CXXXVII. THE CHEMISTRY OF THE WHITE ROTS OF WOOD.

I. THE EFFECT ON WOOD SUBSTANCE OF *POLYSTICTUS VERSICOLOR* (LINN.) FR.

By WILLIAM GEORGE CAMPBELL.

From the Section of Chemistry, Forest Products Research Laboratory, Princes Risborough, Bucks.

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Introduction.

The somewhat arbitrary classification of the wood decays caused by fungi into "brown" and "white" rots has in recent years been the subject of controversy, as it has been suggested that more accurate classification can be based on strictly chemical evidence regarding the effects of the various wood-rotting fungi on wood substance.

So far as "brown" rots are concerned a sufficiently representative number of these would appear to have been studied in order to make it possible, within the limits of available knowledge, to assign a hitherto unstudied fungus to this class on chemical grounds alone, for it has been shown that the chemical effects of "brown" rots on wood substance can strictly be compared to those of simple aqueous acid hydrolysis [Campbell and Booth, 1929]. The specificity of fungi generally with regard to time, mode and position of attack, together with the important question of host selection, has been postulated as a cogent factor in support of the view that the ultimate chemical effects of fungi on wood may be so variable as to preclude the accurate classification of decay into types on chemical grounds. Having in mind, however, the general composition of the skeletal substance of wood and its comparative chemical uniformity throughout the wide range of hardwoods and softwoods for which analytical data have been obtained, it is conceivable that, apart from climatic and other external conditions, the selective capacity of wood-rotting fungi must be controlled largely by the presence or absence in specific woods of substances, other than wood substance proper, which are toxic or otherwise intolerable to certain fungi. This is borne out by the work of Hawley, Fleck and Richards [1924], and of Sowder [1929], and is generally accepted as the explanation of the immunity to decay of notably durable woods. In this connection attention is directed to the conclusions arrived at by Rege [1927] and more recently by Norman [1929, 1] in their estimates regarding the "decomposability" of cellulosic

materials. The decomposability of woods cannot be predicted by any scheme such as those proposed by the above authors, since any prediction as to susceptibility to decay which fails to take into account the nature of the extraneous components of a specific wood and the different types of decay to which wood is subject would in the light of abundant evidence prove to be useless. None of the woods mentioned by Norman [1929, 1] can be regarded as in any sense particularly resistant to fungal decay. The decomposability of wood even when considered quite apart from extractives cannot be predicted by any fixed scheme, for the factors governing the susceptibility of its principal components are as yet but little understood. In certain quarters the presence of lignin in wood is regarded as a factor inhibitory to decay, but this can only hold for fungi of the brown-rot type, and when it is realised that some fungi actually bring about the decomposition of lignin, this belief must unconditionally be abandoned.

It seems certain, however, that the polysaccharides of wood are a potential source of food for fungi, and equally certain that all fungi which attack carbohydrates in preference to lignin do so in the same manner, namely, by hydrolysis of insoluble polysaccharides to soluble monosaccharides. Thus, in so far as the ultimate effects on wood substance are concerned, the brown rots would seem to form a definite class, but since little of a conclusive character is known of the chemistry of the lignin in wood, much experimental work will be necessary before it will be possible with any degree of certainty to state whether this component is always attacked in the same manner by fungi of the "white" rot type.

The "oxidation" or "corrosion" theory regarding the mechanism of white rots has previously been referred to [Campbell and Booth, 1929], but sufficient stress has not been laid on the question as to whether white-rot fungi are capable of deriving nourishment solely from the lignin in wood, or indeed whether attack on the lignin complex is accompanied by simultaneous degradation of polysaccharides. The present investigation has been undertaken to ascertain the detailed effect of *Polystictus versicolor* (Linn.) Fr. in pure culture on wood substance, as it is believed that this fungus produces a white rot. The effect of the decay has been examined in samples of the wood of both beech and ash.

Experimental.

A sample of air-seasoned beech heartwood was ground to pass a 60- and be retained by an 80-mesh screen. After thorough mixing, a portion of the wood flour was removed for analysis (Table I).

A further portion of the original wood was now extracted with 1 % sodium hydroxide for 1 hour at 100° under the conditions laid down by Schorger [1926, p. 506], and, after filtering off the extract, washing with hot water and drying at 105° the residue was analysed (Table I).

