a contaminating effect in that it will combine with the calcium from the wood to form insoluble calcium oxalate which precipitates along with the pectic materials and which is exceedingly difficult to remove since it is so insoluble (64). From the above results and since hydrochloric acid treatment and then ammonium hydroxide extraction allows a successful isolation of pectic materials, it was deemed advisable to omit the ammonium oxalate.
treatment and to use Anderson's method of isolation.

After each chlorination the sawdust was covered with five per cent ammonium hydroxide and allowed to stand for 48 hours. It was not necessary to treat with hydrochloric acid, since during chlorination the wood was sufficiently acid to transpose calcium pectate to pectic acid, which is soluble in ammonium hydroxide.

When the sawdust was made ammoniacal, it became a very dark brown, and the filtrate from the first extraction was almost black, indicating that quantities of the lignin and coloring matter were removed along with any pectic material present. A second extraction with ammonium hydroxide produced a dark colored liquid, but upon acidification so little precipitate was obtained that a third extraction was unnecessary. Only one extraction with ammonium hydroxide was needed after the second chlorination, and that extraction yielded an insignificant amount of material. The filtrates after the first chlorination were combined, and five cubic centimeter portions of the solution were acidified with varying amounts of concentrated hydrochloric acid and allowed to stand while the precipitate settled. In this way it was found that the optimum acidity was 1.2 cubic centimeters of the acid to five cubic centimeters of the solution. When the whole filtrate was made acid in accordance with these results, the coagulation, settling, and completeness of precipitation were the best. The acid was added all at once, because, according to
Martény (62), this procedure gave a more complete precipitation. The precipitate, called "pectin A," was centrifuged out. The thick suspension of dark brown material was washed repeatedly with 85 per cent alcohol. This treatment did not have any noticeable effect in lightening the color of the material. The substance was filtered out, washed with 95 per cent alcohol, and dried on a porous plate and then in a vacuum dessicator overnight. This yielded 17 grams of material. The substance obtained after the second chlorination was treated in an analogous manner and gave 1.7 grams.

To the filtrate from the acid precipitation one volume and then a second volume of 85 per cent alcohol were added. Absolutely no precipitation occurred after either addition, indicating the absence of pectic materials corresponding to small amounts obtained by Martény (62), Richards (64), and Siegle (60) under the same conditions. This absence of material was found also in the acid filtrate from the precipitation of so-called pectic material after the second chlorination.

Attempts to purify the substance called "pectin A" failed. Before purification the dry material was very dark brown, in fact it was almost black. It was treated in the following manner. The material was dissolved in four per cent ammonium hydroxide, filtered to remove any insoluble substances, the filtrate made just acid with hydrochloric acid, and the mixture saturated with bromine for 24 hours.
At the end of this time a little more acid was added to ensure complete precipitation, the excess bromine was removed by adding a volume of alcohol, and the precipitate was centrifuged, washed with alcohol and dried as before. With this treatment more than three-fourths of the material was rendered soluble, and that which was insoluble and precipitated out had not become any lighter. Since bromination had no apparent effect in purification other than to render a large quantity of the substance soluble in some manner, chlorination was tried in an effort to obtain a white product. Although Nanji and Chinoy (58) state that the chlorine water causes a breakdown of the pectin, it was thought that any degradation which might occur had already taken place in the original chlorination of the wood. The dried material was dissolved in four per cent ammonium hydroxide and filtered to remove insoluble substances. The filtrate was made slightly acid, and chlorine gas was bubbled through until the mixture was saturated. Alcohol was added immediately, and the precipitate was allowed to settle. It was filtered, washed, and dried in the usual manner. Again more than three-fourths of the material was rendered soluble, and the precipitate had not become lighter in color. The material after the second chlorination was treated similarly. However, chlorine purification was not attempted, because of the small yield after bromination. Yields after each attempt to purify are shown in Table V. The substances obtained after the first
and second chlorination were combined and subjected to analysis.

