LX. THE BIOCHEMISTRY OF DRY-ROT IN WOOD.

II. AN INVESTIGATION OF THE PRODUCTS OF DECAY OF SPRUCE WOOD ROTTED BY *MERULIUS LACHRYMANS*.

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It was shown by us in a previous communication [1929] that, contrary to the usually accepted view, the action of *Merulius lachrymans* (the fungus responsible for dry-rot) on wood was to destroy the major part of the cellulose fraction of the lignocellulose complex and to leave the lignin component unaffected. It was found that the so-called hemicelluloses, which are present in woody tissues, were also resistant to fungal attack, whereas the hexosans, mannan and galactan were completely destroyed.

We have been able to confirm our previous observations as to the general action of the fungus on wood. The present investigation is concerned with the detailed analysis of the nature of the various fractions that can be isolated from rotted wood, together with a comparison of fractions isolated in a similar manner from sound wood.

EXPERIMENTAL.

The material employed was a 5th grade Norwegian spruce wood that had been rotted by *M. lachrymans*, and the various fractions were isolated according to the details given by Dore [1920]. The whole of the wood used (both sound and rotted material) came from the same source, but it was found that the decayed wood was decomposed to a very much greater extent than the sample used in our previous investigation. On this account a quantitative analysis was repeated, and the results are given below.

Compared with our previous sample, the rotted wood used in this investigation shows a considerable decrease in the alkali-soluble fraction (19.10 % compared with 30.0 %). A decrease is also shown in the lignin content (40.90 % compared with 43.00 %) as well as in the value for cellulose (9.88 % compared with 11.90 %), whereas, on the other hand, the alcohol-soluble and water-soluble fractions both show a marked rise over the percentages previously recorded.

Table I.

Treatment				Sound wood %*	Decayed wood %*
Extracted by	benzene	...	...	1.70	1.48
"	alcohol	...	...	1.60	19.80
"	water	...	...	2.50	8.00
"	5 % NaOH in the cold	...	...	3.80	19.10
Cellulose	...	...	...	55.00	9.88
Lignin	...	...	...	28.00	40.90
Mannan	...	...	...	7.60	0.00
Galactan	...	...	...	0.10	0.00
Total	...	...	...	100.30	99.16
Ash	...	...	...	0.86	0.61
Natural moisture	...	...	...	10.00	17.30

* Calculated on ash-free material dried at 100°.

The isolation of the various fractions described below was carried out on a large scale. The sample of sound wood was reduced to sawdust and the rotted material was ground to a fine powder in a mortar. The wood (both sound and decayed) was then treated in turn with the reagents described in Table I (*i.e.* benzene, alcohol, water and 5 % NaOH), and the cellulose was isolated after chlorination of the extracted samples and removal of the lignin either with sodium sulphite or with alcohol. Each particular sample was then worked up separately, and the various products were isolated and examined.

It will be convenient to detail the nature of the fractions obtained from the rotted and sound material separately and to discuss the theoretical implications later.

Fractions isolated from rotted wood.

Benzene-soluble fraction. After extraction of the decayed material with benzene and distillation of the solvent, the benzene-soluble fraction was obtained as a viscous, rather mobile, brown mass. This was heated on the water-bath with *N*/10 alcoholic caustic potash for 1 hour and the contents of the flask were then acidified with hydrochloric acid. A heavy brown precipitate separated, which on treatment with ether partially dissolved leaving a brown residue. The residue readily reduced Fehling's solution and formed an oxime. The ethereal extract was distilled under reduced pressure; the residue was taken up with a small amount of *N*/10 sodium hydroxide solution, and the sodium salt of a fatty acid was salted out from the alkaline solution with sodium chloride. Unfortunately the amount of fatty acid obtained was too small to allow of identification. The solution, after the initial precipitation of fatty acid and resin, was distilled under reduced pressure and tested with sulphuric acid and β -naphthylamine for glycerol; a positive reaction was obtained.

The benzene-soluble fraction of rotted wood is therefore composed of a fat or fats and two resins, one of which is soluble and the other insoluble in ether.

