

Lectures on
Soil Organic Matter

by

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Foreword

To my friends and the friends of the subject.

In this manuscript are some unpublished results and therefore only for friends and not to use for publication.

I would enjoy having any comments on this material.

W. Flaig

Acknowledgments

This manuscript came about as a result of the kind invitation of Prof. Dr. W. H. Pierre, Head, Department of Agronomy, to give lectures about soil biochemistry. I am very thankful for this opportunity.

I would like to express my best thanks also to my colleague, Prof. Dr. Lloyd Frederick, who stood by me helpfully at all times during the writing of these lectures in the English language and I appreciate his suggestions during our many discussions.

Without the help of his co-workers, Messrs. McIntosh Sims, Horton, Brown, and of the secretaries, Mrs. McLaughlin, Misses Sansgaard and Zart, it would not have been possible to mimeograph the lectures. Also to these, many thanks.

June, 1959

W. Flaig

SOIL ORGANIC MATTER

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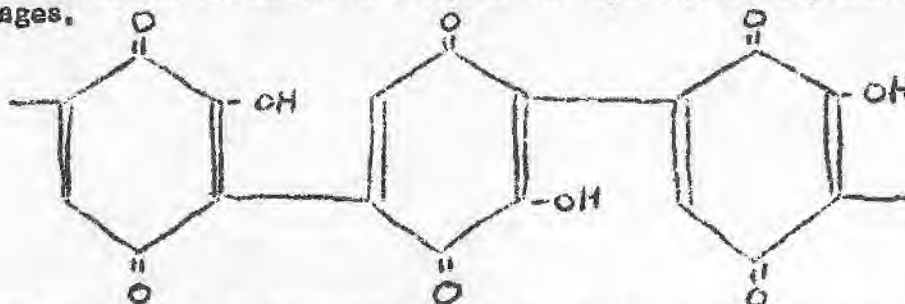
Experiments of the Formation of Synthetic Humic Acids

The first which has begun to synthesize model substances of humic acids has been Eller, W., und Koch, K.: *Synthetic Darstellung von Huminsäuren*. Ber. dtsh. Chem. Ges. 53, 1469 (1920) - Eller, W.: *Kunstliche und Natürliche Huminsäuren*. Brennstoff-Chemie 2, 129 (1921) - Eller, W.: *Studien über Huminsäuren, IV. Darstellung und Eigenschaften künstlicher und natürlicher Huminsäuren*. Ann. Chem. 431, 133 (1923) - Eller, W., Meyer, H., und Saenger, H.: *Einwirkung von Salpetersäure auf Huminsäuren*. Ann. Chem. 431, 162 (1923) - Eller, W., Herdieckerhoff, B., und Saenger, H.: *Einwirkung von Chlor auf Huminsäuren*. Ann. Chem. 431, 177 (1923) - Eller, W.: *Zur Frage der Konstitution der natürlichen Huminsäuren*. Brennstoff-Chemie 6, 55 (1925) - Eller, W.: *Studien über Huminsäuren. Einige Eigenschaften und Reaktionen der Huminsäuren und Humine*. Ann. Chem. 442, 160 (1925).

He oxidized different polyphenols such as hydroquinone, pyrocatechol and pyrogallol in alkaline solution with oxygen. By acidifying he isolated dark brown substances, which he compared with natural humic acids by different chemical reactions. After chlorination of both types of humic acids the properties of the chlorinated substances was nearly the same. Therefore, he believed that the natural humic substances as well as the synthetic would have the same composition, i.e., there would be polymers of hydroxy-p-benzoquinone.

While Eller synthesized polymers of quinones as model substances of humic acids, Erdtman tried to make model substances by step-wise condensation of quinone. (Erdtman, H. G. H.: *Phenol-dehydrierungen, IV. Über die reaktionsfähigen Stellungen des 4,4'-Dimethoxydichinons und die oxydative Kupplung von 4-Athylpyrogallol*. Annalen 513, 240 (1934).