The lack of response of the so-called pectie substance obtained to agents commonly used to purify pectins points to their absence in the ammoniacal extract of the chlorinated wood. Since Schorger (3), as already stated, finds that ammonia has a solvent action on tannins, coloring matters, resins, and lignin as well as on pectins, it is evident that the precipitate under discussion consisted of such substances.
ANALYSES OF THE 'PECTIC SUBSTANCE'

The material obtained after each chlorination was combined and the total weight found to be one gram. The results of all analyses are shown in Table V. A moisture determination was made by drying overnight in an oven at 100 degrees. The per cent ash was found by burning the material with a slow flame and then blasting it to constant weight. The uronic acid content was determined by digesting in 12 per cent hydrochloric acid and by measuring the evolution of carbon dioxide as prescribed by Dickson, Otterson, and Link (63). Pectic substances under these conditions yield from 15 to 20 per cent carbon dioxide. However, during the four hour period of heating with the hydrochloric acid there was no evolution of carbon dioxide, and the so-called pectic material from catclaw wood failed to dissolve or disintegrate in any way. The results of this determination furnish irrefutable evidence for the complete absence of pectic material in the one gram of material left after the so-called purification.

Further evidence was obtained from the results of a calcium pectate determination, which was made according to the method described by Branfoot (30). Where snowy white calcium pectate should have precipitated a dark brown precipitate appeared. This precipitate was the original
### TABLE V

**PECTIC SUBSTANCE**

#### Yields

<table>
<thead>
<tr>
<th></th>
<th>Before purification</th>
<th>Bromination</th>
<th>Chlorination</th>
<th>Total for Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorination 1</td>
<td>17 grams</td>
<td>4.6 grams</td>
<td>0.4 grams</td>
<td>---</td>
</tr>
<tr>
<td>Chlorination 2</td>
<td>1.7 grams</td>
<td>0.6 grams</td>
<td>-----</td>
<td>1.0 gram</td>
</tr>
</tbody>
</table>

#### Analyses

<table>
<thead>
<tr>
<th></th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>5.472%</td>
</tr>
<tr>
<td>Ash</td>
<td>3.328%</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>0.000%</td>
</tr>
<tr>
<td>Calcium pectate</td>
<td>(?)</td>
</tr>
</tbody>
</table>
substance which was merely dissolved in the base used and then reprecipitated on acidification. Calcium chloride had no reactive effect, and simply caused the dark brown suspension to coagulate. This determination is one of the most widely used methods of estimating pectin, and, therefore, the failure to obtain calcium pectate gives conclusive evidence that the material isolated was not pectic in nature.
CONCLUSIONS ON THE "PECTIC SUBSTANCE"

From catclaw wood after chlorination a very dark brown material was obtained by extraction with ammonium hydroxide. The fact that it could not be purified by bromine or chlorine, agents used regularly for purification of pectin, gave definite evidence that the material isolated was not a pectic substance. This was substantiated further by the results of the analyses. There are several possible reasons why no pectin was isolated. It may not have been present in the wood. However, according to Anderson (42), failure to isolate pectic substances is due to incomplete removal of the covering materials. It would seem, though, that chlorination is a drastic enough treatment to remove all matter which would make the pectin unavailable to the solvent used. Furthermore, the appearance of the wood after the two chlorinations indicated that the encrusting materials had been removed and that almost nothing remained but cellulose. It may have been that this treatment was too drastic, since, according to Manji and Chinoy (58), the pectin is broken down by chlorine water. However, wood has been chlorinated by other workers and pectic materials isolated. The most probable explanation is that only a little of the material was present, and, in the presence of such a large amount of lignin and other materials which are extracted by the ammonium hydroxide, it could not be isolated.
EXTRACTION AND PRECIPITATION OF HEMICELLULOSES