Alcohol-soluble fraction. On evaporation of the solvent, there remained a brown, amorphous mass, which partially melted at 60°. It was odourless and

soluble in acetone, glacial acetic acid and sodium hydroxide solution and insoluble in benzene and light petroleum. It was precipitated from glacial acetic acid solution by the addition of water or from sodium hydroxide solution by the addition of hydrochloric acid. It was purified for combustion by dissolving in glacial acetic acid and precipitating with water. The precipitated product was washed and dried *in vacuo*. Analysis indicated the empirical formula, $C_{22}H_{25}O_8$. (Found: C, 63.05 %; H, 6.11 %; $C_{22}H_{25}O_8$ requires C, 63.3 %; H, 5.99 %.) The substance reduced Fehling's solution and the presence of an aldehydic group was confirmed by the preparation of an oxime. Determination of the molecular weight showed that the above formula should be doubled. The substance is therefore not of the nature of the metalignin isolated by Dorée and Barton-Wright [1927], as was previously suggested by us. The substance contained two methoxyl groups (OMe = 9.21 %) and yielded a tetrabenzoyl derivative showing the presence of four hydroxyl groups. (Found: C, 69.0 %; H, 5.6 %; $C_{44}H_{46}O_{12}$ ($O.CO.C_6H_5$)₄ requires C, 69.1 %; H, 5.28 %.) All attempts to separate the material into different fractions failed and from its reactions it appeared to be perfectly homogeneous. Its extended formula can therefore be written $(CH_3O)_2.C_{41}H_{39}O_9(OH)_4.CHO$.

Water-soluble fraction. The water-soluble fraction was acid to litmus and when evaporated to dryness under reduced pressure yielded a dark-brown, viscous mass, which rapidly reduced Fehling's solution. Neither tannins nor organic acids were found to be present, and the nature of the acidity remained undetermined.

The extract was decolorised by boiling with animal charcoal, and methyl alcohol was added to the filtrate, when a copious white precipitate separated. This was found to be entirely composed of glucose (identified by osazone and Bertrand's test). This, with the exception of a trace of acid the nature of which we were unable to determine, was the sole constituent of the water-soluble fraction.

5 % sodium hydroxide-soluble fraction. When the rotted wood, after previous extraction with benzene, alcohol, and water was treated for 36 hours in the cold with a 5 % solution of sodium hydroxide, a dark-brown liquid was obtained. This was filtered off from the residue and together with the first washing was treated with an excess of acetic acid, which produced a flocculent brown precipitate. After standing for 24 hours, the supernatant liquid was poured off, and the precipitate was washed several times with water to free it from acid. It was air-dried for several days and finally dried *in vacuo*. During the course of drying it became very much darker in colour.

The supernatant liquid, which was of a pale straw colour, was dialysed for 8 days to free it from acetic acid and sodium acetate and then evaporated to small bulk under reduced pressure and treated with an equal volume of alcohol. The small amount of dark-brown precipitate was filtered off, washed with alcohol and dried *in vacuo*. The filtrate was evaporated to dryness, when a dark-brown evil-smelling mass was left as a residue.

The alkali-soluble fractions of wood contain the so-called "hemicelluloses," bodies which are composed of hexose and pentose sugars as well as uronic acids of the galacturonic and glycuronic type. A number of investigations have recently appeared on these compounds. O'Dwyer [1926, 1928], for example, was able to isolate two such bodies from beech wood sawdust, to which she gave the names of hemicellulose A and hemicellulose B respectively. Hemicellulose A was insoluble in water, but soluble in dilute solutions of sodium hydroxide, from which it could be precipitated by acids; hemicellulose B was soluble in water, but insoluble in alcohol. The two substances which we have been able to isolate from decayed spruce wood appear to be of the nature of hemicelluloses, but owing to the fact that they showed a number of differences from the products isolated by O'Dwyer, as well as from the corresponding fractions isolated from sound spruce wood, we prefer to name them hemicellulose A_1 and hemicellulose B_1 .

