XXX. THE BIOCHEMISTRY OF DRY-ROT IN WOOD

III. AN INVESTIGATION OF THE PRODUCTS OF THE DECAY OF PINE WOOD ROTTED BY *MERULIUS LACHRYMANS*

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THE previous observation [Barton-Wright & Boswell, 1929, 1931] that the dry-rot fungus *Merulius lachrymans* can be classified as of the "brown rot" type is confirmed. The present communication deals with the detailed constitution of the cellulose-containing, water-soluble and dilute alkali-soluble fractions which were only superficially studied in the earlier work. From the alcohol-soluble fraction a compound has been prepared which forms a 2:4-dinitrophenylhydrazone when treated according to Brady [1931]. This substance had a N-content similar to that of the dinitrophenylhydrazone of the pure substance previously isolated from the alcohol-soluble fraction of spruce wood. These three fractions appear to contain the products of the fungal activity. The benzene-soluble material seems to be unaffected both in amount and in constitution while evidence will be brought forward later that the increase in lignin content of rotted wood as compared with sound wood is solely due to the loss of cellulose degradation products as the gaseous residues of fungal respiration.

The rotted wood used formed part of the panelling on the inside of the brick wall of an outhouse of an empty property. As will be seen from the figures of the bulk analysis the more rotted portion was in an advanced state of decay. The fungal mycelium had passed through the mortar between the bricks and produced, under the ivy covering the outside of the wall, fruit bodies identified as those of *Merulius lachrymans*. The wood of the panelling was a species of *Pinus*, probably *P. resinosa*, the Norway or Red Pine. The rotted wood was separated into two portions according to whether it could or could not be easily powdered by hand in a pestle and mortar. The more rotted portion powdered easily whilst the less rotted parts were reduced in a grinding mill.

The methods of analysis and the preparation of the fractions in bulk were similar to those used previously with the following exceptions. The cellulose was isolated by the technique of Norman and Jenkins [1933] and the lignin by the use of 72 % H_2SO_4 instead of the chlorination method which had previously failed to provide a uniform product. The two samples of rotted wood gave the following analytical values (the analysis of a sound sample of *P. resinosa* is included for purposes of comparison):

Table I

	Sound %	"Rotted" %	"More rotted" %
Extracted by benzene	18.09	3.15	3.51
" alcohol	1.80	7.44	18.05
" water	1.93	6.05	9.20
" 4% NaOH	7.37	29.20	19.91
Cellulose	49.57	27.97	14.36
Lignin	18.99	26.81	38.37
	97.75	100.62	103.40

The content of benzene-soluble material in many soft woods is extremely variable and the wood of *P. resinosa* is no exception. From a series of samples prepared from the same piece of timber 6 × 2 × 12 in. the following values for this fraction have been obtained: 10·6, 2·5, 14·4, 7·8 and 2·6 %. Taking into account the variability of these figures and the fact previously established that the constitution of the benzene-soluble fraction is unaffected by the fungal attack on wood, more comparable results should be obtained by calculating them not on an "ash-free, dried at 100°" basis but on an "ash-free, dried at 100°, benzene-extracted" basis. The following table gives the results of Table I calculated on the benzene-extracted basis.

Table II

	Sound %	"Rotted" %	"More rotted" %
Extracted by alcohol	2·20	7·85	18·77
" water	2·35	6·23	9·59
" 4 % NaOH	8·99	30·08	20·71
Cellulose	60·48	28·81	14·83
Lignin	23·17	27·61	40·42
	97·19	100·58	104·32

The "more rotted" sample is in very decayed condition, the cellulose content having fallen to 14·83 % and the lignin risen to 40·42 %. The rise in the lignin content is a measure of the loss of tissue in the form of gaseous products and only partially an indication of the degree of rotting as the cellulose molecule can be much degraded without any great loss of weight in the rotted wood. This is quite clearly shown in the "rotted" sample. Here the rise in the % lignin is relatively small and yet the cellulose content has been reduced to half with a corresponding rise in the material soluble in dilute NaOH. It is clear therefore that in the early stages of the fungal attack the cellulose molecule is rapidly broken down to smaller units soluble in dilute alkali and that this process, probably hydrolytic, takes place much more rapidly than the oxidation of these small units to the gaseous end products of respiration. This rapid degradation of cellulose to smaller units is probably the reason why even in the earliest stages of rotting the loss of mechanical strength by timber is very great. The accumulation of alcohol-soluble material as the rotting proceeds suggests that this is one of the products of the fungal activity. The fractions which have been examined are described below.

