XVI. THE BIOCHEMISTRY OF DRY-ROT IN WOOD.

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INTRODUCTION.

THE biochemical action of the saprophytic and parasitic fungi which cause decay of lignified tissues is very little understood at the present time. It is usually stated in mycological literature that the action of the fungal hyphae is to cause delignification of the lignocellulose complex by removal of the lignin and to leave a residue of cellulose. These results have been mainly attained by the study of microchemical tests on sound and decayed wood, and no systematic investigation has been made on a macroscopic scale.

Microchemical reactions applied to wood as specific tests for the presence or absence of either lignin or cellulose are of little value, as the characteristic reactions (mainly colour changes) are due to small amounts of impurities in the tissue.

It is well known that wood-destroying fungi are rich in enzymes, and as many as a dozen have been reported from a single species. The early investigations of Czapek [1899] showed that greatly increased quantities of hadromal (coniferyl aldehyde) could be extracted from wood that had been attacked by fungi, and he assumed that they contained an enzyme, hadromase, which split a supposed ether-linkage between the hadromal and cellulose. Somewhat similar results were obtained by Zeller [1916] who isolated an enzyme extract from the mycelium of *Lenzites saepiaria* which was found to act on a substrate of the sapwood of *Pinus echinata*, and an alcoholic extract of the residue gave the phloroglucinol reaction. He proposed the name lignase for Czapek's hadromase.

The investigations of Hartig [1900], based on colour changes with microchemical reagents, showed that the parasites *Polyporus vaporarius*, *P. sulphureus*, and the saprophyte *Merulius lachrymans* removed cellulose from wood and left a residue of wood-gum, while *Trametes pini* was found to remove lignin and leave pure cellulose. After the loss of lignin, the middle lamella (which was assumed to be composed of pectin) dissolved and left isolated tracheids. This latter result is obviously incorrect, as we now know that the middle lamella is composed largely of lignin.

The arguments advanced by Hartig and the majority of subsequent

investigators that decayed wood consists of pure cellulose are based upon staining the residue of this wood with zinc chloriodide—a very unsafe standard. Other workers have also stated that members of the Polyporaceae remove lignin from wood, leaving cellulose. Lindroth [1904] found that the phloroglucinol reaction disappears first in decayed wood and then the Maule reaction also disappears. As the red colour given by wood with phloroglucinol and hydrochloric acid disappears, the colour reactions for cellulose become more intense, until finally pure cellulose is indicated.

Again, Marshall Ward [1897] by the study of colour changes with micro-chemical reagents came to the conclusion that the action of *Sterium hirsutum*, which attacks *Aesculus* (Chestnut), is to destroy the lignin and leave the cellulose. Ward, however, was unable to isolate any enzyme capable of causing delignification.

The evidence for the production of cellulose by *Trametes pini* is founded on stronger grounds than any of the investigations discussed above. Johnson and Lee [1923] found an increase of 15 % in cellulose and a decrease of 30 % in lignin in wood attacked by this fungus.

The first complete examination of decayed wood (Douglas Fir) was made by Rose and Lisse [1917]. Unfortunately the fungus that produced the decay was not identified. There was a marked increase in the solubility of the decayed wood in alkali and a decrease in the amount of cellulose, pentosans and acetyl groups, while the methoxyl content was increased.

From the conflict of evidence on this subject it was considered of interest to carry out an investigation of wood attacked by *Merulius lachrymans*, using modern methods of cellulose analysis. Spruce-wood (*Picea excelsa*) was chosen for the experimental work as its constants are well known and form the requisite standard of comparison for the experimental data. Only completely decayed wood was used to study the action of the fungus.

The action of *Merulius lachrymans* on timber is too well known to need detailed description. The wood loses weight and is converted into the so-called touch-wood, which powders easily to give a brown dust.

It was found that the decayed spruce-wood gave Ditz's test for oxycellulose. A small amount of the wood was suspended in water and methyl orange was added to colour the water yellow. On addition of a few cc. of a saturated solution of sodium chloride, a wine-red colour was developed at once. Sound wood did not give the reaction. A red colour was produced with phloroglucinol and hydrochloric acid. The decayed wood rapidly reduced Fehling's solution, whereas sound wood only brought about reduction after prolonged boiling. Similar results were obtained with decayed beech-wood (hard wood) which had been attacked by *M. lachrymans*. 47.2 % of the decayed wood was soluble in a 5 % solution of sodium hydroxide. The ash-content was 1.07 % and the natural moisture 13.4 %. The technique in general employed was that elaborated by Dore [1920], and in Table I a comparison is made of the values obtained for sound and decayed spruce.

Table I. *Comparison of sound and decayed spruce.*

	Sound wood	Decayed wood
	%	%
Extracted by benzene ...	1.7	1.55
" alcohol ...	1.6	9.46
" water ...	2.5	5.43
" 5 % NaOH	3.8	30.30
Cellulose ...	55.0	11.90
Lignin ...	28.0	43.00
Mannan ...	7.6	0.00
Galactan ...	0.1	0.00
	100.3	101.64
Ash-content ...	0.86	1.07
Natural moisture ...	10.0	13.40

(All analyses are calculated on ash-free material dried at 105°.)

