

CCXV. THE CHEMISTRY OF THE WHITE ROTTS OF WOOD.

III. THE EFFECT ON WOOD SUBSTANCE OF *GANO-
DERMA APPLANATUM* (PERS.) PAT., *FOMES FOMEN-
TARIUS* (LINN.) FR., *POLYPORUS ADUSTUS* (WILLD.)
FR., *PLEUROTUS OSTREATUS* (JACQ.) FR., *ARMIL-
LARIA MELLEA* (VAHL.) FR., *TRAMETES PINI* (BROT.)
FR., AND *POLYSTICTUS ABIETINUS* (DICKS.) FR.

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IN two previous communications [Campbell, 1930; 1931] experimental data have been adduced to indicate that white rot fungi are in no sense uniform with respect to the order in which they attack the principal components of wood. In the present investigation a further series has been examined with a view to determining whether the white rotts can be classified into groups on the basis of their chemical action—individual members which attack wood in the same manner being included in the same group.

EXPERIMENTAL.

The experimental procedure and analytical methods were similar in all respects to those used in previous work except in the case of the decay caused by *A. mellea* which will be discussed later. The fungi to be studied were divided into three groups as follows:

1. *G. applanatum*, *F. fomentarius*, *P. adustus* and *P. ostreatus*.

The material to be decayed by this group of fungi consisted of a well-mixed sample of fresh, air-dried 60–80 mesh beech wood flour. Prior to inoculation each portion of wood flour was submitted to the usual sterilisation treatment, and the decayed wood was analysed after washing free from acid with cold water and drying at 105°.

The effect of 1 % NaOH at 100° was determined as before on both sound and decayed wood. The analytical results which are expressed as percentages by weight of the original oven-dry wood are recorded in Table I.

Table I. *The effect of a series of white rots on the chemical composition of beech wood, together with the effect of decay on the alkali-solubility of the residual wood substance.*

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

	Original wood	Decayed by:			
		<i>Ganoderma applanatum</i>	<i>Fomes fomentarius</i>	<i>Polyporus adustus</i>	<i>Pleurotus ostreatus</i>
<i>Before extraction with 1% NaOH</i>					
Duration of decay	—	2 months	4 months	4 months	4 months
Loss due to decay plus cold water washing	—	10.61	15.75	10.50	8.79
Cold water-soluble	1.03	1.32	1.03	0.89	0.45
Hot water-soluble	1.49	2.65	2.40	2.33	1.29
1% NaOH-soluble	—	—	—	—	—
Cellulose	59.52	55.34	50.70	53.08	55.29
Lignin	20.54	16.81	16.00	18.03	18.60
Methoxyl	5.82	5.03	4.67	5.02	4.41
Total pentosans	22.28	19.46	18.40	19.76	19.86
Pentosans in cellulose	12.65	11.93	9.94	10.60	11.36
Pentosans not in cellulose	9.63	7.53	8.46	9.16	8.50
<i>After extraction with 1% NaOH for 1 hour at 100°</i>					
1% NaOH-soluble	14.57	19.73	18.33	18.75	17.00
Cellulose	58.49	47.12	46.9	50.52	49.99
Lignin	18.96	13.04	12.14	13.26	16.01
Methoxyl	4.66	3.46	3.44	3.71	2.39
Total pentosans	17.07	14.15	12.07	14.28	14.92
Pentosans in cellulose	11.75	7.83	8.51	10.04	9.42
Pentosans not in cellulose	5.32	6.32	3.56	4.24	5.50

In the samples inoculated with *G. applanatum* and *F. fomentarius* moderately large, tough wefts of mycelium formed on the surface of the decaying wood. In each case the weft was removed in a single sheet, carefully washed free from adhering particles of wood and weighed separately after drying at 105°. From 130.32 g. (oven-dry) of original wood decayed by *G. applanatum* 2.76 g. or 2.12% of mycelium was obtained, while in the case of *F. fomentarius* 3.93 g. or 3.02% was obtained from the same weight of wood. In the case of *P. adustus* and *P. ostreatus* the surface growth of mycelium was almost negligible in amount.