Cellulose

The fractions prepared from both wood samples showed signs of fungal attack in that they were greyish in colour, on drying either in air or *in vacuo* became hard amorphous masses and when suspended in either water or dilute alkali formed grey slimy pulps: these changes were particularly marked in the cellulose from the "more rotted" sample. The uronic anhydride and pentosan values were determined as usual by boiling with 12 % HCl, the α -cellulose by treatment with 17·5 % NaOH for 30 min. at room temp. and the i.v. by the method of Bergman & Machemer [1930]. The following table gives the values determined for these two cellulose fractions.

Table III

	"Rotted"	"More rotted"
Uronic anhydride	1·60%	3·00%
Pentosan (calculated according to Angell <i>et al.</i> [1936])	3·50%	2·23%
i.v.	2·20	3·09
α -cellulose	21·1%	39·10%

It might appear that the α -cellulose values of the "rotted" and the "more rotted" samples are contradictory in that the cellulose from the latter contains more of the α -variety than that from the former. If however the α -cellulose is calculated as % of the wood sample as a whole then it is clear that even in the later stages of fungal attack the α -cellulose is attacked but not as rapidly as in the earlier. Of the 28.81 % cellulose in the "rotted" wood 21.1 % is the α -form giving the α -cellulose of the wood sample as 6.08 %, whilst in the "more rotted" wood 14.83 % cellulose has an α -content of 39.1 % giving a value for the whole wood of 5.90 %. It might be doubted whether this difference is significant, but if allowance be made for the fact that the cellulose in the "more rotted" wood is the residue of a much greater amount of sound cellulose than that in the "rotted" wood, actually nearly one-half as much again, then it is clear that the α -cellulose content of the cellulose has been considerably reduced even in the later stages of the fungal attack.

The fall in the pentosan content indicates that the fungus is acting in a manner similar to its attack on the cellulose. In the early stages the pentosan is removed more rapidly than the cellulose, the former by 85 %, the latter by 50 %. The effect of the fungus on the pentosan content of the wood as a whole will be considered at a later stage.

From the i.v. it is possible, following Bergman & Machemer [1930], to calculate the mol. wt./unit reducing group for these two fractions. The values obtained are given below in Table IV and have been calculated for that part of the cellulose fraction which cannot be classified as α -cellulose. In view of the difference of opinion which exists concerning the structure of the α -cellulose molecule the values for the non- α -cellulose fraction have been calculated in two ways: firstly considering the α -cellulose molecule as being a straight chain of approximately 150 glucose anhydride units and therefore having i.v. 0.8; secondly, regarding it as a ring structure with zero i.v.

Table IV

	Mol. wt./unit reducing group	
	"Rotted"	"More rotted"
i.v. 0.8	7782	4386
" 0.0	7194	3945

It appears therefore that parallel with reduction in the α -cellulose the non- α -cellulose is being hydrolysed with the production of units of smaller mol. wt./unit reducing group; the fungal attack is therefore shortening the chain lengths of the cellulose molecules. As the pentose contents of these cellulose fractions are small it is clear that these mol. wt. values illustrate very largely the effect of the fungus on the cellulose molecule. If in addition to the hydrolytic action of the fungus, oxidation occurs so that some of the freed reducing groups are oxidised, then the actual chain lengths of the molecules would be much shorter than represented by the figures in the table. These may therefore be regarded as the maximum values for the mol. wt./unit reducing group. It should be remembered that these fractions are probably mixtures of many chain lengths and the values obtained are therefore only averages of the mol. wt. of the units in the sample.

The values for the uronic anhydride contents are more difficult of interpretation, since the rising values with increasing degree of rotting may be due solely to the inability of the fungus to attack a molecule of the uronic type, the

rise in the content of uronic anhydride being due to the removal of other parts of the fraction. The uronic anhydride content of the normal cellulose was 0.9 % and with such a value for the unrotted material it is impossible to come to any conclusion concerning the origin of the unit in the rotted cellulose.

Alkali-soluble fraction

Two samples were prepared, one from the "rotted" wood and the other from the "more rotted". The powdered wood after extraction with benzene, alcohol and water was kept with 4 % NaOH for 24 hr. in the cold with frequent stirring, the proportions being 1 l. NaOH to 100 g. wood. The alkaline filtrate was acidified with excess glacial acetic acid, the precipitate centrifuged, washed with water and dried by treatment with 95 % alc., abs. alc. and ether successively. The product from the "more rotted" wood was light brown in colour while that from the "rotted" wood was light grey. The product obtained by drying with alcohols and ether, while showing no chemical differences from that prepared by washing with water and dilute alcohol until acid-free and drying *in vacuo*, was very much lighter in colour. The following table gives the values of certain estimated groups.