He formulates the humic acids as polymers of hydroxy benzoquinone with diphenyl linkages.



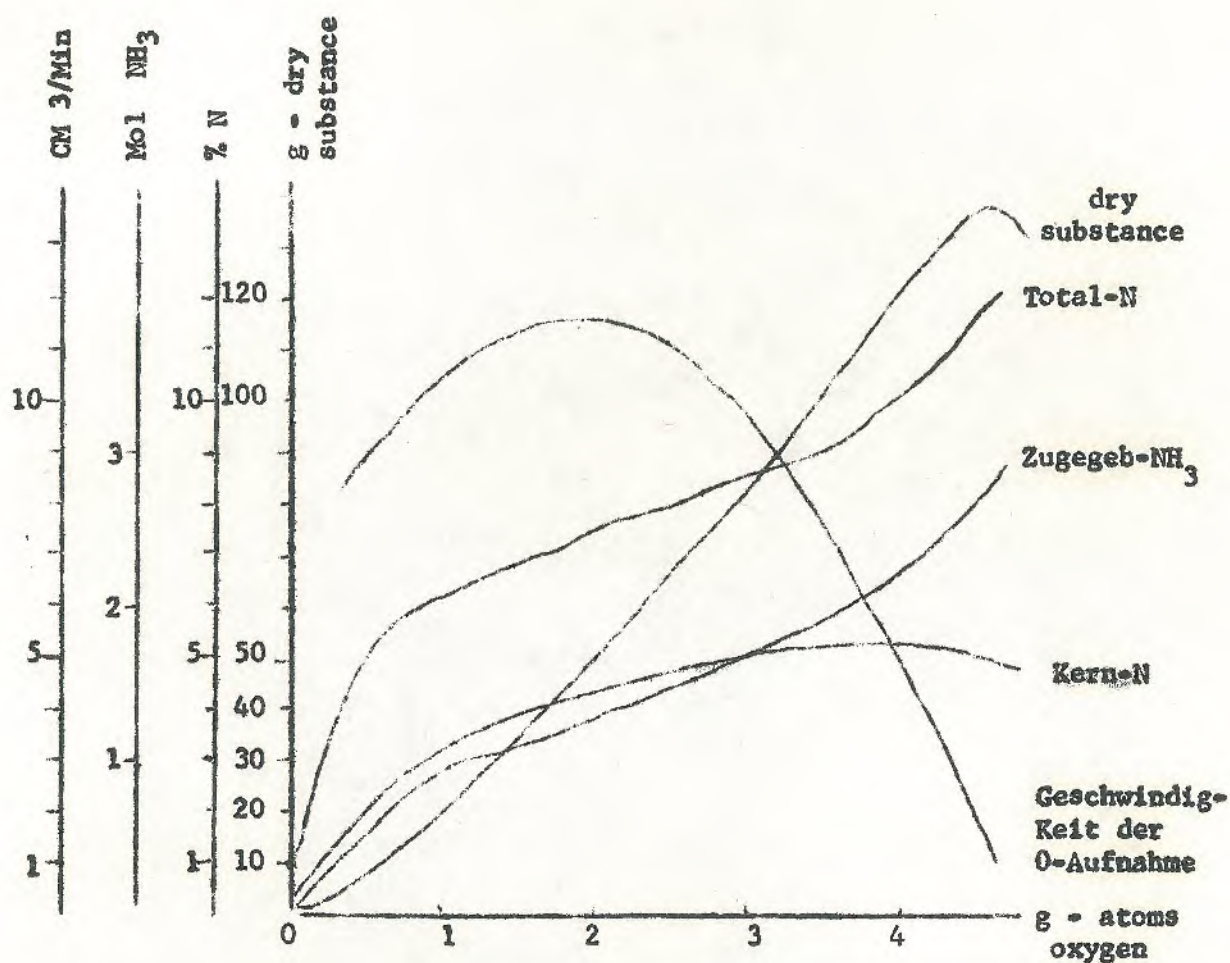
Some years later we made investigations about the oxidation of polyphenols, especially with hydroquinone. Hydroquinone has been oxidized in the presence of ammonia. Ammonia has not been given all at once but stepwise to the reaction solution. At first one mole so that the reaction had always a pH of approximately 10. In the following diagram the results are summarized.