2. Heart rot of cherry wood (*Prunus avium* L.) caused by *Armillaria mellea*.

In a previous communication [Campbell, 1931] the effect of *A. mellea* on beech wood was described in some detail. At the stage of decay which was examined, however, the loss in weight was only 8.66% and no attack on lignin was observed. Since these results were published, material in the form of an air-dry disc of cherry wood showing pronounced heart-rot caused by the same fungus has become available, and this has been examined with a view to gaining information regarding the more advanced stages of decay. In order to obtain an approximate measure of the loss in dry weight sustained by the central portion of the disc during decay it was necessary in the first instance to

determine the specific gravity of both sound and decayed wood. Eight specimens measuring approximately $1.5 \times 1.5 \times 5$ cm. were cut at random from the outer sound heartwood of the disc and four samples were taken from the relatively small decayed central portion. It was considered advisable to determine the volumes of the specimens without wetting them and the author is indebted to Mr E. D. van Rest for drawing his attention to a paper by Hartmann, Westmont and Morgan [1926] from which it was possible to evolve a simple method of determining the volumes of wood samples by means of the displacement of sand. In the paper referred to it is stated that the bulk volume of a specimen of any porous material may be calculated from the difference in volume of sand displaced by the specimen and a metallic standard of approximately the same size and shape. It is shown that when this difference is small it is possible to determine the volume of the specimen with the same degree of precision as it is possible to measure the metallic standard. The fine sand employed in the present experiments was freed from organic matter, sifted to obtain 80–100 mesh material thoroughly washed and dried. The procedure employed was essentially the same as that indicated by Hartmann, Westmont and Morgan [1926] except that the operations were carried out on a much smaller scale. The sand was held in a filter-funnel to the lower end of which was fitted a short length of rubber tubing provided with a spring clip. A small cylindrical glass vessel served to retain the wood specimens and the steel standard, which latter measured $1.48 \times 1.48 \times 5$ cm. at 15° .

It was found possible, with practice, to duplicate the weight of annular sand round either the standard or test pieces to within 0.2 g. in the majority of cases. With sand of bulk sp. gr. 1.455, 0.2 g. corresponds to a volume of 0.137 cc., so that, by accepting in each case the average of the two most closely approximating weights of annular sand it was considered that this method of determining the volumes of test pieces gave sufficiently accurate results.

After the air-dry volumes had been obtained the air-dry and oven-dry weights of each sample were determined. The results of the specific gravity determinations are given in Table II.

After conversion into wood flour of 60–80 mesh the sound and decayed materials were analysed in the usual manner (Table III). In view of the slight divergences in the moisture contents of the sound and decayed samples (Table II), the approximate loss in weight sustained by the wood which was decayed cannot be directly assessed by means of the true specific gravity figures but rather by comparing the averages of the quotients obtained in both series by dividing the dry weight of each sample by its air-dry volume. It can then be assumed, by neglecting the probable effect of partial decay on the swelling properties of wood and the normal slight variation in specific gravity found in all woods in passing from the centre of the tree outwards, that at any given temperature and relative humidity a given volume of the sound cherry heartwood contains more wood substance than the same volume of the

Table II. *Comparison of specific gravity of sound cherry heartwood with that of cherry heartwood of the same tree decayed by A. mellea.*

Specimen No.	Sound wood					
	Volume in cc. (air-dry)	Weight in g. (air-dry)	Weight in g. (oven-dry)	Moisture content % of oven-dry weight	Specific gravity	Wt. (oven-dry)
					Wt. (air-dry)	Vol. (oven-dry)
					Vol. (air-dry)	Vol. (oven-dry)
1	10.657	7.361	6.555	12.3	0.691	0.615
2	6.038	4.528	4.029	12.46	0.750	0.667
3	8.873	5.469	4.862	12.46	0.616	0.548
4	10.149	6.945	6.185	12.28	0.684	0.609
5	11.867	7.448	6.675	11.59	0.628	0.562
6	10.763	7.042	6.304	11.71	0.654	0.586
7	11.647	7.718	6.897	11.91	0.663	0.592
8	9.49	6.519	5.812	12.17	0.687	0.612
Average					0.672	0.599
Decayed wood						
1	12.409	5.735	5.059	13.38	0.462	0.408
2	14.583	7.743	6.819	13.21	0.531	0.468
3	8.763	5.278	4.654	13.38	0.602	0.531
4	7.513	3.228	2.843	13.55	0.430	0.378
Average					0.506	0.446

decayed wood in the proportion of approximately 1.34 : 1, or, alternatively that during decay the central portion of the disc has lost approximately 25 % of its oven-dry weight. By employing this latter figure the analytical data for the decayed wood can be expressed as percentages of the oven-dry weight of the original wood (Table III).

Table III. *The effect of heart-rot caused by A. mellea on the wood of cherry (Prunus avium L.).*

	Original wood %	Decayed wood %	Decayed wood % sound wood
Loss in weight due to decay	—	—	25.00 (approx.)
Cold water-soluble	2.97	4.47	3.35
Hot water-soluble	6.49	7.21	5.41
1 % NaOH-soluble	26.44	29.15	21.86
Cellulose	53.74	53.74	40.30
Lignin	19.32	20.97	15.73
Total pentosans	25.75	27.59	20.69
Pentosans in cellulose	13.20	14.31	10.73
Pentosans not in cellulose	12.55	13.28	9.96

3. *Trametes pini* and *Polystictus abietinus*.