Table V

	"Rotted"	"More rotted"
Uronic anhydride	10.96 %	5.75 %
Pentosan	4.49 %	7.70 %
Methoxyl	4.45 %	6.71 %
I.v.	35.20	50.10
Mol. wt./reducing group	568	399

The methoxyl determinations were made using the semi-micro method of Clarke [1932].

Our previous work had shown that this alkali-soluble material was a mixture of various substances and as a preliminary stage in the investigation the fractions were submitted to mild acid hydrolysis.

10 g. of the "more rotted" sample were hydrolysed at 100° using 16 ml. HCl in 500 ml. water for successive periods of 3 and 6 hr., 2 g. of the residue were treated with fuming HCl in the cold for 18 hr., the solution diluted to 3 % and heated at 100° for 3 hr. The residue was filtered off and regarded as a lignin-like substance. The following table gives the values of the radicals determined in the residues from each of the three successive periods of hydrolysis. The values in brackets indicate the amount of the radical calc. as % original material. The pentosan values were calculated following the formula suggested by Angell *et al.* [1936].

The filtrates after removal of the residues from the acid hydrolysis were neutralized with BaCO₃. Those from the dilute acid hydrolysis yielded a small quantity of precipitate, pale purple in colour and that from the strong acid hydrolysis a small quantity of an almost black substance. The organic material was separated from the BaCO₃ by evaporating the solution to dryness *in vacuo* and extracting the residue with 75 % alcohol, evaporating the extract to dryness and extracting the residue with a higher concentration of alcohol. This process was continued until BaCO₃-free hydrolysis products were obtained in either 95 % or absolute alcohol. The products from the dilute acid hydrolysis were obtained in two fractions, one soluble in absolute, and the other in 95 % alcohol. The former gave crystals of cadmium xylobromide when treated in the usual manner and from the latter were obtained with phenylhydrazine characteristic

crystals of mannose phenylhydrazone, M.P. 190°. By titration with Fehling's solution the mannose was estimated as 0.39 g. or 3.9 % of the original material.

It appears therefore that dilute acid hydrolysis liberated mannose, xylose and an unknown acid, the last being obtained as a Ba salt.

Table VI

	Original substance	After hydrolysis		
		3 hr. (100°)	9 hr. (100°)	Fuming HCl (15°)
% of original	100	82.5	80.8	49.5
% OMe	6.71	9.2 (7.3)	9.03 (7.3)	11.84 (5.86)
% uronic anhydride	5.75	4.46 (3.68)	4.94 (3.99)	0.00
% pentosan	7.70	0.00	0.00	0.00
I.V.	50.1	24.8	23.3	32.2
Mol. wt./reducing group	399	807	858	621

The products of strong acid hydrolysis were obtained in solution in 95 % alc. The solution contained glucose (isolated as glucosazone). By titration with Fehling's solution the glucose content of the solution was calculated as 0.4 g. The total loss on hydrolysis by fuming HCl was 3.13 g. and of this 1.60 g. were obtained as glucose.

The alkali-soluble material from the "more rotted" wood consisted therefore of three parts; first xylose, mannose and an unidentified acid obtained by dilute acid hydrolysis, secondly a fraction hydrolysed by fuming HCl yielding about 50 % of the hydrolysis product in the form of glucose and containing also a considerable quantity of a uronic acid (12.08 %), and thirdly a lignin body containing about 12 % OMe.

The examination by acid hydrolysis of the alkali-soluble material from the "rotted" wood was carried out as described above for the material from the "more rotted" sample. 4 g. were submitted to a single dilute acid hydrolysis before treatment with fuming HCl. The following table gives the values obtained for the different radicals estimated in the residues from the hydrolysis. The figures in brackets represent these values calculated as % original material.

Table VII

	Original material	After hydrolysis	
		4 hr. (100°)	Fuming HCl (15°)
% of original	100	69.2	19.55
% uronic anhydride	10.96	6.00 (4.15)	0.00
% pentosan	4.59	0.00	0.00
% OMe	4.52	5.14 (3.56)	11.54 (2.27)
I.V.	35.2	21.8	12.01
Mol. wt./reducing group	568	917	1667

The filtrates were worked up as before. That from the dilute acid hydrolysis yielded an unidentified acid, isolated as a Ba salt, xylose and mannose as sugars, the latter amounting to 0.58 g. The fuming acid hydrolysis yielded glucose to the extent of 0.64 g. (32.2 % of the total material hydrolysed); this fraction also contained 19.5 % of uronic anhydride.