Hemicellulose A_1 . This was a dark-brown amorphous body, insoluble in water, but slightly soluble in alcohol. It was readily soluble in weak solutions of sodium hydroxide and could be reprecipitated from alkaline solution by the addition of acid. It reduced ammoniacal silver nitrate but not Fehling's solution.

20 g. of the material was hydrolysed with 200 cc. of 3 % sulphuric acid for 6 hours. At the end of this time the residue was filtered off, and the requisite amount of barium hydroxide was added to neutralise the sulphuric acid. The precipitate of barium sulphate was filtered off, and the filtrate was found to be still slightly acid. A further quantity of barium hydroxide was added until the solution was alkaline, and the precipitate was filtered off and preserved. The filtrate was evaporated under reduced pressure to a thin syrup and extracted several times with absolute alcohol and then with 80 % alcohol.

The two alcoholic extracts were tested for mannose as the hydrazone, arabinose as the osazone, xylose as the cadmium xylobromide salt, galactose as mucic acid and glucose as the osazone. The portion soluble in absolute alcohol gave the characteristic crystals of cadmium xylobromide and showed the presence of xylose in the complex. No other sugar was found to be present in this portion. The extract obtained with 80 % alcohol gave galactosazone melting at 189–190°. The presence of galactose was confirmed by the preparation of mucic acid.

The percentage of uronic acid in the complex, estimated by the method of Nanji, Paton and Ling [1925], was 10.73 %. The second barium precipitate mentioned above was treated with the calculated quantity of sulphuric acid to decompose the barium salt of the uronic acid. The barium sulphate was filtered off, and the filtrate was saturated with cinchonine at 100°. On cooling, large crystals of cinchonine sulphate separated out, owing to the presence of a slight excess of sulphuric acid in the mixture; at the same time, a mass of fine needle-shaped crystals was also obtained. The fine needles were centrifuged off, freed from cinchonine sulphate by washing with alcohol and twice

recrystallised from water (m.p. 211–214°). We have been unable to find in the literature a uronic acid the cinchonine salt of which melts at this temperature. Up to the present time, the only uronic acids that have been isolated from hemicelluloses are galacturonic and glycuronic acids. It is possible that we are here dealing with a penturonic acid, that derived from arabinose or xylose. Gymnospermous woods have not been investigated with regard to the nature of their hemicellulose content, and we are at present engaged in making a fuller examination of this point, as well as in preparing pure samples of the uronic acids from xylose and arabinose for purposes of comparison.

When treated with fuming hydrochloric acid (sp. gr. 1.20) hemicellulose A₁ after 20 hours yielded a lignin-like body having OMe 12.4 %. The original sample before acid treatment had OMe 6.68 %.

The nature and amounts of the products obtained from hemicellulose A₁ are shown in Table II. An attempt was made to determine the nature of the

Table II.

	%
Xylose	1.17
Uronic acid (nature unknown)	10.73
OMe of hemicellulose A ₁ before treatment with HCl	6.68
OMe of residue after treatment with HCl (<i>sp. gr.</i> 1.2)	12.40
OMe of residue calculated on original material	6.15
OMe hydrolysed by HCl	0.53
Residue insoluble in HCl	49.64

linkages between the component parts of the molecule. Portions of 5 g. of the hemicellulose A₁ were hydrolysed with 3 % sulphuric acid for 1, 3, 5 and 10 hours respectively and the methoxyl content, furfuraldehyde and carbon dioxide values were determined. The figures, calculated as a percentage of the original material, are given in Table III. It is clear that the linkages between

Table III. *Effect of acid hydrolysis for varying periods on hemicellulose A₁.*

Fraction	Original material %	1 hour %	3 hours %	5 hours %	10 hours %
Xylose	1.17	0.00	0.00	0.00	0.00
Uronic acid	10.73	4.10	4.40	4.40	4.89
Methoxyl	6.68	6.52	6.45	6.30	6.00

the component parts of the complex differ from one another. Part of the methoxyl is hydrolysable by dilute acid, and the ease of hydrolysis suggests that the linkage is of an ester type, while the remainder of the methoxyl is attached to the portion insoluble in fuming hydrochloric acid, *i.e.* the lignin-like fraction, and the linkage here is of a more resistant nature, probably of an ether type.