The effect of *T. pini* was observed on a sample of silver fir (Table IV). In this case insufficient material was available for a detailed examination of the effect of 1 % NaOH on the sound and decayed wood. In the case of the decay caused by *P. abietinus* the starting material was 60–80 mesh Norway spruce and in this case both decayed and sound wood were analysed before and after extraction with 1 % NaOH for 1 hour (Table V).

Table IV. *Analysis of 60-80 mesh silver fir wood before and after decay by Trametes pini.*

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

	Original wood	Decayed wood
Duration of decay	—	17 months
Loss due to decay <i>plus</i> cold water washing	23.01	—
Cold water-soluble	1.02	0.92
Hot water-soluble	1.87	2.5
1 % NaOH-soluble	12.0	12.98
Cellulose	57.46	46.47
Lignin	29.35	22.87
Methoxyl	4.81	3.7
Total pentosans	9.41	6.76
Pentosans in cellulose	3.18	2.61
Pentosans not in cellulose	6.23	4.15

Table V. *The effect of Polystictus abietinus on the chemical composition of the wood of Norway spruce, together with the effect of the decay on the alkali-solubility of the residual wood substance.*

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

	Before extraction with 1 % NaOH		After extraction with 1 % NaOH for 1 hour at 100°	
	Original wood	Decayed wood	Original wood	Decayed wood
Duration of decay	—	5 months	—	—
Loss due to decay <i>plus</i> cold water washing	—	6.79	—	—
Cold water-soluble	1.67	1.64	—	—
Hot water-soluble	2.77	4.7	—	—
1 % NaOH-soluble	—	—	13.54	20.27
Cellulose	60.54	55.08	55.72	51.08
Lignin	26.22	23.16	23.85	17.36
Methoxyl	4.7	3.98	4.17	2.92
Total pentosans	10.28	8.87	8.59	6.22
Pentosans in cellulose	4.12	3.73	4.16	3.59
Pentosans not in cellulose	6.16	5.14	4.43	2.63

DISCUSSION OF RESULTS.

The results in Table I reveal that each fungus in the group has attacked carbohydrates as well as lignin. Well-defined differences with regard to the proportions of each constituent attacked, however, are at once apparent. For instance, *G. applanatum* and *P. adustus* acting on wood taken from the same original sample have, over widely different times, brought about an equal loss in weight and yet the latter has caused a greater depletion of cellulose than the former. This is in part compensated for by the fact that *G. applanatum* has a more pronounced action on lignin. Further differences are observed in the manner in which these two fungi attack the furfuraldehyde-yielding complexes of the wood. *G. applanatum* produces a relatively greater amount of depletion of the pentosans not associated with the cellulose than of the pentosans in the cellulose, whereas the reverse is true in the case of *P. adustus*. In the case of *G. applanatum* 2.12 parts of the 10.61 % loss in weight caused by the decay can be directly accounted for by the formation of a weft of mycelium

on the surface of the decaying wood while only a negligible amount of surface mycelial growth was observed on the wood decayed by *P. adustus*. These considerations point to pronounced differences in the biological activities of the two fungi although in a general sense they have the same ultimate chemical action on wood.

Comparison of the wood samples decayed by *P. adustus* and *P. ostreatus* respectively for the same length of time reveals that the two fungi deplete lignin to approximately the same extent although *P. ostreatus* causes a smaller total percentage loss of wood substance. At the same time at the stages of decay examined cellulose appears to be more resistant to *P. ostreatus* than to *P. adustus*. Slight differences in the respective effects of the two fungi on the furfuraldehyde-yielding complexes are also observed despite the fact that the depletion of total pentosans is the same in both cases.

In Table I the greatest degree of decomposition of the beech wood has been brought about by *F. fomentarius* but the net chemical effect only differs from that of *G. applanatum* in a few particulars. Much more cellulose has been decomposed while the extent of lignin depletion is not much more than in the decay caused by *G. applanatum*. The attack on pentosans is slightly more severe and the pentosans in the cellulose suffer proportionately greater depletion than the pentosans not associated with the cellulose. *F. fomentarius* resembles *G. applanatum* in that a considerable amount of mycelium is laid down, 3.02 parts of the 15.75 % loss in weight of the original wood being accounted for in this way.