The fractions soluble in dilute alkali from both samples of rotted wood appear to have the same general constitution but differ in the proportions in which the parts are present. If any part of the material hydrolysed by dilute acid is the

product of fungal activity it would be the pentose fraction; the part hydrolysed by the fuming HCl is a product of fungal action as it has no counterpart in sound wood.

An attempt was made to resolve this fraction further by methylating the alkali-soluble material after dilute acid hydrolysis to remove the sugars and the unidentified acid. 10 g. were hydrolysed with 1% HCl for 6 hr. at 100° and the residue methylated with methyl sulphate and strong alkali. At the end of the reaction the solution was acidified with glacial acetic acid and the residue filtered off. This residue was remethylated and after five methylations 5.65 g. residue, OMe 28.99%, were obtained on acidification of the final reaction mixture. No further increase in the OMe content could be obtained with methyl sulphate, and Purdie's reagents could not be used owing to the insolubility of the residue in any solvent and the consequent difficulty of freeing the methylated product from Ag₂O. The residue, light brown in colour, was extracted with CHCl₃ until no further solution occurred. The residue, dark brown in colour, OMe 24.64%, was subjected to mild acid hydrolysis using 1% HCl at 100°. Hydrolysis was negligible, only a trace of brown gum being obtained from the filtrate. The residue was subjected to hydrolysis by cold fuming HCl and again the amount of hydrolysis was negligible. This residue evidently represents a fully methylated lignin-like body and is 39.5% of the original material.

The filtrates from the first three methylations were brought to a slight acidity by addition of NaOH, reduced to small bulk *in vacuo*, the crystals of sodium acetate filtered off and the solution remethylated. The resulting solution was brought to a slight acidity, evaporated to a small bulk and completely extracted with CHCl₃. To this CHCl₃ extract was added that from the lignin-like residue described above, the solution dried over anhydrous Na₂SO₄ and the solvent removed *in vacuo*. The product, a brown gum, dried to a hard varnish, OMe 32.04%; after methylation with Purdie's reagents OMe 34.53%, representing the highest value obtainable. This fraction was hydrolysed with fuming HCl at 15° for 20 hr., during which time the solution turned dark brown in colour; the solution was aerated to remove part of the acid, diluted with water and the residue filtered off. The solution was neutralized with BaCO₃ and evaporated to dryness *in vacuo*. The CHCl₃ extract of this semi-crystalline mass was evaporated to a thin syrup and crystallized after several months *in vacuo*. The mixture of crystals and syrup was extracted with dry ether leaving a small amount of brown residue. On evaporation a mixture of crystals and an ether-soluble gum were obtained. After two crystallizations from dry ether m.p. 115–118° and OMe 40.6%.

It was obvious that while a fraction had been separated by methylation comprising a unit containing glucose, trimethyl glucose having been obtained by hydrolysis of part of the methylated body, this method did not liberate the glucose-containing fraction unassociated with the lignin-like body. It was therefore decided to attempt a separation by acetylation.

5 g. portions of the alkali-soluble material from the more rotted wood were acetylated using three different methods: (1) acetic acid, acetic anhydride and a trace of conc. H₂SO₄ at 100°; (2) pyridine and acetic anhydride in the cold; (3) Barnett's method as used by Bell [1932]. In all cases an insoluble residue remained after treatment. The soluble portion was recovered by precipitation in warm water, the precipitate was washed with water on the filter until acid-free and dried with ether. The fractions were all pale fawn in colour giving dark brown solutions with dilute alkali. The amounts of acetylated material obtained by the first and third methods were much greater than that with the use of the

second. The following table gives the acetyl content and the i.v. of the acetylated fractions together with the same values for a similar fraction prepared from the alkali-soluble material after dilute acid hydrolysis.

Table VIII

Acetylated alkali-soluble fraction			
	Acetyl value	i.v.	Mol. wt./reducing group
Method (1)	46.6 %	25.7	778
„ (2)	44.3 %	41.9	479
„ (3)	47.7 %	47.5	421
Acetylated alkali-soluble fraction after dilute acid hydrolysis			
Method (3)	43.7 %	13.5	1481

An attempt to resolve the acetylated alkali-soluble fraction by fractional precipitation was unsuccessful.