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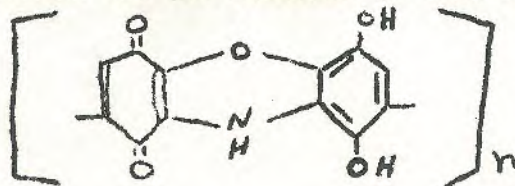
In one curve the addition of ammonia is shown in connection with the oxygen obtained. The reaction product has been precipitated by acidifying the mixture with hydrochloric acid. After separating and washing, the reaction product has been analyzed in the following way:

1. The total content of nitrogen had been determined with Kjeldahl.
2. By boiling with 2N sodium hydroxide ammonia has been distilled off.

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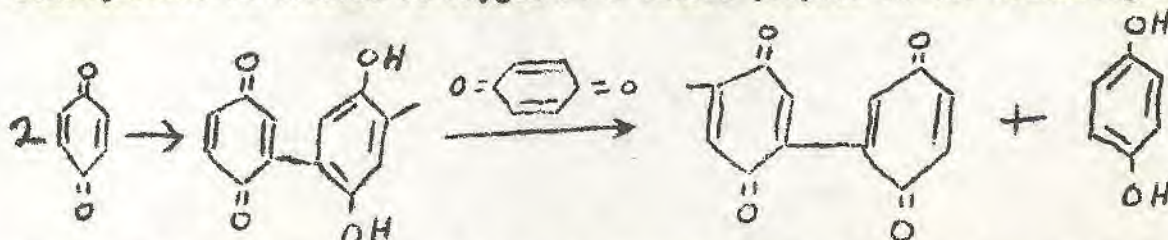


The diagram shows that an increase of dry weight occurs up to an uptake of a little bit more than 4 gr-atoms oxygen. The nitrogen content increases to the uptake of 4 gr-atoms oxygen. The decrease of dry matter after uptake of more than 4 gr-atoms oxygen shows at the same time an increase of distillable ammonia. It seems that after the uptake of 4 gr-atoms oxygen a ring cleavage takes place and ammonia compounds are formed. The so-called "kern" nitrogen or heterocyclic bound nitrogen increases to the uptake of approximately 4 gr-atoms oxygen and then decreases. The highest value of heterocyclic bonded nitrogen is nearly 6%. According to this diagram the following formula for the reaction product can be given:



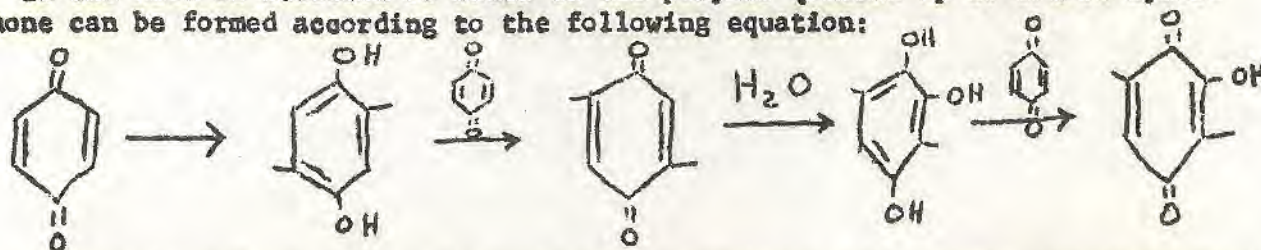
In this case the linkage is discussed as an oxygen bridge. By investigation with model substances we have learned that this oxygen bridge can be oxidized very easily. With ferric chloride or potassium ferric cyanide, 4,4'-dihydroxy diphenyl ether can be oxidized to 2 molecules quinone. These considerations give only an idea about one possibility of the formation of humic acids. The oxygen bridge can be compared with the Thiele-reaction. In both cases the addition of acid substances occur. By the combination of the addition of ammonia or primary amines - but never secondary amines - the oxygen bridge is stabilized.