After the last extraction with ammonium hydroxide following the chlorination procedure, the wood was washed thoroughly with water. To extract the hemicelluloses, the wood was covered with four per cent sodium hydroxide and allowed to stand for 48 hours. The wood was filtered and the extraction repeated. This last extraction yielded such small amounts of precipitate upon acidification that a third extraction was not thought necessary to complete the removal of the hemicelluloses. The filtrates from the two extractions were combined. After the second chlorination the first treatment with sodium hydroxide gave very slight amounts of hemicellulose on acidifying, so a second extraction was not made. The filtrates after the first and second chlorination were not combined because those after the second treatment with chlorine have been found to contain oxy cellulose (18). In each case the wood and the filtrates were considerably lighter in color than those obtained with the extraction of ammonium hydroxide which indicates that most of the soluble portion of lignin was removed with the ammonia solution. After the last extractive treatment the sawdust was neutralized with hydrochloric acid, washed thoroughly, dried in air, and set aside. It was now a light cream color, whereas, at the start, it had been a dark red-brown. This color
change indicates that the purpose of chlorination, which is to remove lignin and encrusting materials, had been accomplished successfully.

The sodium hydroxide extracts were made acid, and alcohol was added until no more precipitate formed. It was found that the amount of alcohol required for this was two volumes. The precipitates were allowed to stand until settling was complete, and then the large volume of supernatant liquid was siphoned off, and the hemicelluloses centrifuged, washed with alcohol, filtered, and dried on a porous plate and then in a vacuum dessicator overnight.
PURIFICATION AND FRACTIONATION OF HEMICELLULOSES

The medium brown, impure hemicelluloses from the first and second chlorination were purified by dissolving them in a 100 times their weight of four per cent sodium hydroxide, filtering to remove insoluble material, making the solution slightly acid, and saturating with bromine for 24 hours. The bromine was used up during the course of the reaction, so successive additions had to be made. Two volumes of alcohol were then added to precipitate the hemicelluloses remaining in solution. When settling of the precipitate was complete, the supernatant liquid was siphoned off and the hemicelluloses centrifuged, washed, and dried in the usual manner. With one bromine treatment a light tan product resulted. When brominated a second time, the hemicelluloses were rendered snow white and free from lignin.

Fractionation of the hemicelluloses was carried out in the following manner. The pure white substance was dissolved in a 100 times its weight in four per cent sodium hydroxide and filtered to remove any insoluble material. Tests were made on the filtrate to determine the acidity most favorable for precipitation of the first fraction. It was found that one cubic centimeter of concentrated hydrochloric acid to five cubic centimeters of the solution gave a precipitation which was complete, leaving an absolutely
clear supernatant liquid. The hemicelluloses after each chlorination were precipitated from solution with this acidity. After standing for 24 hours the hemicellulose was centrifuged, washed with 95 per cent alcohol until free of chloride, and dried in the usual manner. The hemicellulose from the first chlorination was called fraction A, while that from the second chlorination was designated fraction A'. The filtrates from the precipitation of the first fractions were tested with one, one and a quarter, one and a half, and two volumes of 85 per cent alcohol. It was found that an increase in the amount of alcohol used merely gave a progressive increase in the amount of precipitate, giving no indication that a separation into definite chemical entities was being effected. However, was this the progressive precipitation of a single substance or of a mixture of hemicelluloses of different compositions? In an attempt to establish this point a portion of the acid soluble hemicellulose was precipitated by adding one volume of alcohol to the filtrate from fraction A. The precipitate formed was centrifuged, washed free from chloride, and dried as before. This hemicellulose was called fraction B. The corresponding hemicellulose obtained after the second chlorination was called fraction B'. To the filtrates from fraction B and fraction B', a second volume of alcohol was added. This completely precipitated the remaining portion of hemicellulose from solution and arbitrarily divided the acid soluble part into two fractions. These last precipitates
were labeled fraction C and fraction C*. If the analyses of fraction B and fraction C differed even slightly it would point to a mixture in the acid soluble portion of the hemicelluloses obtained after chlorination. The yields of the various fractions are given in Table VI.

