3. 2-Phenylpyrroline is formed by the action of heat on phenyl cyclopropyl ketimine. Curves are given which show the effect of temperature and the presence of the ketimine hydrochloride on the stability of the ketimine base.

4. The facts enumerated in 1, 2 and 3, together with a general study of ketimine stability, have led to the proof that de Booseré's so-called ethyl cyclopropyl ketimine hydrochloride, which is described as remarkably stable toward hydrolysis, is in reality a pyrroline derivative. The true ethyl cyclopropyl ketimine hydrochloride is sensitive to hydrolysis, as are the other true ketimine hydrochlorides.

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[CONTRIBUTION FROM THE NEW JERSEY AGRICULTURAL EXPERIMENT STATION]

PROCESSES INVOLVED IN THE DECOMPOSITION OF WOOD WITH REFERENCE TO THE CHEMICAL COMPOSITION OF FOSSILIZED WOOD[1]

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Our information concerning the chemical processes involved in the decomposition (or so-called "decay") of wood, fossilization being merely a type of decomposition, is very meager. The question as to whether decomposition, which is never complete and which results only in a partial reduction in the bulk of woody substance, consists in the gradual utilization by microörganisms (fungi and bacteria) of all the chemical constituents of wood or whether certain complexes are decomposed more readily than others, has aroused considerable discussion. The nature of the processes involved in the decomposition of wood and other plant substances has found special application in connection with the recently proposed theories of the origin of coal and peat, largely centering upon the role of celluloses and lignins in this process.

It is commonly assumed that the disintegration of wood consists, first, in the chemical simplification of the various constituents from a greater to a lesser complexity, finally leading to their transformation into carbon dioxide, ammonia, water and, under anaërobic conditions, also into hydrogen and methane. The assumption that a number of intermediary substances are formed in this process has never been sufficiently substantiated.

Investigations reported elsewhere[2] on the decomposition of plant residues by microörganisms brought out the fact that the water-soluble plant constituents are the first to be attacked, long before the hemicelluloses

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[1] Journal series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

[2] S. A. Waksman and F. G. Tenney, *Soil Science*, **24**, 275–284 (1927); **26**, 155–171 (1928).

and celluloses are acted upon. No intermediary products at all or only in mere traces were found in the decomposition of celluloses by pure cultures of fungi[3] under controlled laboratory conditions; practically all of the carbon of the celluloses decomposed by these organisms was accounted for either in carbon dioxide given off or in the cell substance synthesized by the organisms. In view of the fact that this microbial cell substance frequently contains a large amount of cold and hot water-soluble material, the increase observed by Bray and Andrews in the water-soluble constituents as a result of decomposition of wood by fungi may be due to this cause rather than to the formation of intermediary products. The reasons submitted by Hawley and Wise[4] why these results must be considered in the light of the formation of intermediary compounds in the destruction of lignins and celluloses in wood are not very convincing. If one only recalls that large quantities of fungus mycelium are synthesized in the process of decomposition of the wood and that this mycelium is rich not only in water-soluble substances but also in various hemicellulose-like or gum-like substances, we can account even for the increase in the alkali-soluble materials as a result of wood decomposition.

In connection with a study[5] of the formation of "humus"-like substances from pure celluloses by microörganisms, it has been shown that the organic matter resulting from the repeated decomposition of celluloses, under controlled laboratory conditions and in pure sand but in the presence of additional inorganic nitrogen compounds and other minerals necessary for the growth of the microörganisms decomposing the celluloses, was a product which contained 23.4–30.2% of cold water-soluble, 6.9–13.4% of hot water-soluble and 23.8–25.4% of alkali-soluble (after cold and hot water treatment) material, all of which was a part of the cells of living and dead microörganisms.

There is no doubt that when celluloses are decomposed by anaërobic bacteria large quantities of organic acids and alcohols are produced, but these should be considered as final products of anaërobic metabolism rather than as intermediary products; these acids and alcohols will be decomposed further when conditions are made aërobic and the reaction is favorable.