Table I. *Analysis of original 60–80-mesh beech heartwood before and after alkali extraction.*

(Results expressed as percentages by weight of original oven-dry wood.)

	Original wood	After alkali extraction
Cold water-soluble ...	0·64	—
Hot water-soluble ...	1·65	—
1 % NaOH-soluble ...	16·63	—
Cellulose	57·49	58·67
Lignin	23·79	19·79
Total pentosans	26·95	19·02
Pentosans in cellulose ...	15·5	18·42
Pentosans not in cellulose	11·45	0·60
Methoxyl content ...	6·33	4·81

The effect of Polystictus versicolor *(Linn.) Fr. on beech wood.*

Portions of the wood sample were sterilised by steaming at 100° for 30 mins. on each of three successive days and then incubated with *P. versicolor* at 20°. At the end of 6 and 10 weeks respectively, samples were taken for analysis. The wood was found to be distinctly acid to litmus and was accordingly collected on a linen filter, washed with cold water till acid-free and dried and sterilised at 105°. The analyses of the decayed residues are given in Table II. The effect of 1 % sodium hydroxide on these residues was then determined as for the original wood (Table II).

Table II. *Analysis of 60–80-mesh beech heartwood after decay by* P. versicolor *and washing with cold water.*

(Results expressed as percentages by weight of the original oven-dry wood.)

	Decayed wood		Decayed wood after alkali extraction	
Duration of decay ...	(1) 6 weeks	(2) 10 weeks	(1) 6 weeks	(2) 10 weeks
Loss due to decay ...	5·68	20·15	—	—
Cold water-soluble ...	1·58	0·71	—	—
Hot water-soluble ...	2·88	1·88	—	—
1 % NaOH-soluble ...	18·32	15·03	—	—
Cellulose	55·77	48·55	55·19	47·02
Lignin	21·05	16·91	16·31	13·56
Total pentosans	22·16	18·36	17·77	15·35
Pentosans in cellulose ...	13·94	13·63	16·52	14·07
Pentosans not in cellulose	8·22	4·73	1·25	1·28
Methoxyl content ...	5·38	4·42	4·31	3·58

The effect of mild aqueous acid hydrolysis on the original beech wood.

100 g. of the original beech wood were now incorporated with 2 litres of 0·25 % aqueous hydrochloric acid and maintained at 100° for 80 mins. After removal of the acid and thorough washing with hot water the residue was dried at 105° and analysed (Table III).

Table III. *Analysis of original 60–80-mesh beech heartwood after hydrolysis with 0·25 % HCl at 100° for 80 mins.*

(Results expressed as percentages by weight of original oven-dry wood.)

Loss due to hydrolysis	5·5
1 % NaOH-soluble	23·81
Cellulose	52·66
Lignin	22·69
Total pentosans	22·10
Pentosans in cellulose	12·27
Pentosans not in cellulose	9·83
Methoxyl content	4·68

The effect of an acid-alcohol solution on the original beech wood.

100 g. of the original wood were incorporated with a solution consisting of 1 litre of 0·25 % aqueous hydrochloric acid and 1 litre of 97 % ethyl alcohol and maintained at 100° for 80 mins. The extract was filtered off and the residue was washed with ethyl alcohol, then with hot water until free from acid, dried at 105° and analysed (Table IV). The effect of 1 % sodium hydroxide on this residue was also determined (Table IV).

Table IV. *Analysis of original 60–80-mesh beech heartwood after treatment with one volume 0·25 % HCl, one volume 97 % alcohol at 100° for 80 mins.*

(Results expressed as percentages by weight of original oven-dry wood.)

	Residue	Residue after alkali extraction
Loss due to treatment ...	5·2	—
Cold water-soluble ...	0·40	—
Hot water-soluble ...	1·28	—
1 % NaOH-soluble ...	16·02	—
Cellulose	56·66	56·94
Lignin	20·96	17·72
Total pentosans	23·38	18·20
Pentosans in cellulose ...	15·14	17·45
Pentosans not in cellulose	8·24	0·75
Methoxyl content ...	5·27	4·77

The effect of P. versicolor (*Linn.*) *Fr. on ash heartwood.*

A sample of ash heartwood, the analysis of which is recorded in Table V, was divided into a series of small blocks each of which was inoculated with

Table V. *Analysis of 60–80-mesh ash heartwood before and after decay by* P. versicolor.