An examination of the figures shows that a decrease of 60 % has occurred in the cellulose, while the lignin has increased by 57 %. It was found in the course of the analysis of the cellulose that the lignin in the wood had in some way been altered. When the wood was placed in chlorine gas to chlorinate the lignin prior to its removal by boiling with sodium sulphite solution, instead of the usual golden-yellow colour being formed, the whole mass turned a dull brick-red. A further point in this connection is the complete removal of the hexosans, mannan and galactan. The portion soluble in 5 % sodium hydroxide solution (after removal of the fractions soluble in benzene, alcohol and water respectively¹) represents, in part at any rate, the hemicellulose components of the wood. These bodies are soluble in dilute caustic alkali and are readily hydrolysed by acids. They contain "uronic" acids of the type of galacturonic acid [Nanji, Paton and Ling, 1925], as well as pentose and hexose residues. It has been shown by O'Dwyer [1926] that the hemicellulose fraction of beechwood consists of two substances, hemicellulose A, which is precipitated from alkaline solution by acid, and hemicellulose B, soluble in water but insoluble in alcohol. Hemicellulose A contains xylose and 11 % of glycuronic acid, whereas hemicellulose B contains arabinose and 63 % of galacturonic acid as well as small amounts of galactose. Dorée and Barton-Wright [1927] have also shown that two hemicelluloses are present in spruce-wood. That a part at least of the sodium hydroxide-soluble fraction of decayed wood consists of hemicelluloses was indicated by the presence of increased amounts of uronic acids. These on distillation with 12 % hydrochloric acid do not give a quantitative yield of furfural; but the carbon dioxide evolved by the decarboxylation of their carboxyl group affords an exact measure of their amount. The yield of uronic acid from the good wood was 2.688 %, and from the decayed wood 4.84 %; an increase of 82 %. The greater part of the alkali-soluble fraction was precipitated by the addition of acetic acid, and the remainder, after concentration, by adding alcohol [cf. O'Dwyer, 1926]. The increase in solubility of the decayed wood in NaOH solution compared with that of sound spruce

¹ The total of the values found for the benzene, alcohol, water and 5 % sodium hydroxide extract (47.2 %) agrees well with the solubility of the wood in 5 % sodium hydroxide alone (46.71 %), without preliminary extraction with organic solvents and water.

is very high and cannot be entirely accounted for by the presence of hemicelluloses. It is possible that the remainder of the alkali-soluble fraction is composed of the metabolic products of the fungus and the contents of the fungal hyphae.

Comparison of the figures for the methoxyl content of sound and decayed wood shows an increase in the latter of 77 % (sound wood, OMe = 5.44 %; decayed wood, OMe = 9.67 %). A small part of the methoxyl content is located in the hemicellulose fraction [see O'Dwyer, 1928], while the rest occurs in the lignin.

We have obtained evidence (which we hope to publish in a later communication) that the major part of the alcohol extract is composed of lignin. It is possible that it is of the nature of the metalignin isolated by Dorée and Barton-Wright [1927], which has the molecular composition $C_{20}H_{20}O_6$ and is the simplest type of lignin known.

DISCUSSION.

Direct determination of the cellulose and lignin in decayed wood shows decrease of the former and increase of the latter, and estimation of the methoxyl groupings confirms the increase of the lignin. These results show very clearly that no delignification of the wood is brought about by the fungus, but rather that the main attack is confined to the cellulose. The hexosans, mannan and galactan, are also removed, and we would therefore suggest that *M. lachrymans* first attacks these two easily hydrolysed bodies in the course of its metabolic activities and then confines its attack to the cellulose.

It is difficult to account for the increase in the hemicellulose fraction of the decayed wood. The hemicelluloses are readily hydrolysed substances, and the main hydrolytic products are sugars. On the other hand uronic acids are also produced, and it may well be due to the presence of the latter that the fungus does not affect the hemicelluloses.

The influence of the hydrogen ion concentration of the medium on the development of the mycelia of wood-destroying fungi has only been examined in a few cases, but Meacham [1918] found that the first marked deflection in the growth curve occurred at an acidity of $N/350$ HCl and was limited by $N/50$. The hydrogen ion concentration, however, does not appear to be the chief factor determining a desirable medium. According to Zeller, Schmitz and Duggar [1919], no general statement can be made on the relationship between the p_H of the medium and the growth of wood-destroying fungi.

Merulius lachrymans is very sensitive to the presence of acids. Wehmer [1911, 1912] has found that both gallic and tannic acids are markedly toxic to the fungus, and it is for this reason that coniferous (soft) wood is very much more rapidly attacked than a hard wood such as oak. Concentrations of 1–2 % of these acids at once stop all mycelial growth, and coniferous wood can be made resistant to *Merulius* by impregnation with tannic acid. The presence of acids produced by hydrolysis would seriously hamper the fungus in its

attack on the wood, and it may be on this account that the hemicelluloses are not affected. On the other hand, the hydrolysis of the galactan, mannan and cellulose fractions leads to the production of no growth-inhibiting products, but provides the requisite carbohydrate material for active metabolism. With the destruction of the cellulose matrix of the tracheids the touch-wood or tinder-wood condition, which is so typical of wood affected by *M. lachrymans*, is obtained.

SUMMARY.

The effect of the fungus *Merulius lachrymans* (the cause of dry-rot in wood) is to remove the galactan, mannan and cellulose fractions in spruce-wood; the hemicelluloses and lignin are not affected, *i.e.* no delignification of the woody tissues takes place.

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