The soluble products from all the methods contained pentose and those from methods (1) and (3) contained glucose, isolated after fuming HCl hydrolysis of 1 g. samples of each acetylation product. The residue from method (2) contained pentose and glucose and the pentose content could not be further reduced by treatment with these mild reagents whilst more powerful reagents removed not only the pentose but also other parts of the residue. As it was intended to estimate the mol. wt./reducing group for the glucose-containing units in the alkali-soluble fraction it was desirable that every trace of pentose should be removed. It is clear that these acetylation methods have not effected a complete separation of the pentose- and glucose-containing units and that mild acid hydrolysis cannot be avoided and is the only way of removing the pentose fraction completely. As the use of a trace of conc. H_2SO_4 as a catalyst might be regarded as likely to result in considerable acetolysis and pyridine was too feeble a catalyst, Barnett's method was used in spite of the objection that the chlorine tends to chlorinate the lignin part and render it more soluble in the acetylating reagents. The product of Barnett's method on the residue from the dilute acid hydrolysis of the alkali-soluble fraction was a pale yellow amorphous mass, uronic anhydride content 4.28 %, m.p. 246° . The acetyl and iodine values are given in Table VIII. On hydrolysis with fuming HCl the residue was black in colour and amounted to 9.43 % and on working up the filtrate glucose was identified as osazone; titration with Fehling's solution gave 0.543 g. glucose (41.4 % of the acetate hydrolysed). The nature of the residue is unknown. Assuming that it contained no reducing groups when it was in the form of the acetyl derivative and that the glucose molecules are in the form of a chain then the minimum mol. wt. of the glucose chain (deacetylated) would be 756 representing a chain of 4 or 5 mols. If the residue contained reducing groups the chain lengths would be very much larger. Of the acetyl derivative hydrolysed about 95 % has been accounted for in the fractions acetyl, glucose, uronic anhydride and residue.

The alkali-soluble material from the "rotted" wood was acetylated by the methods used in examining the "more rotted" fraction. The following table gives the values obtained.

Table IX

Acetylated alkali-soluble fraction			
	Acetyl value	i.v.	Mol. wt./reducing group
Method (1)	35.6 %	31.1	643
„ (2)	33.4 %	45.2	443

Barnett's method (method 3) was used on the residue from the dilute acid hydrolysis of the alkali-soluble fraction. The product obtained had acetyl value 41.3 %, i.v. 13.5, giving mol. wt./reducing group value 1481, a uronic anhydride content 0.96 % and m.p. 152°. Method (1) did not result in any fractionation of the alkali-soluble material as the whole of it passed into solution and the product was not resolved by fractional precipitation. Both the residue and the soluble portion obtained by method (2) contained pentose but only the former contained glucose. The product of method (3) on the residue from dilute acid hydrolysis was hydrolysed using fuming HCl for 20 hr. at 15°. The residue amounted to 13.6 % and the filtrate contained 0.578 g. glucose representing 45.7 % of the acetate hydrolysed. The whole of the acetyl derivative is accounted for in the fractions acetyl, glucose, uronic anhydride and residue, the nature of the last being unknown. Making the same assumptions as before, the mol. wt. of the glucose chain (deacetylated) is calculated as 751 giving a chain of 4 or 5 mols minimum length.

Water-soluble fraction ("more rotted" only)

The solution had strong reducing powers, was acid to litmus and on evaporation gave a brown, deliquescent mass drying after several weeks *in vacuo* to an amorphous mass. With HCl and phloroglucinol the absorption band spreading from the *D* line towards the *E* suggests the presence of a pentose. The pentose content was 7.7 % and the uronic anhydride value 10.0 %. The aqueous solution gave no precipitate on neutralizing with Ba(OH)₂, but a considerable excess of the latter produced a bulky, fawn-coloured precipitate, which was filtered off and dried *in vacuo*, Ba 28.8 %. On addition of 2 vol. 95 % alc. a further precipitate was obtained, Ba 28.5 %. The filtrate from the Ba precipitations was treated with dil. H₂SO₄ to remove the excess of Ba. The filtrate had strong reducing powers and on removal of the solvent yielded a pale yellow gum which after many months *in vacuo* dried to a resinous mass.

As the two Ba precipitates were alike in properties and Ba content they were united. 2 g. of the Ba precipitate treated with cinchonine sulphate failed to give any crystalline material other than cinchonine and it was impossible to prepare any pure fraction from the remaining residue.