(Results expressed as percentages by weight of oven-dry material.)

	Original wood	Decayed wood
Loss due to decay ...	40·76	—
1 % NaOH-soluble ...	26·64	24·74
Cellulose	49·37	55·87
Lignin	22·11	22·90
Total pentosans	23·56	24·46
Pentosans in cellulose ...	11·98	14·19
Pentosans not in cellulose	11·58	9·27

P. versicolor after the usual sterilisation. Decay was allowed to proceed to an advanced stage. After removal of the surface mycelial growth, the blocks were dried at 105° and weighed. The decayed material was now ground to pass a 60- and be retained by an 80-mesh screen and, after thorough mixing, analysed in the usual manner (Table V).

Since the average loss in weight of the blocks was 40·76 % of their original dry weight this figure was employed in order to calculate the analytical data for the decayed wood as percentages of the oven-dry weight of the original wood (Table VI).

Table VI. *Analysis of 60–80-mesh ash heartwood decayed by* P. versicolor.

(Results expressed as percentages by weight of the original oven-dry wood.)

1 % NaOH-soluble	14·65
Cellulose	33·11
Lignin	13·26
Total pentosans	14·49
Pentosans in cellulose	8·41
Pentosans not in cellulose	5·98

Discussion.

Comparison of the data in Table I shows the effect of 1 % sodium hydroxide on the original wood. After alkali extraction the yield of cellulose as determined by the Cross and Bevan method is actually greater than that obtained from the original wood. The pentosan content of the cellulose is enhanced as a result of the alkali treatment, but at the same time it can be seen that part of the cellulose complex is soluble in alkali since the percentage increase in yield of this component is less than the increase in its pentosan content. This peculiar effect of alkali appears to be characteristic of hardwoods since similar results have been obtained in the case of oak [Campbell and Booth, 1930]. The results indicate that during the alkali treatment the furfuraldehyde-yielding complexes which are not associated with the cellulose in the original wood are involved in some change which results in the solution of one portion in the alkali and the association of another portion with the cellulose.

Various sources of error in the estimation of the pentosan content of wood by the phloroglucinol method have long been recognised. These have been discussed by Hawley and Wise [1926, p. 155] who have concluded that up till 1926 no entirely satisfactory method of estimating pentosans had been devised. Some of the known facts concerning the sources of error in the phloroglucinol method have recently been recapitulated by Norman [1929, 2], who has suggested certain corrections in view of the fact that, on distillation with 12 % hydrochloric acid, plant constituents other than pentosans yield appreciable amounts of furfuraldehyde as well as other bodies which are precipitated by phloroglucinol. Moreover, there is evidence in favour of the view that the furfuraldehyde phloroglucide precipitate has no fixed chemical composition

[Hawley and Wise, 1926, p. 155], and that extraction of this precipitate with alcohol does not appear to remove all the hydroxymethylfurfuraldehyde phloroglucide [Hawley and Wise, 1926, p. 157]. It would appear, therefore, that no modification of this method of determining pentosans can ever be universally accepted. Antoniani [1928] has suggested that hydroxymethylfurfuraldehyde can be removed from the hydrochloric acid distillate by redistillation, but again a correction is necessary for the diminution of furfuraldehyde which ensues. This author also concludes that the furfuraldehyde phloroglucide is not always of exactly similar composition.

It is therefore considered that in wood analysis the pentosan determination of Schorger [1926, p. 532] should remain in use until an acceptable substitute is forthcoming, since from the purely statistical standpoint this determination has been shown in previous work to be of unquestionable value.