Later on Erdtman (Z. Pflanzenern., Dung., und Bodenkunde 69, 38 (1955)) made further investigations about polymer quinones. He describes the polymerization of benzoquinone in absence of oxygen as a linear polymer in the following way:



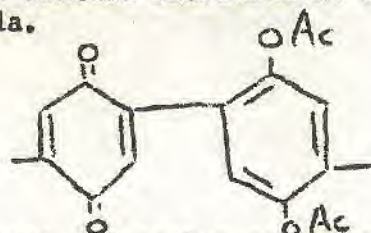
The redox normal potential of these polymers which consists partly of hydroquinone parts is lower than that of p-benzoquinone. Therefore, the dehydrogenation of such polymers by p-benzoquinone which has not reacted must form up to 50% hydroquinone. All intermediate steps of such an oxidation are possible and therefore the amount of hydroquinone can be between 0 and 50%.

In the case of addition of water in the polymer quinone up to 67% of hydroquinone can be formed according to the following equation:



Erdtman investigated the polymerization of quinone with alkali and determined the percentage amount of hydroquinone. In strong alkaline solutions he could determine up to 60% hydroquinone and in weak alkaline condition (sodium acetate or 0.1 N sodium hydroxide) between 14 and 20% hydroquinone. Therefore, he believes that the formed quinone-humic acid are combined with C-C connections. The formed polymers are soluble in alkali by formation of green or green-brown solutions which absorb oxygen very easily. The formation of deep brown solutions occur.

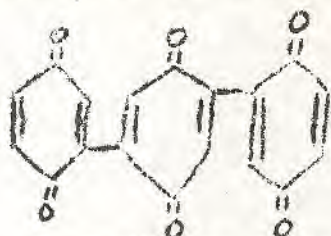
By methylation with dimethyl sulfate in alkali in absence of oxygen products are formed with a methoxyl content of 20 to 25%. By further methylation with diazomethane the methoxyl content increases to 25%. A product of this kind could have the following formula.



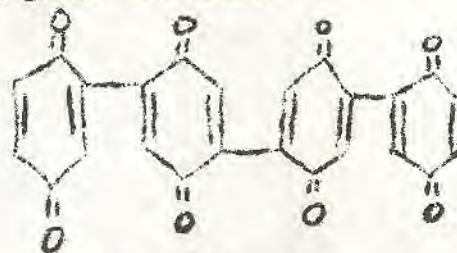
The content of hydrogen and carbon correspond to this formula. Similar results were found by reducing acetylation. By hydrolysis of the leucoacetates in absence of air with alkali solutions a green color could be obtained. With absorption of oxygen the color becomes brown.

In the case of diquinone in the absence of air Erdtman got brown solutions and has been able to isolate in small amounts dihydroquinone (approximately 1%). By treatment of diquinone with alkali in the presence of hydroquinone, the solutions had a dark green color.

Similar results have been obtained with tri- and tetraquinone. In absence of air quinones gave with alkali brown solutions and a mixture of the corresponding quinones with the corresponding hydroquinones gave green solutions.



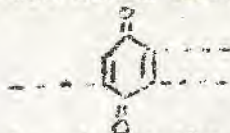
Trihydroquinone



Tetraquinone

Trihydroquinone has been made by condensation of mono-iodo-hydroquinone-dimethyl ether with 2,5-di-iodo-hydroquinone-dimethyl ether according to Ullmann-reaction. The tetraquinone has been made by condensation of mono-iodo-hydroquinone-dimethyl ether with 2,5,2',5'-tetramethoxy-4,4'-di-iodo-diphenyl. In both cases the ethers have been demethylated to the corresponding phenols and oxidized with chromic acid.