Further examination of the analytical data reveals that each of the four fungi under consideration brings about an increase in the alkali-solubility of the residual wood substance at the stages of decay examined, but reference to a group of data recorded by Hawley and Wise [1926, p. 298] shows clearly that for equal percentage losses of wood substance all the white rots examined so far induce much smaller increases in alkali-solubility than the brown rots. It has been indicated previously [Campbell, 1930; 1931] that herein lies one of the principal chemical bases of differentiation between the two types of wood decay. In brown rots increase in alkali-solubility is roughly proportional to the amount of cellulose depletion and does not reach a maximum until a comparatively late stage of decay. In the white rots under consideration there is no proportionality between alkali-solubility and loss of cellulose or any other constituent.

The data in Table III illustrate the type of difficulty which is likely to arise in attempting to compare sound wood with wood which has been decayed by a white rot where the loss in weight due to decay is not known. It is suggested, however, that the method here used to determine loss in weight is sufficiently accurate for the purpose of the investigation, since it serves to confirm a previous conclusion [Campbell, 1931] that the decay caused by *A. mellea* must on purely chemical grounds be regarded as a variety of white rot. When the analytical data for the decayed wood are calculated as per-

centages by weight of oven-dry sound wood, it is observed that a stage has been reached at which lignin is decomposed, although the constituent which has been most severely attacked is undoubtedly the cellulose. As in the early stages of decay the pentosans associated with the cellulose have suffered depletion as well as the pentosans not in the cellulose. Finally, a definite decrease in alkali-solubility has taken place. At the stage under review the decay caused by *A. mellea* is closely similar in its effects to other white rotts which have been studied.

The results in Tables IV and V illustrate the respective effects of two white rotts on gymnospermic woods. *Trametes pini* has figured largely in the literature on wood decay as a typical white rot fungus in that it has been supposed to confine its attack to the lignin of wood and to leave the cellulose unchanged. The data in Table IV indicate clearly that no such specific action takes place and that the white rot caused by *T. pini* is comparable in general with other white rotts which have been studied. The results appear to agree with the findings of Hawley, Wiertelak and Richards [1930] with regard to the same fungus.

Thaysen and Bunker [1927, p. 129] have quoted evidence in support of the view that *P. abietinus* causes a rot in which cellulose is depleted in the early stages but in which lignin is not attacked until decay is well advanced. The data in Table V, however, do not bear this out. At the early stage of decay under investigation, the attack on lignin is proportionately greater than the attack on cellulose, and of the two groups of pentosans those which are not associated with the cellulose are more susceptible to attack. In this case there is a comparatively sharp rise in alkali-solubility as cellulose and more especially lignin become more soluble in alkali as a direct result of attack. Unlike most of the other examples studied the pentosans not in the cellulose are more soluble in alkali than those which are associated with the cellulose.

CONCLUSIONS.

Sufficient data have now been obtained to show that as a class the white rot fungi are not consistent in the manner of their attack on wood, for although none has yet been found to have a specific action on lignin there is no general uniformity with regard to either the order or proportion in which the major wood components are decomposed. The evidence suggests that the white rotts should be subdivided on chemical grounds into the following three groups.

Group I.

White rotts in which lignin and pentosans are attacked in the early stages and in which the incidence of the attack on the cellulose proper is delayed. Representative fungus: *Polystictus versicolor* [Campbell, 1930; Lutz, 1931].

Group II.

White rots in which cellulose and its associated pentosans are attacked in the early stages and in which the incidence of the attack on lignin and pentosans not associated with the cellulose is delayed. Representative fungus: *Armillaria mellea* [Campbell, 1931].

Group III.

White rots in the early stages of which both lignin and cellulose are attacked but in varying proportions. Representative fungi: *Polyporus hispidus* [Campbell, 1931], *Ganoderma applanatum*, *Polyporus adustus*, *Pleurotus ostreatus*, and *Polystictus abietinus*.

It is considered that an early stage of decay might reasonably be regarded as one at which the observed loss in weight calculated as a percentage of the oven-dry original wood is approximately between 5 and 10. There is, therefore, insufficient evidence on which to classify *F. fomentarius* and *T. pini*, although the probability is that they will be found to fall into Group III. From an examination of the data obtained by Falck and Haag [1927] with respect to *Fomes annosus* it appears that this fungus also belongs to Group III, since if the specific gravity figures recorded by these authors be used in the manner previously indicated to calculate the results for the decayed wood as percentages of the weight of oven-dry sound wood it can be seen that the cellulose content as well as the lignin content decreases in the early stages of decay. In the same way it can be shown that the percentage alkali-solubility begins to decrease after the loss in weight of the original wood exceeds 20 to 25 % of the oven-dry original wood.