Comparison of Tables I and II shows the effect of *P. versicolor* on the principal components of beech wood. It can be seen that even though the decayed residue had been washed with cold water prior to analysis it still retained more water-soluble material than the original sound wood. An increase in water-soluble material is a common result of fungal decay. The data further show that all of the remaining wood components have suffered depletion during decay, but that in the incipient stages depletion is most pronounced in the pentosans not in the cellulose. The extent of lignin depletion is relatively greater than that of the cellulose, and in the initial stages of decay the loss in this latter constituent is practically accounted for by the depletion of its pentosan content. The fall in the methoxyl content is small in comparison to that of the lignin content. Few data relative to this type of decay have hitherto been available, for, although an analysis has been made of apple wood infected with the same fungus [Hawley and Wise, 1926, p. 300], the absence of lignin determinations makes it impossible to define the type of decomposition. Thus in Tables II, V and VI data have been introduced to show that as the decay caused by *P. versicolor* becomes more advanced, decomposition of the cellulose proper takes place in addition to that of the other major wood components. The results in Table V reveal that in consequence of decay there is an apparent accumulation of cellulose, and a depletion of pentosans not in the cellulose, but it is seen from Table VI that accumulation of cellulose does not take place. On the contrary, the data reveal that of the total loss in weight of 40·76 % sustained as a result of decay, 16·26 % is accounted for by cellulose depletion and 8·85 % by lignin depletion. Further, it can be seen that in the advanced stages of decay the pentosans not in the cellulose are still depleted to a greater extent than the pentosans in the cellulose, but although these components are the first to be attacked by the fungus, the rate at which they are decomposed is diminished as decay proceeds, and preferential attack is made on the cellulose and lignin.

With regard to the alkali solubility of wood decayed by *P. versicolor*, comparison of Tables I and II reveals that in the initial stages of decay the alkali solubility of the residue is only slightly greater than that of the original wood.

Further examination of the data shows that the cellulose in partly decayed wood is more susceptible to alkali than the corresponding component in sound wood, but that after 10 weeks' decay the alkali solubility of this component of the decayed residue begins to decrease. The alkali solubility of lignin is practically the same in both sound and decayed wood, but the furfuraldehyde-yielding complexes in decayed wood are less soluble in alkali than the corresponding components in sound wood. By comparison with previous data [Campbell and Booth, 1929] it can be seen that where the losses in weight due to incipient decay are approximately the same, a "brown" rot is responsible for a much greater increase in alkali solubility of the wood residue than is the rot produced by the fungus under investigation. In this latter type of decay the total alkali solubility of the residual cell wall substance tends to decrease as decay becomes more advanced, the decrease in solubility of the furfuraldehyde-yielding complexes being greater than the total increase in solubility of cellulose and lignin. If it is conceded that the water-soluble material in wood is also soluble in alkali it can be seen that, if the total percentage of water-soluble material be subtracted from the percentage of alkali-soluble material in sound and decayed wood respectively, the resultant figure for the alkali solubility of the decayed wood is actually less than that for the original sound wood. In the advanced stages of decay (Tables V and VI) a large decrease in alkali solubility is obtained.

From the foregoing evidence it is apparent that the decay of wood caused by *P. versicolor* differs radically in its effects from those of the more widely studied class of "brown" rots.

Consideration of the ultimate chemical nature of the decay.

The production of acids in the course of the decay of wood by fungi raises the important question as to whether such acids are in the first instance secreted by the fungus as a direct means to effect decay, or whether they are merely formed during decay as decomposition products of wood substance. In the case of the brown rots, where decomposition has been shown to be strictly comparable in effect to mild aqueous acid hydrolysis [Campbell and Booth, 1929], it has not yet been shown experimentally whether the decay is attributable to a hydrolytic enzyme, an acid secreted by the fungus, or both of these agencies, but in the present study it is apparent that despite the observed acidity of the decayed wood, acid hydrolysis cannot solely be responsible for the decay. Comparison of the data in Tables II and III shows that where the respective losses in weight due to decay by *P. versicolor* and an acid hydrolysis are approximately the same, the compositions of the respective residues betray the characteristic differences in the effects of the two processes on wood substance.