The uronic anhydride content exhibited a peculiar feature, namely, a large initial decrease (7 %) after hydrolysis for one hour, but showed an increase during the remaining period. This result again suggests that there is more

than one type of linkage present in the complex. During the first hour, a weak linkage is destroyed, but during the remaining period over which hydrolysis is continued, a more resistant linkage is attacked, and one which prevents complete decarboxylation from taking place when uronic acids are estimated by the method of Nanji, Paton and Ling. The mild acid hydrolysis breaks this linkage slowly, and the yield of carbon dioxide recorded on decarboxylation represents the sum of the yields of gas from the acid set free by the mild acid hydrolysis and that set free by 12 % hydrochloric acid in the course of estimation. In order to nullify the effect of the latter hydrolysis, the time during which the estimations were carried out was kept constant. In this way the effect of the mild hydrolysis became apparent.

The fraction of rotted wood soluble in sodium hydroxide solution can therefore be divided into two portions, a part insoluble in fuming hydrochloric acid, which contains methoxyl groupings, but yields no carbon dioxide or furfuraldehyde when treated with hydrochloric acid, and may well be a body of lignin-like nature; and a second part composed of a mixture of pentose (xylose) hexose (galactose) and an unknown uronic acid. These constituents may be removed separately from the complex, together with a certain amount of methoxyl residues. The linkages between the xylose, galactose and uronic acid have not been completely defined by the methods employed, but it is evident that the pentose is more loosely combined in the complex than galactose and that the uronic acid is linked in such a manner that it is shielded from decarboxylation. This might be achieved by esterification or by the linkage of one of the other components of the complex to one of the carbon atoms in the uronic acid molecule so that a protective action is exercised over the carboxyl group. The possibility of esterification preventing decarboxylation is remote. Until we have carried out further work on the point, the first alternative will have to stand.

Hemicellulose B₁. Hemicellulose B₁, isolated in the manner described above, was a dark-brown powder, readily soluble in water. Unfortunately the amount available was too small to allow of any extensive observations being made on its properties. It contained 7.12 % of pentose; the methoxyl content was 3.19 % and the uronic acid value 7.68 %. When treated with hydrochloric acid (sp. gr. 1.2) for 24 hours it gave an insoluble residue of 31.88 %. It thus appears that here, as in the case of hemicellulose A₁, the true hemicellulose portion of the molecule is linked with a lignin-like fraction.

Cellulose fraction. The cellulose was isolated from decayed wood by chlorination after previous extraction in the cold with 5 % sodium hydroxide and subsequent removal of the chlorolignin by repeated extraction with boiling alcohol. It could also be isolated by boiling the chlorinated product with sodium sulphite. Both methods gave similar results. The residue, after extraction of the lignin with alcohol, was air-dried, when it was obtained as a hard dry mass, which could be reduced to a fine powder by grinding in a mill. It showed little fibrous structure. Small portions of the material were treated

with calcium hypochlorite, but in no case was any bleaching action observed. Uronic anhydride amounted to 3.23 % and pentose to 0.39 %. The physical nature of the material, together with the increased uronic acid value compared with cellulose isolated from normal wood (see below), suggested that the cellulose had suffered oxidation under fungal action; tests for oxycellulose were therefore carried out. The material rapidly reduced Fehling's solution and showed a marked affinity for basic dyes. With α -naphthol and sulphuric acid it developed a violet colour and with thymol and sulphuric acid a rose tint. Subjection to the Cannizzaro reaction abolished the reducing properties. The residue after treatment with strong sodium hydroxide solution was filtered and washed free from alkali and then tested with Fehling's solution; no reduction occurred. The filtrate, when acidified with hydrochloric acid, yielded a white precipitate which also failed to reduce Fehling's solution. The filtrate was neutralised and tested with Fehling's solution, but showed no reducing properties. Presumably an aldehydic grouping present in the cellulose of the decayed wood had suffered simultaneous oxidation and reduction under the influence of the Cannizzaro reaction, giving rise to an insoluble alcohol and the sodium salt of an insoluble acid. These tests indicate that part at least of the cellulose contained in rotted wood consists of oxycellulose. Treatment with 17.5 % sodium hydroxide solution according to the method of Schorger gave a yield of 22.9 % of α -cellulose. No attempt was made to estimate either β - or γ -cellulose.