We have no justification as yet for speaking therefore of the "simplification" of complex plant constituents when these plants are decomposed by fungi. There is more sound evidence that the chemical processes involved in the decomposition of wood by microörganisms consist merely in the complete destruction of the more readily available substances, followed

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[3] H. Heukelekian and S. A. Waksman, *J. Biol. Chem.*, **66**, 323–342 (1925).

[4] L. F. Hawley and L. F. Wise, "The Chemistry of Wood," The Chemical Catalog Company, Inc., New York, **1926**, p. 301.

[5] S. A. Waksman, *Cellulosechemie*, **8**, H. 9/10 (1927).

by a slow but gradual disappearance of those that are less readily decomposed; the more resistant plant constituents contribute to the formation of the residual (or fossilized) material.

Chemists are willing to agree that the disintegration of wood is largely a result of the activities of bacteria and fungi and that the nature of decomposition depends upon the nature of the organisms that bring about the process. Unfortunately, the chemistry of wood, as well as of natural organic materials in general, still represents a series of complicated problems, and, unless these are first carefully worked out, one could not expect to unravel the chemistry of decomposed wood, as well as of the residual soil humus and peat, and understand the nature of the chemical and biological processes involved in their formation from the natural materials.

It is sufficient to call attention to the chemical composition of the lignins and celluloses in wood, which make up, in the form of lignocelluloses, 65 to 70% of the constituents of the wood. The chemical nature of these substances obtained from wood is influenced quite markedly by the method used in their preparation. When one attempts to determine lignin quantitatively, he is faced with an array of methods which give varying results not only quantitatively but even qualitatively, yielding substances which vary considerably in their chemical nature and their biological behavior. The quantitative determination of cellulose is also apt to give varying results, depending on the method employed, especially if one does not use sufficient care in accounting for the admixture of hemicelluloses, lignins and cutins. It is not remarkable therefore that the results obtained on the decomposition of plant materials are very conflicting, and it is only within very recent years that definite light has been thrown upon the chemical nature of the processes involved.

Rose and Lisse[6] have shown that when wood is rotted by fungi there is a gradual decrease in the cellulose and pentosan content and an increase in the lignin, as determined by the methoxyl content, and the amount of alkali-soluble materials. Lignin, which is more resistant to decomposition by microörganisms, gradually accumulates, while the celluloses and hemicelluloses disappear.

These results were utilized by Fr. Fischer[7] to prove his theory that coal originates from the lignins of plant materials, since they resist decomposition, while the other plant constituents, namely, the celluloses, hemicelluloses, proteins, etc., are gradually broken down by microörganisms.

Lamarlière[8] has shown previously that coniferous wood freshly removed from peat beds preserves, the microscopic structure, but a chemical analysis

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[6] R. E. Rose and W. M. Lisse, *J. Ind. Eng. Chem.*, **9**, 284–287 (1917).

[7] Fr. Fischer and H. Schrader, *Brennstoff-Chem.*, **2**, 37–45 (1921).

[8] L. G. de Lamarlière, *Compt. rend. acad. sci.*, **131**, 511–512 (1900).

shows that the celluloses are largely gone, while the amorphous residue dissolves rapidly, after treatment with chlorine, in alkalies.

The nature and rapidity of decomposition of wood depend upon the nature of the infecting organism. Bray and Andrews[9] found that certain hymenomycetes causing brown rot of wood may bring about a loss of 10 to 50% of the wood in six to ten months. The cellulose may be reduced from 60 to 6% in one year, while the loss in the lignins was only 3%; this small loss all can be accounted for by a loss in the methoxyl content (2.8%).

However, although the lignins are not decomposed as rapidly as the celluloses, hemicelluloses and proteins, they will still undergo some decomposition, slow to be sure, under aerobic conditions. The existence of organisms capable of attacking lignins has now been definitely established.[10]

As a matter of fact, it has been demonstrated[10a] that decomposition of wood, under aërobic conditions and largely through the agency of fungi, can either consist in a rapid disintegration of the celluloses with the accumulation of lignins or in a gradual decomposition of both the celluloses and the lignins and frequently even in an accumulation of cellulose. These two processes were termed by Falck as "destruction" and "corrosion." They account for the differences observed in the chemical composition of the wood decomposed by the brown and white rot-producing fungi.