None the less it is conceivable that the acid produced during decay may play some part in the decomposition of the wood substance, but its normal hydrolytic effect must be modified by some other agency. In seeking a means whereby

an acid hydrolysis of wood can be so modified as to result in lowered alkali solubility of the residue, marked attack on lignin, and depletion of pentosans not in the cellulose which is more intense than the depletion of pentosans in the cellulose, reference must be made to previous work [Campbell, 1929] in which it is shown that these results can be produced by the incorporation of an alcohol with the hydrolysing medium. A detailed study of the wood-acid-alcohol reaction has revealed the variations in effect obtainable on wood substance with different proportions of acid and alcohol, as well as when the duration and temperature of the reaction are altered. For comparative purposes therefore the effect of an acid-alcohol solution has been determined on the original beech wood used in this investigation and the data in Table IV show that, although the extent of decomposition obtained by this treatment was not so great as that produced in the sample of decayed wood, the order of the effect of incipient decay on wood substance is distinctly similar to that of the acid-alcohol solution. The lower alkali solubility in Table IV is undoubtedly explained by the fact that most of the water-soluble material has been washed out of the residue. With regard to the manner in which ash wood (Tables V and VI) has been decomposed by the same fungus it is interesting to note that when wood substance is subjected to an acid-alcohol solution under conditions calculated to produce a similar loss in weight [Campbell, 1929], the manner in which the principal wood components are decomposed, as evinced by the comparative compositions of original wood and residue, is similar in effect to the action of *P. versicolor*. It is thus conceivable that a closer understanding of the wood-acid-alcohol reaction and its ultimate mechanism may lead to a true chemical definition of this type of decay.

Experimental evidence at this stage does not warrant the conclusion that the decay ultimately consists in the simultaneous action of an acid and an alcohol on wood substance, for, although there is evidence that acid is produced during the decay, the manner in which an alcohol could be produced is not clear.

The view has been expressed by Wehmer [1927] that in white rots lignin is depleted by a process of oxidation. Much support would be gained for this theory if it could be shown experimentally that the wood-acid-alcohol reaction were similar in effect to a catalytic oxidation of wood substance in the presence of acid. Dorée and Cunningham [1913] have shown that acetic and formic acids are formed when moist wood is oxidised by ozonised oxygen, and, theoretically, it is not improbable that an oxidase secreted by a fungus might produce similar results. In such an event, further simultaneous action of oxidase and acids might complete the process of decomposition. Such a theory would appear to be compatible with experimental evidence, for it would explain the early attack of *P. versicolor* on the lignin and pentosans, and the later attack on the cellulose of wood substance. Acids might be formed by the action of an oxidase on lignin and pentosans, and then react together with the oxidase to bring about the later stages of the decay in which cellulose is decomposed.

SUMMARY.

1. The chemical effect of *P. versicolor* (Linn.) Fr. on the wood of beech and ash has been examined in detail.

2. The decay is characterised by the following salient features.

(*a*) The decayed wood is more soluble in water than the original sound wood.

(*b*) In the early stages of decay the residue is slightly more soluble in alkali than the original wood, but alkali solubility tends to decrease as decay becomes more advanced.

(*c*) The pentosans of wood substance and lignin are the first components to be attacked, and of these the pentosans not in the cellulose suffer most depletion.

(*d*) As decay becomes more advanced the rate at which pentosans are depleted is diminished, and preferential attack is made on the cellulose proper as well as on the lignin.

3. It is concluded that the fungus causes a white rot on the wood of beech and ash.

4. The effects of the decay on wood substance are closely comparable to those produced by acid-alcohol solutions under certain conditions.

5. The theory is advanced that the fungus secretes an oxidase which acts upon lignin and pentosans to produce acid. Further simultaneous action of oxidase and acid completes the process of decomposition in which the cellulose proper is depleted.

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REFERENCES.

Antoniani (1928). *Nature*, **122**, 903.
Campbell (1929). *Biochem. J.* **23**, 1225.
—— and Booth (1929). *Biochem. J.* **23**, 566.
—— —— (1930). *Biochem. J.* **24**, 641.
Dorée and Cunningham (1913). *J. Chem. Soc.* **103**, 677.
Hawley, Fleck and Richards (1924). *Ind. Eng. Chem.* **15**, 699.
—— and Wise (1926). Chemistry of wood. (Chemical Catalog Co. Inc.)
Norman (1929, 1). *Biochem. J.* **23**, 1367.
—— (1929, 2). *Biochem. J.* **23**, 1353.
Rege (1927). *Anal. App. Biol.* **14**, 1.
Schorger (1926). Chemistry of cellulose and wood. (McGraw Hill Book Co.)
Sowder (1929). *Ind. Eng. Chem.* **21**, 981.
Wehmer (1927). *Ber. deutsch. bot. Ges.* **45**, 536.