Lignin fraction. The alcoholic solution obtained from the extraction of chlorolignin was distilled under reduced pressure until the chlorolignin began to be deposited as a hard brown mass on the bottom of the flask. This was removed, ground to a fine powder and washed with a small amount of water until free from sodium chloride (which remained from an earlier brine treatment of the chlorinated wood).

The chlorolignin thus isolated could be separated into two fractions: one soluble in glacial acetic acid and one soluble in sodium hydroxide solution. The chlorolignin was first treated with glacial acetic acid in the cold or occasionally with gentle warming, until after repeated extraction no more would pass into solution. The residue was washed free from acid and treated with a 1 % solution of sodium hydroxide. The acid fraction was isolated by pouring into a large volume of water, when a flocculent brown precipitate settled out. This was filtered off, washed, and dried *in vacuo*. The portion soluble in dilute sodium hydroxide solution was precipitated by the addition of dilute hydrochloric acid, washed free from acid and dried *in vacuo*.

Glacial acetic acid fraction. Further purification for combustion was effected by re-dissolving in glacial acetic acid and again precipitating with water. (Found: C, 47.6, 47.4 %; H, 3.72, 3.6 %; Cl, 19.22, 19.07 %.) No formula can be constructed from these figures and the acetic acid-soluble fraction is evidently a mixture. Further evidence that a mixture had been obtained can be seen from the figures found for the combustion of the benzoyl derivative,

in which the percentages for hydrogen are higher than for the original product. (C, 54.64, 54.22 %; H, 4.48, 4.33 %; Cl, 11.91, 11.61 %.)

Sodium hydroxide-soluble fraction. This also proved to be a mixture and the figures found for carbon on combustion showed an even greater variation than those obtained for the acetic acid-soluble fractions. (C, 43.05, 43.15, 48.1, 48.9 %; H, 4.09, 3.85, 3.68, 4.07 %; Cl, 15.87, 15.30 %.)

Fractions isolated from sound spruce wood.

For the purpose of comparison, fractions were now isolated from sound spruce wood in exactly the same manner as from the decayed material. The wood was reduced to sawdust by running the end of a block against a fast-running circular saw. In this way 1400 g. of material were prepared. This sawdust was reduced to wood flour of the same grade as the rotted sample by grinding in a mill and was extracted successively with benzene, alcohol, water and 5 % sodium hydroxide solution in the cold. The cellulose was isolated by chlorination of the wood after alkaline extraction followed by boiling with alcohol or sodium sulphite.

Benzene-soluble fraction. On evaporation of the solvent, a practically colourless, odourless, slightly viscous resinous mass remained. This was boiled for one hour with *N*/10 alcoholic caustic potash; the contents of the flask were then acidified with hydrochloric acid and the precipitate of fatty acids and resins filtered off. On extraction with ether, the resins entirely passed into solution, together with a portion of the fatty acids. The ethereal extract was evaporated to small bulk, and the residue was dissolved in a small amount of sodium hydroxide solution, from which the fatty acids were salted out, leaving the resins in solution. The filtrate, after initial acidification with hydrochloric acid, was distilled under reduced pressure and tested for glycerol with β -naphthylamine and sulphuric acid: a positive reaction was obtained.

The benzene-soluble fraction isolated from sound wood, therefore, like that of the rotted material, is composed of fats and resins.

Alcohol-soluble fraction. After removal of the alcohol by distillation under reduced pressure, a small amount of a dark-brown, oily liquid remained. This was found to be insoluble in glacial acetic acid and was too small in amount for identification.

Water-soluble fraction. On evaporation of the aqueous extract under reduced pressure, a dark-brown, slightly viscous mass remained. This reduced Fehling's solution and tests with methylene blue showed it to be composed of tannins and reducing sugars in small amount.