When a comparison is made of sound and decomposed wood, using the same method of chemical analysis, we can readily recognize the chemical changes that have taken place as a result of the decomposition of the various constituents of the wood by different groups of microörganisms. A typical analysis is given in Table I, where the chemical composition of healthy chestnut and cypress wood is compared with the composition of two samples of decomposed wood, one of which was non-fibrous in nature, having undergone the so-called process of "destruction," and the other fibrous in nature, having been decomposed by the fungi bringing about "corrosion," according to Falck's nomenclature. These results show that while in the case of certain processes of wood decomposition the celluloses have practically all disappeared (even more so than the hemicelluloses) and most of the decomposed wood consists of lignin which has resisted decomposition, in other cases both the lignins and celluloses, as well as the hemicelluloses, have all undergone a gradual decomposition. It is unfortunate that in this particular experiment the samples of decomposed wood were selected at random from decomposing trees in a New England forest and were not studied under controlled laboratory conditions. However, in other investigations on the decomposition of plant materials[11]

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[9] M. W. Bray and T. M. Andrews, *Ind. Eng. Chem.*, **16**, 137–139 (1924).

[10] (a) R. Falck, *Ber.*, **60**, 225–232 (1920); (b) S. A. Waksman, F. G. Tenney and K. R. Stevens, *Ecology*, **9**, 126–144 (1928).

[11] Results to be published soon in *Soil Science*.

TABLE I

CHEMICAL COMPOSITION OF DECOMPOSED WOOD AS COMPARED WITH HEALTHY WOOD

On percentage basis of dry material

Chemical complexes	Sound wood Chestnut	Sound wood Cypress	Rotted wood Non-fibrous decomposed wood[a]	Rotted wood Fibrous decomposed wood[b]
Ether-soluble	2.66	3.53	1.48	6.91
Cold and hot water-soluble	7.08	3.18	1.26	5.61
Alcohol-soluble	3.27	1.92	5.05	3.30
Hemicelluloses	15.23	11.16	4.72	14.43
Celluloses	32.58	37.62	2.16	27.78
Lignins[c]	22.05	28.21	71.14	23.61
Protein	0.54	0.66	1.31	2.18
Ash	.54	.76	0.65	2.25
Total accounted for	83.95	87.04	87.77	86.07

[a] Wood which has undergone the so-called process of "destruction."

[b] Wood which has undergone the so-called process of "corrosion."

[c] Free from ash and nitrogen.

which were carried out under controlled laboratory conditions, it has been found that under anaërobic conditions lignins do not decompose at all or only in mere traces due to the absence of specific organisms, while, under aërobic conditions, the lignins are slowly decomposed, but here as well they are found to be the most resistant group of plant constituents.

It is interesting to observe in Table I the increase in the protein content of the decomposed wood over the sound wood; this is not necessarily due to the accumulation of the proteins because they are more resistant to decomposition by microörganisms than the celluloses, but to the fact that the products of protein decomposition are immediately reassimilated by the microörganisms bringing about the decomposition and are thus preserved. This has been amply demonstrated in this Laboratory for the decomposition of various plant substances by pure and mixed cultures of microörganisms under controlled conditions.

The chemical nature of the transformation of the wood constituents, especially the celluloses and lignins, has attracted considerable attention. According to Wehmer,[12] the wood-destroying fungus *Merulius lacrymans* brings about the decomposition of the cellulose and the transformation of the lignin of the wood into "humin," a part of which is soluble in alkalies and precipitated by acids.

According to Kürschner,[13] various wood-destroying fungi, such as *Merulius lacrymans*, attack largely the cellulose in the wood, while the lignins are transformed into a complex mixture of changed and unchanged depolymerized substances of a humic-like nature. Strache[14] analyzed the inner portion of a 1000-year old pine and found that only traces of cellulose

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[12] C. Wehmer, *Ber.*, **48**, 130–134 (1915); *Brennstoff-Chem.*, **6**, 101–106 (1925).