**TABLE VI**

**Yields of the various hemicelluloses**

<table>
<thead>
<tr>
<th></th>
<th>grams</th>
<th>percent in the wood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First chlorination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction A</td>
<td>0.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Fraction B</td>
<td>1.0</td>
<td>0.066</td>
</tr>
<tr>
<td>Fraction C</td>
<td>1.1</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>Second chlorination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction A'</td>
<td>0.2</td>
<td>0.055</td>
</tr>
<tr>
<td>Fraction B'</td>
<td>0.3</td>
<td>0.020</td>
</tr>
<tr>
<td>Fraction C'</td>
<td>0.4</td>
<td>0.037</td>
</tr>
</tbody>
</table>
The small amounts of the various fractions obtained did not allow an extensive analysis of all the hemicelluloses. Those after the second chlorination were not investigated. The results of all analyses are shown in Table VII. The percent moisture was found by drying the material in an oven overnight at 100 degrees, and ash determinations were made by burning the substance slowly and then blasting the residue to constant weight. The uronic acid content was estimated as carbon dioxide (65), and from this the equivalent weight, that is, the weight of the probable recurring unit of the complex hemicellulose, was calculated on the assumption that there was one uronic acid and, consequently, one carbon dioxide evolved per unit. The equivalent weights of fraction B and fraction C were the same, indicating that, on an average, the carbohydrate content was identical. This was not surprising, since in the fractionation, no sharp separation could be made between these two fractions. The per cent methoxyl was determined according to Zeisel's method (65), and the number of methoxyl groups per uronic acid calculated. Fraction A, which may have been a definite chemical entity as indicated by the sharp and complete precipitation of the fraction, contained as nearly one methoxyl per uronic acid as can be determined within the limits of experiment. On the other
### Table VII

**Analyses of the Hemicelluloses**

<table>
<thead>
<tr>
<th></th>
<th>Fraction A</th>
<th>Fraction B</th>
<th>Fraction C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cent Moisture</td>
<td>4.025</td>
<td>7.120</td>
<td>4.025</td>
</tr>
<tr>
<td>Per cent Ash</td>
<td>0.4445</td>
<td>0.3703</td>
<td>0.4375</td>
</tr>
<tr>
<td>Per cent Carbon dioxide</td>
<td>1.333</td>
<td>1.002</td>
<td>2.014</td>
</tr>
<tr>
<td>Per cent Methoxyl</td>
<td>0.928</td>
<td>2.158</td>
<td>5.136</td>
</tr>
<tr>
<td>Specific Rotation</td>
<td>-183.8</td>
<td>-91.75</td>
<td>-75.92</td>
</tr>
<tr>
<td>Per cent Xylan</td>
<td>99.58</td>
<td>92.48</td>
<td>99.48</td>
</tr>
<tr>
<td>Methoxyl group per uronic acid unit#</td>
<td>1.040</td>
<td>1.508</td>
<td>3.195</td>
</tr>
<tr>
<td>Per cent methylated uronic acid ##</td>
<td>0.56</td>
<td>0.44</td>
<td>0.61</td>
</tr>
<tr>
<td>Total percentage ###</td>
<td>99.96</td>
<td>99.96</td>
<td>100.00</td>
</tr>
<tr>
<td>Equivalent weight ¥</td>
<td>5159</td>
<td>9154</td>
<td>2195</td>
</tr>
<tr>
<td>Number of pentose units per uronic acid unit</td>
<td>22.59**</td>
<td>15.12**</td>
<td>14.98**</td>
</tr>
</tbody>
</table>

Note: All calculations were made on an ash and moisture free basis

# Methoxyl group per uronic acid unit = \( \frac{\% \text{ Methoxyl} \times 44}{\% \text{ CO}_2 \times 51} \)

## Per cent methylated uronic acid = \( \frac{\% \text{ CO}_2 \times \text{Mol. Wt. of methylated uronic acid in question}}{44} \)

### Total percentage = sum of per cents of xylan and methylated uronic acid

¥ Equivalent weight = \( \frac{\% \text{ CO}_2 \times 44}{100} \)

Number of pentose units per uronic acid unit

* = Eq. Wt. - Mol. Wt. of methylated uronic anhydride / Mol. Wt. of anhydro-pentose

** = Eq. Wt. - Mol. Wt. of methylated uronic anhydride / Mol. Wt. of anhydro-xylose
hand, fraction B and fraction C, which contained the same average number of carbohydrate units per equivalent weight, were found to differ significantly in methoxyl content. Fraction C contained an average of more than two methoxyls per uronic acid, while fraction B contained definitely less than two methoxyl groups per uronic acid. This situation could best be explained by assuming that these fractions contained mono-, di-, and tri-methoxyl-uronic acid, mixed in proportions to give the analytical results.