5 g. of the Ba precipitate were dissolved in water acidified with dilute acetic acid and the Ba removed by the addition of the calculated amount of H₂SO₄. The filtrate after removal of BaSO₄ reduced Fehling's solution strongly, was dark brown in colour and was decolored with charcoal. Attempts to precipitate this fraction with NaHSO₃, to form an oxime, a phenylhydrazone or a semicarbazone were without success, but with Brady's reagent a copious amorphous red precipitate appeared. After standing for 4 hr. this was collected and washed with 2*N* HCl and with water until acid-free; the crude product had m.p. 178°. This compound was exhaustively extracted with hot absolute alcohol leaving a brown insoluble residue, m.p. 280° dec. With Neuberg's test a violet colour was given indicating that the substance was a bis-dinitrophenylhydrazone. Found (micro): C, 41.0; H, 3.49; N, 19.25 %; C₂₀H₂₀N₈O₁₃ requires C, 41.3; H, 3.44; N, 19.31 %. The substance forming the dinitrophenylhydrazone would be C₈H₁₂O₇. The alcohol-soluble material was obtained by evaporating the solution *in vacuo* to small bulk, filtering and pouring into an equal bulk of 2*N* HCl when a red precipitate separated. The washed and dried precipitate, m.p. 147°, gave a violet colour with the Neuberg test. Found (micro): C, 45.10; H, 4.09; N, 17.82 %; C₂₄H₂₆N₈O₁₃ requires C, 45.43; H, 4.10; N, 17.67 % which as a bis-compound represents an original substance of C₁₂H₁₃O₇. The filtrate after the

removal of the precipitate resulting from the addition of Brady's solution was set aside and a further precipitate settled out very slowly. After 3 months the precipitate was filtered off and exhaustively extracted with boiling absolute alcohol in a Soxhlet extractor; the amount of substance removed was small and the residue was a bright red amorphous mass M.P. 182°. Found: C, 45.80; H, 3.41; N, 18.40 %. The preparation gave a violet colour with alcoholic NaOH; bis-dinitrophenylhydrazone $C_{23}H_{21}N_3O_{12}$ requires C, 45.9; H, 3.49; N, 18.6 %. The original compound would be $C_{11}H_{13}O_6$.

The filtrate after removal of the Ba-precipitated fraction of the water-soluble material was evaporated to a stiff gum, from which no crystalline fraction could be obtained. However prepared the substance precipitated by Brady's method was red and amorphous. The material was soluble in water and was purified by dissolving it in the smallest volume of pure pyridine, diluting with an equal volume of water and pouring into 2*N* HCl with good stirring and ice cooling. The red precipitate which formed was collected and washed with dil. HCl and dried *in vacuo*: M.P. 116°. Found: C, 51.98; H, 5.31; N, 13.03 %. From the colour reactions with Neuberg's reagent the compound appears to be a mono-dinitrophenylhydrazone and $C_{19}H_{23}N_4O_8$ requires C, 52.41; H, 5.29; N, 12.87 %. The original substance would be $C_{13}H_{19}O_5$.

An attempt to resolve the water-soluble fraction was made by hydrolysis; 2 g. of the $Ba(OH)_2$ precipitate were dissolved in acidified water, the Ba removed with the requisite amount of H_2SO_4 , the solution acidified to 2 % HCl and heated for 6 hr. at 100°. A dark brown lignin-like substance was filtered off and both it and the solution had lost their reducing powers. The ease with which these water-soluble substances form lignin-like bodies is of interest in view of the formation of a lignin-like substance which appears in the alcohol-soluble fraction under the action of the fungus. The alkali-soluble fraction also contains a lignin-like substance which may be of fungal origin.

Alcohol-soluble fraction

Following the method outlined in our earlier work this fraction was purified by solution in glacial acetic acid and precipitation of the pure material by pouring the acid solution into a large volume of cold water. After two precipitations the OMe content was 8.92 %. Using Brady's more recent technique a dinitrophenylhydrazone was prepared. This product was non-crystalline, had M.P. 195° and dissolved sparingly in alcoholic NaOH with a red colour. Found: N, 5.55 %; $C_{50}H_{64}O_{19}N_4$ requires N, 5.52 %. This confirms our previous finding that the alcohol-soluble fraction contains a substance with the formula $C_{44}H_{50}O_{16}$ and that this substance contains only one reducing group.