[13] K. Kürschner, *Z. angew. Chem.*, **40**, 224–232 (1927).

[14] H. Strache, *Brennstoff-Chem.*, **9**, 21–22 (1927).

were left, while the lignin had changed, in the absence of oxygen, into "humic acid," which was thus considered to be the first step in the formation of lignite and brown coal. The residue of the action of *Merulius lacrymans* upon pine wood was found by Schwalbe and Ekenstam[15] to consist of 73% of lignin, 15% of cellulose, 8% of other carbohydrates and 4% of resins; the fats and pentosans were all destroyed; 64.2% of the lignin was soluble in 5% sodium hydroxide solution and it had less methoxyl (10.2%) than lignin of fresh wood. Brandl[16] recorded the presence of 24.90% of acid-hydrolyzable material, including 8.1% of cellulose, and 75.1% of non-hydrolyzable material in decomposed oak wood; the latter fraction was separated into lignin (36.35%) and humic acid (38.75%) on the basis of solubility in sodium bicarbonate solution.

Marcusson,[17] in an attempt to prove by chemical theories and facts the idea of the older botanists and geologists that coal originates largely from celluloses, claims that as a result of decomposition of plant materials the celluloses are changed to oxycelluloses by the action of air, light and moisture; the oxycelluloses are then transformed into "humic acids," which are soluble in dilute alkalies. The role of microörganisms in the processes of decomposition is not considered at all. According to Marcusson, the results of Rose and Lisse[6] could not be used in proving Fischer's theory concerning the role of lignins in coal formation, since the former did not determine the actual lignin content of the various forms of wood, but merely measured the methoxyl content and the amount of alkali-soluble material. Since the methoxyl content of various lignin preparations varies and since it is also present in other substances, as pectins, it need not be taken as an index of the lignin content of wood at various stages of decomposition. Marcusson found that when healthy and rotten wood were treated first with a 1% solution of sodium hydroxide and the lignin determined on the insoluble residue by the Willstätter method, the cellulose was found to decrease from 58% in healthy wood to about 6.0% in the fully rotted wood, while the lignin content was practically the same, ranging from 20 to 23%. The results of Rose and Lisse are explained by Marcusson by the fact that pectins present in the middle lamella and certain waxes also contain methoxyl groups, while the increase in the alkali-soluble materials is explained by the assumption that glucuronic acid, a constituent of oxycellulose, is thereby made soluble.

Without going into a detailed review of the extensive literature on the origin and chemistry of soil "humus," peat and coal, which is found elsewhere,[18] the results of some experiments will be presented here to show that

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[15] C. G. Schwalbe and A. Ekenstam, *Cellulosechemie*, **8**, 13–15 (1927).

[16] A. Brandl, *Brennstoff-Chem.*, **9**, 89–94 (1928).

[17] J. Marcusson, *Z. angew. Chem.*, **39**, 898–900 (1926); **40**, 48, 1105 (1927).

[18] S. A. Waksman, *Soil Science*, **22**, 123–162 (1926).

Marcusson's explanations will not stand any broad generalization and are merely isolated instances which have no application at all to the formation of "humus," peat and coal. The methods of analysis of the various plant constituents are reported in detail elsewhere.[19]

Table II gives an analysis of two samples of wood obtained from an undrained lowmoor peat bog, at Newton, N. J., at a depth of 7–8 feet, and of fossilized wood of the Pleistocene period kindly supplied to the authors by Dr. David White of the U. S. Geological Survey. The composition of these can be compared with that of healthy wood reported in Table I.