Calculations from the carbon dioxide determinations showed that fraction A contained 22.5 pentose units per mono-methoxyl uronic acid. A xylan determination (66) run on this fraction corroborated this result, giving 21.66 pentoses per uronic acid. Fraction B and C were found to contain approximately 15 pentose units per methylated uronic acid. A four per cent solution of the hemicelluloses in two per cent sodium hydroxide was used in determining the specific rotations. In general, the rotations of the acid insoluble hemicelluloses are smaller than that of the acid soluble hemicelluloses (17), (59), (68), (69).

The hemicelluloses from cateclaw wood conformed to this general picture. This difference in rotation between fraction B and fraction C could not be accounted for in this way, since they contained the same average number of carbohydrate units. Since the addition of three methoxyl groups to free glucuronic acid increases its specific rotation by 29
degrees (6), it is scarcely logical to assume that the addition of one to two methoxyl groups to one out of every 16 carbohydrate units in the hemicellulose could raise the rotation of fraction C 16 degrees above fraction B. On the other hand the difference in rotation is in the right direction to correspond to this observed difference in the constitution of these two fractions of hemicellulose.
HYDROLYSIS OF FRACTION A

The hemicellulose fraction A was the only one which was obtained in large enough quantities to permit hydrolysis. The hydrolytic procedure was carried out as follows: 6.2 grams of fraction A were mixed with 100 cubic centimeters of four per cent sulfuric acid and heated on a boiling water bath for 20 hours. The light brown solution was then made almost neutral with barium hydroxide, care being taken to keep on the acid side of neutral to prevent the destruction of the sugars which occurs in a basic solution. Barium carbonate was added and the mixture boiled to complete the neutralization. Charcoal was added and the mixture boiled for several minutes to decolorize it. Barium sulfate, charcoal, and any excess barium carbonate were centrifuged out, and the supernatant liquid filtered to remove any small crystals of barium sulfate remaining in the solution. The filtrate was concentrated down under reduced pressure to a syrup which was then diluted to a concentration which gave a granular precipitate when a drop of the solution was placed in a little alcohol. The syrupy solution was poured drop by drop and with vigorous stirring into 95 per cent alcohol to precipitate the barium salt as a granular mass. The salt was reprecipitated twice from alcohol to remove any free sugar. The filtrate from the barium salt precipitation was
concentrated down under reduced pressure to incipient crystallization. On cooling this set to a dry mass of perfectly white crystals. The sugar crystals were washed from the flask with 95 per cent alcohol, dried on a porous plate and then in the oven at 90 degrees for four hours.
IDENTIFICATION OF HYDROLYTIC PRODUCTS

The sugar obtained in crystalline form melted at 151 degrees, and gave the Bertrand's test (67) for cadmium bromide xylonate. The specific rotation in a two per cent water solution was +19.87°. The specific rotation of pure xylose is +19.13°; the melting point is 163 degrees. The absence of galactose and glucose was shown by the negative mucic acid and potassium acid saccharate tests. Arabinose was absent since the attempt to make the alpha benzyl phenyl osazone failed. Therefore, the only sugar found in the sugar crop was xylose.

The alcohol used to wash the xylose contained any mother liquid which might have been present in the crystalline mass. The concentrate of this solution gave a positive Bertrand's test for xylose, and negative tests for galactose, glucose, and arabinose. Besides the negative potassium acid saccharate test for glucose, the syrup would not ferment with yeast. The yeast had not been poisoned by any barium salt which might have been present, because, when no fermentation occurred after 24 hours, a very small amount of glucose was added, and fermentation was observed in a short time.