It should not be overlooked that the mere fact that it is possible to subdivide the white rots on the lines indicated above cannot be taken to indicate that each fungus induces a set of chemical reactions peculiar to the group to which it belongs. It would obviously be difficult at very advanced stages of decay to differentiate between one white rot and another by chemical means, and it is therefore highly probable that the ultimate primary reactions involved are the same in all three groups but that the main variants are the incidence in point of time of the primary reactions, the course of secondary reactions, if any, and the rates of the several reactions. It has been pointed out elsewhere [Campbell, 1932] that the principal enzymic reactions which are generally associated with the decay of wood by fungi are hydrolysis and oxidation. In brown rots hydrolysis appears to be the only primary reaction [Campbell and Booth, 1929] but it is suggested that in white rots decomposition is brought about by the combined action of oxidation and hydrolysis. In the present state of knowledge it appears that it is only on this basis that the observed behaviour of white rot fungi can be explained. The different rates of attack on the major wood components must be due in large measure to differences in the incidence and extent of secretion of at least two kinds of

enzymes with widely differing propensities. This contention derives support from the observation of Nutman [1929] that *Polyporus hispidus* secretes both hydrolysing and oxidising enzymes, while according to Lutz [1931] the same holds true for *Polystictus versicolor*, but, in addition, this fungus is capable of inducing at least one secondary reaction which results in the formation of alcohol from the products of the primary hydrolysis.

Consideration of the effect of white rot fungi on the pentosans of wood presents some difficulty. In brown rotts, for instance, it is a general rule that of the two categories of pentosans those which are associated with the cellulose are the more susceptible to attack, but in white rotts it appears that sometimes the one category and sometimes the other suffers relatively greater depletion without any reference to the extent of depletion in either the cellulose proper or lignin. In view of the above suggestion that both oxidising and hydrolysing enzymes play a part in all white rotts it is not improbable that the true effect of decay on the wood pentosans is to some extent masked, for on the one hand it is known that oxycellulose yields a certain amount of pentose on hydrolysis, and on the other hand there is the possibility in some cases of the formation during decay of insoluble by-products which yield furfuraldehyde on hydrolysis with hydrochloric acid.

There appear to be good grounds for the conclusion that in white rotts the solubility of the decayed wood in alkali rises to a maximum at a much earlier stage of decomposition than in brown rotts. In certain instances [Campbell, 1930; 1931] this has been ascribed to the fact that the pentosans in decayed wood which are not associated with the cellulose are less soluble in alkali than the corresponding complexes in sound wood, but it is obvious from the results of the present investigation that this is not true for all white rotts, since here it has only been found to hold for beech wood decayed by *G. applanatum* and *P. ostreatus*. These results appear to confirm the suggestion made in the preceding paragraph concerning the formation of by-products during decay. It is therefore obvious, in view of the general effect observed on the total alkali-solubility of wood decayed by white rot fungi, that the solubility of some major constituent other than pentosans must be consistently affected. It has been shown [Campbell and Booth, 1929] that in brown rotts hydrolysis has the direct effect of increasing the alkali-solubility of cellulose, and Bray [1924] has shown that in the same type of decay the increase in the total alkali-solubility of the residual wood is proportional to the loss in cellulose. The suggestion accordingly presents itself that in white rotts the full effect of hydrolysis on the alkali-solubility of cellulose is either masked or partially inhibited by the action of oxidases.

SUMMARY.

1. A detailed chemical examination of a further series of white rotts of wood tends to strengthen a previous conclusion that fungi of the white rot type invariably decompose carbohydrates as well as lignin, although there is

no general uniformity with regard to either the order or proportion in which the several wood components are decomposed.

2. The white rots can be subdivided on chemical grounds into three distinct groups as follows.

(i) White rots in which lignin and pentosans are attacked in the early stages and in which the incidence of the attack on cellulose is delayed.

(ii) White rots in which cellulose with its associated pentosans is attacked in the early stages and in which the incidence of the attack on lignin and the pentosans not in the cellulose is delayed.

(iii) White rots in the early stages of which both lignin and cellulose are attacked but in varying proportions.

3. It is suggested that the ultimate primary reactions involved in all white rots are oxidation and hydrolysis and that the main variants on which the above subdivision depends are the incidence in point of time of the primary reactions, the course of secondary reactions if any, and the rates of the several reactions.

4. In white rots the total alkali-solubility of the decayed wood as a percentage of oven-dry sound wood rises to a maximum and begins to decline at a much earlier stage than in brown rots.

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