Sodium hydroxide-soluble fraction. The residue of the sound wood after extraction with benzene, alcohol and water was allowed to stand in a 5 % solution of sodium hydroxide for 36 hours in the cold. At the end of this period the residue was filtered off yielding a golden-brown solution. The first washings from the residue were added to the filtrate and the whole was acidified with acetic acid. A small amount of a light-coloured precipitate

separated out. The supernatant liquid was removed and the precipitate was washed free from acid and dried *in vacuo*. Unlike the corresponding precipitate from decayed wood, it underwent no darkening in colour on drying. The filtrate, after acidification, was dialysed for 8 days to remove excess of acetic acid and sodium acetate and was then evaporated to small bulk under reduced pressure. When alcohol was added, a slight precipitate was formed, but this was too small in amount to collect.

The first precipitate which separated on acidification of the alkaline extract presumably corresponds with the hemicellulose A of O'Dwyer and the second, which formed on the addition of alcohol, to her hemicellulose B. In any case her nomenclature will be followed here for the sake of convenience and to save confusion.

Hemicellulose A. The fawn-coloured precipitate formed on the addition of acid to the alkaline extract of sound wood was treated with fuming hydrochloric acid (sp. gr. 1.2) for 24 hours and left a residue of 47.7 %, which had OMe 12.72 %. The original material had OMe 7.87 %, and contained 3.61 % of uronic anhydride and 5.94 % of pentose.

12 g. of the material were hydrolysed with 3 % sulphuric acid for 5 hours at 100°. At the end of this time, the residue was filtered off, washed and dried and amounted to 55.9 % of the original material. The filtrate was neutralised with barium hydroxide in exactly the same way as for the fraction isolated from decayed wood, and the precipitate of barium sulphate was removed and preserved. The residue of the neutralised filtrate after evaporation was extracted first with absolute alcohol and then with 80 % alcohol, and the two extracts were tested for mannose, galactose, arabinose and xylose. The absolute alcoholic extract was found to contain xylose and the 80 % alcoholic extract galactose, while the barium sulphate precipitate contained the unknown uronic acid described above for hemicellulose A₁. The constitution of hemicellulose A is summarised in Table IV. Owing to the small amount of

Table IV.

	%
Xylose	5.90
Uronic acid	3.61
OMe	7.89
Residue after treatment with HCl (<i>sp. gr.</i> 1.2) ...	47.70
OMe in residue after treatment with HCl	12.72
OMe calculated on original material	6.07
OMe readily hydrolysed by HCl $\left(7.89 - \frac{12.72 \times 47.7}{100}\right)$	1.82

material available it was impossible to carry out a series of hydrolyses over varying periods of time, as in the case of hemicellulose A₁. We have, on this account, no evidence as to the nature of the linkages between the component parts of the complex.

Cellulose. The cellulose was isolated after alkaline extraction of the sound wood and chlorination, by dissolving out the chlorolignin either with alcohol

or with alkaline sodium sulphite. The percentages obtained were the same in both cases; 55.0 % as compared with 9.88 % in rotted wood.

The cellulose was a white fibrous substance, which did not reduce Fehling's solution and showed none of the properties of oxycellulose. It yielded 80.65 % of α -cellulose and 8.97 % of pentosan, calculated from the furfuraldehyde precipitated as phloroglucide, after making allowance for the uronic acid content. The percentage of uronic acid was 0.95 %.

Lignin fraction. The chlorolignin was isolated from the sound wood in the same manner as described above for decayed material. Separation into two fractions was again effected by means of glacial acetic acid and 1 % sodium hydroxide solution. On combustion the samples gave the following values:

Glacial acetic acid fraction: C, 47.89, 48.77, 52.9, 42.1, 43.1 %; H, 4.54, 4.94, 4.79, 4.66, 5.42 %; Cl, 18.77 %; OMe, 4.7 %.

Sodium hydroxide-soluble fraction: C, 49.53, 53.14, 50.77 %; H, 4.03, 4.87, 4.77 %; Cl, 12.93, 12.83 %.