Erdtman presumes that the polymerization goes not only in linear direction but also tri-dimensional products are found according to the following possibilities.

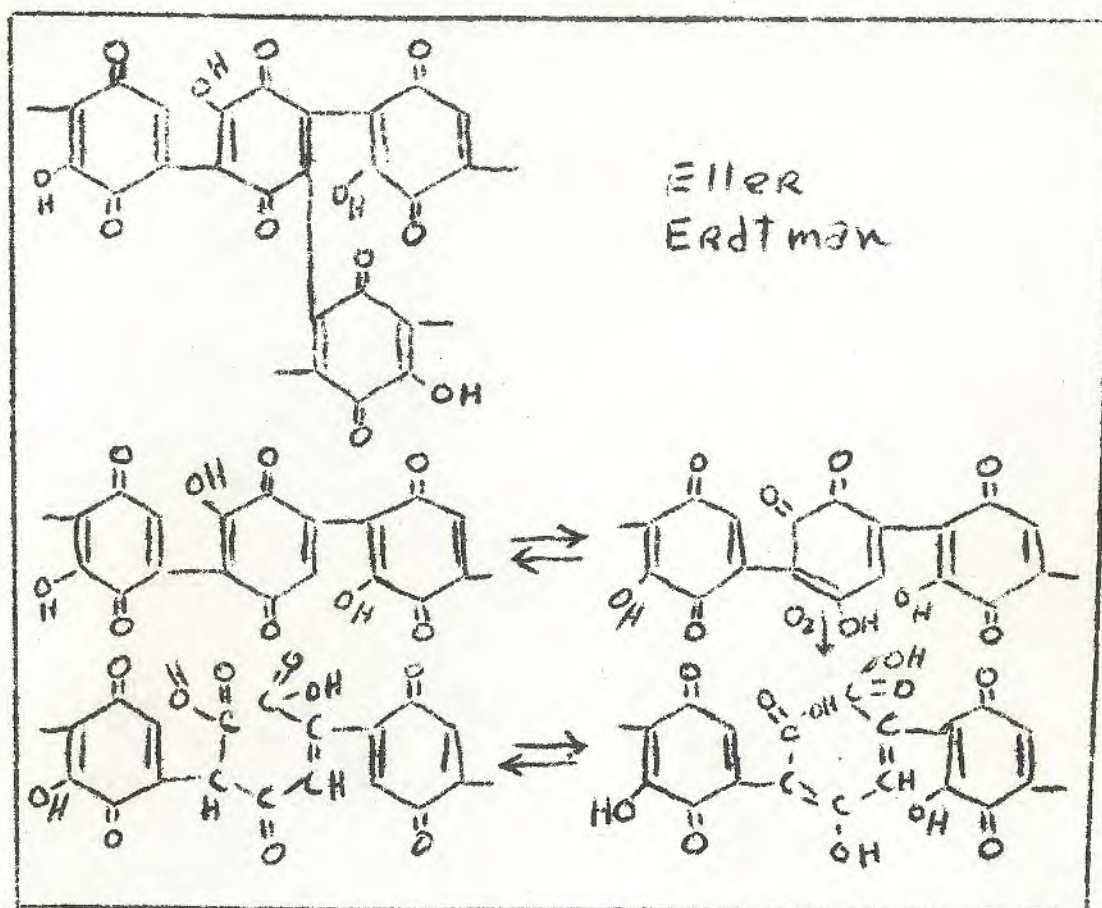


Till now it is not known how large the difference between the reaction velocity to linear polymers and tri-dimensional polymers is. But it seems that the polymerization to tri-dimensional components is much slower than to linear polymers. We discussed therefore the formation of tri-dimensional polymers in the following way.