The methoxyl-uronic acid from woods has never been identified (16), (18), (27) by any workers except O'Dwyer (5). This is one of the reasons for believing that the
methoxyl group is attached to the uronic acid. The uronic acid in the barium salt of catechol gave negative results for both galacturonic and glucuronic acids with the mucic acid and potassium acid saccharate tests, indicating that in this case, too, the methoxyl group was attached to the uronic acid.
CONCLUSIONS ON THE HEMICELLULOSES

From the cateclaw wood after two chlorinations small amounts of hemicelluloses were extracted with four per cent sodium hydroxide. They were purified by bromination and fractionated into fraction A, fraction B, and fraction C. Fraction A precipitated sharply when the alkali extracts were made acid, but fraction B and fraction C did not give a distinct separation when one and then a second volume of 85 per cent alcohol were added to the filtrate from the acid precipitation. The fractions after the second chlorination were not investigated. Only one hemicellulose, fraction A, was obtained in quantities large enough to permit hydrolysis. On hydrolysis of this fraction only one sugar was found and identified. It was shown to be xylose by Bertrand's test, by its melting point, and by its specific rotation. The ease and completeness with which the sugar fraction crystallized strongly confirmed the conclusion that xylose was the only sugar obtained from hydrolysis of the hemicellulose.

From the uronic acid and pentosan determinations fraction A was shown to contain approximately 22 anhydro-xylose units and, from the methoxyl determination, to contain exactly one methoxyl group per uronic acid. Fraction B and fraction C proved to be mixtures. The sugar moiety was not
identified but the carbon dioxide evolution when boiled with 12 per cent hydrochloric acid showed that the ratio of non-
urone content to uronic acid was identical in both fractions.
If the non-urone portion is calculated as xylose, the result
gives 15 anhydroxyloses per uronic acid. The difference in
fraction B and fraction C lay in the degree of methylation.
The partial separation of the acid soluble hemicelluloses
through fractional precipitation with alcohol showed that
the more soluble portion was the more highly methylated. If
fraction B contained mostly molecules with one methoxyl group
mixed with molecules having two and three methoxyl groups per
uronic acid, and if fraction C contained a preponderance of
molecules with three and two methoxyl groups per uronic acid,
the methoxyl content found by analysis would be explained.
Since the uronic acid from other woods has not generally
been identified due to the failure of mucic acid and potassium
acid saccharate tests for galacturonic and glucuronic acid,
and since a methoxyl group has been found to be present, it
is conceded that the methoxyl group is attached to the uronic
acid. The hemicelluloses from catclaw acted in an entirely
similar manner; hence, it was assumed that the methoxyl groups
were attached to the uronic acid.

The composition of the hemicelluloses from catclaw corre-
sponds with that of hemicelluloses from other hardwoods, ex-
cept that the pentose content is higher than in most. White
birch (69) hemicelluloses contained from nine to 18 xyloses,
black locust heartwood (59), from 12 to 19; black locust sap-
wood (17), from 11 to 18; and lemon wood (68), from eight to
16. The anhydroxylose units in catclaw wood were 15 and 22.
As has been found in other woods (38), one must conclude that
the acid soluble hemicelluloses from catclaw are higher in
uronic acid content and are more highly methylated than the
acid insoluble fraction. The acid insoluble hemicellulose
has a lower uronic acid content and a higher equivalent weight
than the acid soluble hemicellulose.
SUMMARY

1. Catoelaw wood, *Acacia greggii*, after treatment with alcohol, acetone, water, sodium hydroxide and ammonia to remove the readily available coloring matter, lignin, hemicelluloses and pectic materials, was delignified by means of chlorine.

2. No pectic materials were isolated after chlorination.

3. Small amounts of polyuronide hemicelluloses were isolated.

4. The recurring unit in the acid insoluble hemicellulose was a polyanhydro-xylo-mono-methoxyl-uronic acid with 22 xylose units.

5. The recurring unit in the acid soluble, alcohol insoluble hemicellulose contained 15 anhydro-pentose units joined to mono-, di-, and tri-methoxy-l-uronic acid.
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