From the wide variations in these figures, it is clear that, as in the case of the chlorinated products isolated from decayed wood, we are dealing with mixtures and not homogeneous products. The main difference lies in the fact that the samples isolated from fungus-infected wood possess a higher percentage of oxygen than the corresponding samples from sound material.

DISCUSSION.

It is necessary to emphasise the fact that throughout the course of this investigation only completely decomposed material was employed. We are, on this account, unable to advance any views as to the exact course of fungal metabolism at each stage in the progress of decay. It is clear, however, that the presence of the fungus in the wood produces far-reaching and profound changes in the wood components, especially in the two most important fractions of the lignocellulose complex, namely, the cellulose and lignin.

The fact that the cellulose has fallen by 81 % in the rotted material and that the residue remaining is much altered in nature, indicates that the chief action of the mycelium is exercised on this particular component of the woody tissues. Two reactions appear to have progressed simultaneously here: a hydrolysis of the cellulose to glucose and an oxidation to oxycellulose. Presumably the comparatively large amount of glucose located in the water-soluble fraction of the rotted wood was originally derived from the cellulose and was left as an unconsumed residue by the fungal mycelium in its passage through the wood. In any case, it is the α -cellulose fraction of the total cellulose of the wood that is chiefly affected by the presence of the fungus, as there is a fall from 47.6 % to 2.26 % in α -cellulose, calculated on the total wood.

It was shown by us in our previous communication that the hemicellulose fractions of the wood were apparently unaffected by fungal infection; although at the time we were unable to account for the increased solubility of decayed

wood in sodium hydroxide solution. It is very unfortunate in this connection that different workers have defined the term hemicellulose in different ways. Candlin and Schryver [1928], for example, have introduced the term "polyuronide" to include substances containing linked uronic acids and sugars, whether the latter be pentoses or hexoses. Such a definition includes pectins as well as hemicelluloses of the type described by O'Dwyer and gums of the gum arabic type recently described by Norman [1929]. More recently, Norris and Preece [1930] have agreed with the classification put forward by Candlin and Schryver and have further suggested that the term hemicellulose should be restricted to bodies which give no uronic acids but only sugars on hydrolysis. It appears to us superfluous to introduce the term polyuronide in this connection, since it brings into the same class such diverse bodies as the pectins and hemicelluloses isolated by O'Dwyer, while the terms hexosan and pentosan still appear to be quite adequate to describe compounds only containing hexoses or pentoses, and some synthetic name may be derived for products consisting of mixtures of these two classes of sugars. The term hemicellulose is used here for products soluble in dilute sodium hydroxide solution, but insoluble in sodium carbonate solution, which on hydrolysis yield hexose and pentose sugars as well as uronic acids. Using such a definition, it will be seen that the hemicellulose products of wood are practically unaffected by *M. lachrymans*. In both sound and rotted wood, the same products are obtained on hydrolysis, namely, xylose, galactose and an unknown uronic acid and in much the same proportions. A residue insoluble in fuming hydrochloric acid is also found in both cases and it is curious that no mention is made by either O'Dwyer or Norman that a residue remains when this material is hydrolysed by weak acid. The fact that a residue of nearly 50 % remains after treatment with fuming hydrochloric acid for 24 hours considerably invalidates the Willstätter and Zechmeister [1913] method of estimating lignin in woody tissues.

It is peculiar that the fungus fails to remove the hemicellulose fraction of the wood, since the hemicelluloses contain both hexose and pentose sugar in their composition. On the other hand, *M. lachrymans* is markedly sensitive to the action of acids and even such weak acids as tannic and gallic acid inhibit its growth and produce a toxic effect. Since uronic acids are produced by the hydrolysis of hemicelluloses, it may well be on this account that the fungus fails to attack the hemicellulose fraction.