TABLE II

CHEMICAL COMPOSITION OF TWO SAMPLES OF FOSSILIZED WOOD

Percentage of dry material

Material	Wood from peat	Fossilized wood (oak)
Ether-soluble material	1.54	0.84
Cold and hot water-soluble organic matter	0.87	0.56
Alcohol-soluble	1.34	1.82
Hemicelluloses	8.15	3.79
Celluloses	6.12	3.38
Lignins (free from nitrogen and ash)	65.02	70.74
Protein	5.37	2.21
Ash	3.85	4.40
Total accounted for	92.25	87.74

The results show that in the wood from peat and in the fossilized wood there is a considerable reduction to an almost complete disappearance of the water-soluble substances; there is also a reduction in the ether-soluble substances and hemicelluloses and a marked decrease in the celluloses. The lignin and ash content have increased to such an extent that they account for three-quarters of the total wood material or of 80% of the constituents accounted for. The proteins also have increased very markedly, from about 0.6% in healthy wood to 2.21% in the fossilized wood and 5.37% in the wood from the lower layers of the peat bog. These results are in full accord with those obtained in this Laboratory experimentally, under controlled conditions, on the decomposition of natural plant materials by microörganisms, which can be summarized by a rapid reduction in the water-soluble substances, decomposition of hemicelluloses and celluloses as well as of certain ether-soluble substances, and an increase in the lignin, ash and protein content. The changes in the nature and quantity of the latter are especially illuminating, since they depend not only on the nitrogenous constituents but also on the nature and amount of the celluloses and hemicelluloses in the plant material, organisms active in the decomposition processes and the conditions under which decomposition is carried out.

[19] S. A. Waksman and K. R. Stevens, *Soil Science*, **26**, 113–138 (1928).

To demonstrate whether Marcusson is justified in assuming that, after treatment of rotted wood with an alkali solution to remove the so-called "oxycelluloses," the same percentage of lignin will be found in the decomposed as in the healthy wood, the wood from peat and the fossilized wood were subjected to treatment with ether for fifteen hours in Soxhlets, followed by 95% alcohol for one hour at boiling temperature, then boiling for five hours with a 2% hydrochloric acid solution. The lignin content was determined (by adding to a weighed quantity of dry residual material 10 volumes of 80% sulfuric acid, allowing to stand in the cold for two hours, then diluting with fifteen volumes of water and heating at 120° for one hour)[20] in the wood thus treated as well as in the original material and in the wood treated with a 2% sodium hydroxide solution for one hour on a water-bath. The lignin was filtered off, dried and weighed. The ash and nitrogen content of the lignin residue were determined and subtracted to give the amount of lignin.

The results presented in Table III show that the lignin content of untreated wood is somewhat higher than that of wood which has been previously extracted with ether, alcohol and dilute acid. The lignin content of the wood extracted with a 2% sodium hydroxide solution is considerably lower than the lignin of the wood not so treated; however, when the alkaline extract is neutralized with hydrochloric acid and the precipitate formed thereby dried and weighed, it is found that the sum of the weight of the precipitate and the weight of the lignin in the alkali-treated wood is almost the same as the weight of the lignin in the untreated wood. In other words, the alkali treatment merely removed a part of the lignin, or a modified form of lignin, as a result of the activities of microörganisms. Hawley and Campbell[21] found that when Sitka spruce is extracted with 1% sodium hydroxide solution on a boiling water-bath for one hour, the lignin content decreased from 29.3 to 27.7%. Others found that with the advance of decomposition there is an increase in the amount of lignin soluble in alkali bicarbonate or cold alkali hydroxide solutions (so-called "humic acids"). The accumulation of lignin as a result of disintegration of wood under anaërobic conditions is due entirely to its resistance to decomposition under these conditions and not to its formation in the process of decomposition.

TABLE III

LIGNIN CONTENT OF FOSSILIZED WOOD, SUBJECTED TO DIFFERENT PRELIMINARY TREATMENTS (PERCENTAGE OF TOTAL ORIGINAL DRY MATERIAL)

Material	Wood from peat bog	Fossilized wood
Untreated	72.76	84.58
Treated with ether, EtOH and 2% HCl	65.02	70.74
With 2% NaOH for 1 hour at 100°	54.52	56.61
Ppt. of alkaline extract with HCl	10.94	20.38

[20] It may be stated here that the lignin obtained by this method compares very well quantitatively with the amount of lignin found by the original hydrochloric acid method of Willstätter or any of its modifications.

[21] L. F. Hawley and W. G. Campbell, *Ind. Eng. Chem.*, **19**, 742–744 (1927).