DISCUSSION

Of the two samples of rotted wood examined the "rotted" sample is regarded as being only slightly attacked as the lignin content is very little greater than that of the sound wood. As will be shown in a later communication the lignin is not attacked by the fungus and therefore the amount of this fraction in any sample of rotted wood calculated as % of the sample is a guide to the loss of tissue through the respiratory action of the fungus. The "more rotted" fraction shows a much greater degree of fungal attack, the lignin content having risen to a high value. Even in the "rotted" fraction the destruction of the cellulose has proceeded to a very considerable degree, for while the actual loss of wood as gaseous products of respiration is probably less than 15 % of the original wood

the loss of cellulose is over 50 %, the cellulose content having fallen from 60.5 to 28.8 %. In the "more rotted" fraction where the actual loss of weight of the timber has probably been just over 40 % the cellulose content has fallen to less than 25 % of its original value. This makes it clear that in the earliest stages of the fungal attack the cellulose is degraded to a smaller molecular unit, which ceases to be recorded as cellulose, at a much greater rate than these smaller units are oxidized to the gaseous end products of respiration. This degradation of the cellulose is even more clearly seen when the actual constitution of the cellulose fraction itself is studied. The α -cellulose content of the wood samples has fallen from about 48 % of the whole sample for the sound wood to about 6 % of each of the two samples of rotted wood. The speed with which this α -cellulose is attacked is remarkable when account is taken of the small loss of tissue due to the action of the rot in the "rotted" wood sample. Further, the average mol. wt. calculated from the iodine values of the non- α -cellulose fraction of the "rotted" and "more rotted" celluloses have been determined and these values show clearly that besides the reduction in the amount of cellulose and of α -cellulose the non- α -cellulose fraction is being reduced in molecular size. The average chain length of the molecules of the non- α -cellulose in the "rotted" fraction is about 44 glucose anhydride units while that of the "more rotted" sample is about 24 such units. From the uronic anhydride values there is no evidence of extensive side chain oxidation during this degradation and as with increasing degree of rotting the i.v. increase probably the reducing groups at the ends of the molecules are not being oxidized as the degradation proceeds. It appears therefore that this breakdown α -cellulose $\rightarrow$ non- α -cellulose $\rightarrow$ non-cellulose fraction is a process of hydrolysis in which the first stage takes place with extreme rapidity. These observations are correlated with the fact that timber rotted by *M. lachrymans* loses a great portion of its mechanical strength during the earliest stages of the fungal attack.

When the pentosan content of the cellulose is considered it is clear that this fraction is removed very rapidly by the fungus, the value falling from 10.33 % of the cellulose of the sound wood to 3.5 % of that of the "rotted" and to 2.23 % of that of the "more rotted". If these values are calculated as % of the wood sample then it becomes clear that the removal of the pentosan from the cellulose in the early stages of the fungal attack is much more rapid than the degradation of the cellulose to a non-cellulose fraction. The cellulose content of the wood falls by about 50 % while the content of pentosan associated with the cellulose falls to about 15 % of its value in sound wood. In the later stages of the attack this preferential attack on the pentosans is maintained but the rate is rather slower for while the cellulose content falls by about 50 % the pentosan falls to about one-third of the value for the "rotted" wood. This preferential loss of pentosan may be due to its smaller mol. wt. as compared with that of the cellulose with the result that the hydrolytic activity of the fungus reduces it more rapidly to a molecular size such that it is removed by some of the solvents before the cellulose fraction is estimated.

The fall in the cellulose content of the rotted woods varies inversely with the content of material soluble in dilute alkali. This dilute alkali-soluble fraction when examined by progressive hydrolysis yielded three fractions, one hydrolysed by dilute acid giving mannose, xylose and an unidentified acid, a second, which had a uronic anhydride content and on hydrolysis with fuming HCl gave glucose and a third insoluble fraction which resembled lignin. Part of the xylose in the first fraction probably represents the pentosan split from the cellulose by the fungus as the pentosan content of the alkali-soluble fraction of the sound wood