Campbell and Booth [1929] have investigated the action of *Trametes serialis* on Sitka spruce and like other investigators have observed an increase in the solubility of the rotted material in dilute solutions of caustic alkalis, as well as a decrease in cellulose content. From the sum of the percentages of the fractions in the rotted wood, they have put forward the suggestion that a substance is formed, which, while insoluble in water, is not estimated as either cellulose, lignin or pentosan and is possibly of the nature of a carbohydrate degradation product. From a comparison of the effect of the fungus

on sawdust with the action of an aqueous acid hydrolysis at 100° they have formulated the view that, in the earlier stages at least, the effect of infection of woody tissues by a "brown-rot" type of fungus is to be regarded as strictly comparable with aqueous acid hydrolysis and consider that support for this suggestion is to be found in the acid nature of rotted wood. In the absence of any experimental work on the nature of the alkali-soluble fraction of rotted material, it appears somewhat premature to advance any theory to account for the effects of fungi of the "brown-rot" type. In the later stages of decay, our results indicate that oxidation plays a very much more prominent part in the process than anything akin to an aqueous acid hydrolysis.

Although the total lignin content of wood is unaffected by *M. lachrymans*, nevertheless, the fungus produces a number of changes in this component of the lignocellulose complex. One curious feature of the problem is the occurrence of a definite compound of composition $C_{44}H_{50}O_{16}$ in the alcohol-soluble fraction of decayed wood. This substance shows the reactions ordinarily associated with an "alkali" lignin and very closely approximates in composition to the lignin isolated by Powell and Whittaker [1924, 1925] from flax by treatment of the fibres with 8–12 % solutions of sodium hydroxide under pressure for several hours. We had hoped that this product would have been a substance of simpler composition and possibly of the nature of "metalignin" isolated by Dorée and Barton-Wright [1927], which possessed the molecular composition, $C_{20}H_{20}O_6$.

It is difficult to account for the presence of this lignin in the alcohol-soluble fraction. It was the only homogeneous lignin product we were able to isolate throughout this investigation. The majority of investigators of the chemistry of lignin have assumed that the lignin fraction of the lignocellulose complex is a homogeneous body and numerous formulae have been put forward from time to time. From the results of our present work, this homogeneity does not appear to exist. The chlorolignins that were isolated, for example, from both sound and decayed wood were all mixtures. It is possible that lignin, using the name generically, is composed of closely related isomerides, perhaps differing only in methoxyl content, and, since they are colloidal in nature, it may be on this account that apparent homogeneity has been found, where in fact none existed. But whether lignin be a single substance or a mixture, it is highly susceptible to oxidation and is able to take up large amounts of oxygen [*cf.* Dorée and Hall, 1924; Dorée and Barton-Wright, 1929]. Apparently a similar result is brought about by the action of a "brown-rot" fungus on wood. The chlorolignins isolated from decayed wood all possessed a higher oxygen content than similar bodies isolated from sound material.

The action of *M. lachrymans* on wood is therefore to consume the hexosans, mannan and galactan, and then to attack the cellulose fraction of the complex. The latter is converted to glucose, the α -cellulose being primarily affected, while a small residue is left as oxycellulose. The hemicelluloses are not destroyed and the lignin fractions, though not removed, suffer oxidation.

SUMMARY.

1. A detailed investigation of the action of *Merulius lachrymans* (the fungus of dry-rot in wood) has been made on spruce wood. The action of the fungus is first to remove the easily hydrolysable fractions, mannan and galactan, and then to attack the cellulose. The latter is converted into glucose and consumed as such. At the same time a small residue of oxycellulose is left.

2. A homogeneous lignin body was extracted from the rotted wood with alcohol, which showed the molecular composition $C_{44}H_{50}O_{16}$ and possessed the extended formula $(OCH_3)_2 \cdot C_{41}H_{33}O_9(OH)_4 \cdot CHO$. From its reactions it appeared to be similar to the lignins which have been isolated from woody tissues by the action of caustic alkalis under pressure.

3. The hemicellulose fractions of spruce wood are unaffected by the fungus. An unknown uronic acid was isolated from the hemicellulose A fraction. The suggestion is put forward that this is a penturonic acid.

4. The total lignin content of the wood is practically unaltered, but marked oxidation has taken place, *i.e.* there is little or no delignification of the wood.

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