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One part of the hydroxy-p-quinones reacts in a tautomeric form as hydroxy-o-benzoquinone which is split up by further oxidation. Compounds are formed which are very reactive and adapted for linkage.

Recently Scheffer and his co-workers made further investigations about the oxidation of hydroquinone and formation of synthetic humic acids. (F. Scheffer und W. Ziechmann: *Über Synthesehuminsäuren aus Polyoxybenzolen*, Z. Pflanzenernähr., Dung., Bodenkunde 80, 129-133 (1958), Scheffer, F. W. Ziechmann und H. Scholz: *Über Synthesehuminsäuren aus Polyoxybenzolen 2. Mitteilung, Quantitative Untersuchungen zur Oxydation von Hydrochinon im homogenen System mit Alkalischer Reaktion*, loc. cit. 80, 134-149 (1958)). In these investigations they are mostly interested to determine the oxidation of hydroquinone by variation of sodium hydroxide and oxygen uptake. Furthermore, they determined the formed CO₂ and the amount of synthetic humic acids. They believe that, during oxidation of hydroquinones to humic acids or their precursors, oxygen uptake, condensations (formation of ether linkages and carbon linkages) and polymerization reactions occur side by side and one after the other.



It may be that the oxidation of hydroquinone also occurs in neutral solution. The rate of oxidation depends on the pH value and is faster in alkaline solution, as we know according to the work of Weisberger several lectures before. But most of the reactions for oxidizing hydroquinone to synthetic humic acids has been done in alkaline solutions with pH values which never occur in the soil. The average pH values in the soil are weak acid. Another question is this: it is right to use as model substances unsubstituted polyphenols, especially with OH group in 1,4-position?