calculated as % of the whole sample is 0.53 while that for the "rotted" is 1.35 and for the "more rotted" 1.69. This fraction of the rotted woods appears therefore not only to contain the breakdown products of the cellulose itself but also of its associated pentosans. An attempt to isolate this fraction which is only hydrolysed by strong acid from the lignin residue was made by methylating the residue from dilute acid hydrolysis but without success. The product obtained certainly yielded trimethyl glucose on hydrolysis but it also contained a large amount of a lignin-like substance. However by acetylating a similar residue using Barnett's method it was possible to prepare from both samples of rotted wood a fraction which contained a certain amount of uronic anhydride, only a very small part however of that present in the material acetylated, and yielded on hydrolysis with fuming HCl glucose and a small quantity of a dark coloured residue. The small amount of uronic anhydride in the acetyl derivative may be due to the destructive action of the reagents on the molecule. The nature of the residue is unknown and it may be that it is not present in the material originally acetylated but is a product of the acetylation and the strong acid hydrolysis. From the acetyl, uronic anhydride and iodine values, the amount of glucose obtained on hydrolysis and the amount of the residue it is possible to obtain a value which probably represents the minimum mol. wt. of the glucose chain in the acetylated compounds. For both samples of rotted wood this value is approximately 750 representing a chain of 4 to 5 glucose anhydride units. This must be regarded as the minimum, since if there is in the acetylated material any reducing group other than that at the end of the chain of glucose molecules then the actual chain length would be much greater than the calculated value. It is impossible to make a close comparison between the values so obtained and those calculated from the i.v. of the residues of the alkali-soluble material after dilute and strong acid hydrolyses, because it is quite obvious that one fraction is not exactly the acetyl derivative of the other. A calculation of the mol. wt./reducing group of the fraction removed by strong acid hydrolysis for both the "rotted" and the "more rotted" woods shows, however, that the values obtained are of the same order as those from the acetyl derivatives. For the fraction from the "rotted" wood the value is 612 and for the "more rotted" 1250.

It is obvious from a comparison of the uronic anhydride content of the alkali-soluble fraction of the "rotted" wood with that calculated from our previous communication for sound wood that considerable oxidation has been involved in the formation of this unit. Calculated as % of the whole wood sample the uronic anhydride content of the alkali-soluble fraction of the sound wood is 0.36 % while for the "rotted" wood the content of this fraction is 3.30 %.

From the water-soluble fraction of the "more rotted" wood, which alone has been studied, certain substances with powerful reducing properties have been isolated as 2:4-dinitrophenylhydrazones. The acidic properties of the crude extract have not been elucidated. This fraction shows considerable differences from that isolated in our previous work. No part of it is precipitable with methyl alcohol and it gives no crystalline substance with phenylhydrazine hydrochloride under the usual conditions. In our last publication part of the water-soluble fraction was reported as containing glucose; this was deduced from the crystalline form of the osazone prepared as usual. Since that time I have had the opportunity of examining many aqueous extracts from rotted woods and from a small number have isolated an osazone of crystalline form similar to that of some preparations of glucosazone. In no case however was the amount large enough to enable a detailed examination to be made, but

when sufficient was obtained to determine the M.P. of a pure sample it was found to be far from that of glucosazone, being in fact *c.* 138°.

Only the empirical formulae of the substituted phenylhydrazones have been determined and in no case does the formula represent a molecule containing as few as 6 C atoms. If these then are intermediate products or even by-products in the breakdown of cellulose in fungal respiration, the fungus must attack, by other than hydrolytic methods, units consisting of two or three glucose anhydride molecules; the presence of a molecule containing C₁₃ suggests that the minimum unit attacked is a trisaccharide. These substances may not be the breakdown products of cellulose but units synthesized by the fungus from much smaller compounds during its respiratory activities.

The identity of the bodies yielding furfuraldehyde and CO₂ on distillation with 12% HCl has not been established.

The lignin fraction of the alkali-soluble material is of interest since part at least of it must be a product of fungal activity; as prepared from both the "rotted" and the "more rotted" woods it contains about 12% OMe. In addition this fraction from the latter wood can be methylated to a total content of about 24%.

It can be concluded that the breakdown of the cellulose to smaller units is essentially hydrolytic and proceeds at a very rapid rate in the earliest stages of fungal attack. The molecular size of the units produced by hydrolysis is unknown but there is evidence which suggests that the size of the unit which the fungus attacks oxidatively is not less than a trisaccharide. Exact information on this point is lacking since it is not possible from the work done to decide whether certain fractions are intermediate stages in the respiratory process or waste products.

SUMMARY

1. Two fractions of rotted wood have been prepared from a piece of pine wood panelling (probably *P. resinosa*) rotted by *Merulius lachrymans*.

2. The properties of the alcohol, water and dilute NaOH extracts and of the cellulose in each were examined in detail.

3. Conclusive proof is given that the early stages of the attack on the cellulose are hydrolytic and that this process is extremely rapid even in the earliest stages. The molecular size of the final product is uncertain but there is a suggestion that it is not smaller than a trisaccharide.

4. Certain reducing compounds have been isolated from the water-soluble fraction. It is uncertain whether these, together with the alcohol-soluble material, are intermediate stages in the respiration of the fungus or waste products.

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