To study the effect of alkali treatment upon the acid lignin obtained from natural and from decomposed plant material, several plant-lignin and peat-lignin preparations were subjected to treatment with 2% sodium hydroxide solution, either in the cold for twenty-four hours, or for one hour on a steam-bath, or for thirty minutes at 120° (1-g. portions of lignin were extracted with 100 cc. of the sodium hydroxide solution). The solutions were then filtered and the residues washed, dried and weighed; hydrochloric acid was then added to the solutions and the precipitates formed were filtered, washed, dried and weighed. The results are reported in Table IV.

TABLE IV

INFLUENCE OF ALKALI TREATMENT UPON LIGNIN FROM STRAW AND FROM PEAT

One gram of lignin extracted with 100 cc. of 2% NaOH solution

		Acid lignin from rye straw	Lignin-like complexes from lowmoor peat treated with 2% HCl	Lignin-like complexes from lowmoor peat treated with Et$_2$O, EtOH and 2% HCl	Lignin-like complexes from highmoor peat treated with Et$_2$O, EtOH and HCl
24 hrs. cold	Undis. res., g.	0.420	0.496	0.420	0.757
	Acid ppt., g.	.428	.292	.428	.057
1 hr. 100°	Undis. res., g.	.212	.350	.271	.441
	Acid ppt., g.	.548	.332	.445	.327
30 min. 120°	Undis. res., g.	.041	.389	.220	.193
	Acid ppt., g.	.663	.405	.523	.534

These results show that acid lignin prepared from rye straw can be dissolved almost completely in 2% sodium hydroxide solution if a sufficiently high temperature and pressure are used.[22] However, only 65 to 75% of the lignin which has been thus dissolved is reprecipitated from the alkali solution on neutralization with hydrochloric acid.

The lignin prepared from peat behaves in this respect very similarly to the lignin of rye straw, with the exception that in this case the amount recovered on acidification is somewhat less.

These results prove further that treatment of rotten wood with a hot alkali solution does not merely remove the oxycelluloses, as assumed by Marcusson, but has a decided effect in bringing the lignin itself, either in an unmodified or in a modified form, into solution.

The possible reason for the discrepancy in the results reported by Rose and Lisse, Bray and Andrews and others, as well as in this paper, on the one hand, and those of Marcusson, on the other hand, may be due to differences in the nature of the organisms concerned in the processes of decomposition. It has been definitely established that under aërobic conditions some fungi and other organisms[2,10a,23] are capable of attacking

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[22] M. M. Mehta, *Biochem. J.*, **19**, 958–978 (1925).

[23] A. W. Schorger, "The Chemistry of Cellulose and Wood," McGraw-Hill Book Co., New York, **1926**.

lignins as well as celluloses. However, under anaërobic conditions no organisms have been found so far which would be capable of decomposing this group of chemical substances. This is largely the reason for the accumulation of lignins under the processes of formation of peat,[20] coal and fossilization of wood, but not in certain aërobic processes of rotting of wood by certain fungi belonging to the group of *Basidiomycetes*.

Although the results presented in this paper are not primarily concerned with the decomposition processes in peat bogs, it may be stated here that evidence will soon be submitted that peat bogs are teeming with life (largely bacterial) and are not sterile, as commonly assumed. The reason for peat formation and its chemical nature as well depend largely upon the nature of these organisms and their activities under anaërobic conditions.

Summary

Decomposition of wood under anaërobic conditions, as in peat bogs or in the process of fossilization, brings about the disappearance of the celluloses and hemicelluloses and a marked accumulation of the lignin complexes.

The nature of the chemical processes involved in the decomposition of wood under aërobic conditions depends largely upon the microörganisms concerned in the process.

There is no justification in assuming that in the decomposition (rotting) of wood, oxycelluloses are formed as an intermediary step in the formation of "humus" from celluloses.

Evidence presented in this paper, as well as by other investigators, definitely establishes the fact that the processes of decomposition of wood are largely microbiological in nature and the nature of decomposition will depend upon the organisms active in the process, as influenced by the environmental conditions and the chemical composition of the plant constituents.

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