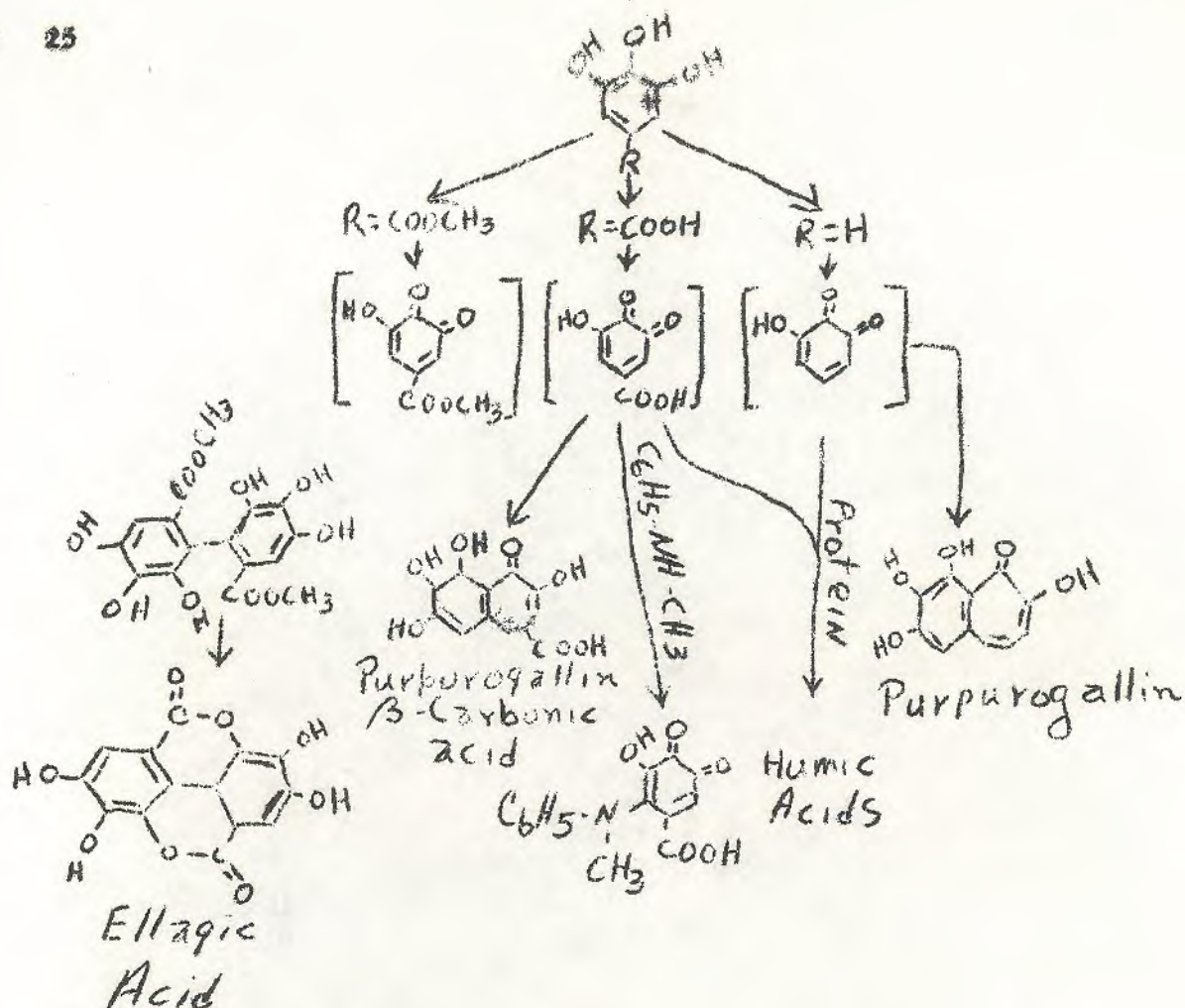
We tried to find out ways to prepare humic acids under more or less physiological conditions, using lignin fragments. Some of these investigations may be mentioned. (Steinmetz, A.: Modellversuche zur Beteiligung von Polyphenoloxidasen bei der Bildung natürlicher Huminsäuren - Diss. Braunschweig 1956, 97 pp.).

We oxidized model substances as well as lignin fragments with phenol oxidases isolated from mushroom with oxygen in a range of pH between 4.5 and 6.5.

In the case of model substances it has been interesting that 2,5-di-tert.butylhydroquinone will not be oxidized to the corresponding benzoquinone. But the unsubstituted hydroxy quinone can be oxidized to p-benzoquinone with difficulties.

In the case of 3,5-di-tert.butylpyrocatechol the corresponding o-benzoquinone could be isolated. We succeeded also by the oxidation of 4,6-di-tert.butylpyrogallol to isolate the yellow dimeric 3-hydroxy-4,6-di-tert.butyl-o-benzoquinone and its further oxidation products 2,4-di-tert.butyl-4-oxalo-crotonic acid anhydride.

In the case of gallic acid or pyrogallol the following reaction has been found:



In the presence of protein or amino acid, humic acids have been found which are very similar to those which can be isolated on the soil. By hydrolysis the same amino acids are found which have been in the added protein. The residue of the hydrolysis shows the same behavior as that of natural humic acids.

By using primary amines as methyl-aniline the corresponding 3-hydroxy-o-benzoquinone derivative can be found. It is interesting that by this reaction also purpurogallin and purpurogallin-β-carbonic acid could be found.

Furthermore, the formation of di-phenyl derivatives as ellagic acid could be shown. It seems to me that this is the first case that indeed model substances of humic acids could be prepared. The first steps of the reaction are known by the mentioned investigations with model substances.

Furthermore, it must be remarked that the dimerization reactions in the case of the formation of humic substances must be investigated much more than till now. There are:

1. Dimerization to diphenyl derivatives.
2. Diensynthesis as in the case of the formation of purpurogallin derivatives; this type of reaction has been discussed in detail.