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In this manuscript are some unpublished results and therefore only for friends and not to use for publication.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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1.

INTRODUCTION TO THE WORK OF THE INSTITUTE FOR SOIL BIOCHEMISTRY

F. A. L. Braunschweig - Völkenrode

At first I will give you a survey of the work of the Institute of Soil Biochemistry. You can see in which matters we are especially interested.

S. 1 A/1

Soil organic matter is in the center of our interest. We try to elucidate the chemical constitution of the different compounds. Some of these substances are low - others are high molecular. We work in a way similar to that used by others for the elucidation of natural substances and high molecular substances. We also use "model substances." Generally model substances are such kinds of substances whose chemical and sometimes also physical properties are very similar to those of the natural products.

Furthermore we endeavor to find out which chemical reactions occur during the alteration of the different organic residues of the organisms and compounds.

The inorganic soil colloids are only of interest to us insofar as there are interactions with the organic components. One of the propositions for investigations of that kind is the knowledge of their structure and of the reactive groups.

One part of the organic components in the soil is decomposed by the micro-organisms; in another one synthesized. According to the work of many authors it can be noted that the largest part of the organic components in the soils is altered by the micro-organisms. Therefore, these are very important for the processes which occur in the soil.

Another important problem is to study the physiological effect of the different organic compounds on the plant growth. This is generally the main task and relates especially to this subject which we call "Soil Biochemistry." It is a very complicated problem. The different disciplines of natural science as chemistry, physics, physical chemistry, physiology, microbiology in some cases, botany, also mineralogy and so on are needed to get results.

To find out the influence of soil organic matter on plant growth we work with fractions of humic substances as well as model substances. We do so because the chemical composition of organic matter in soil is nearly unknown at present. Moreover, it is easier for the first step of investigation to work with substances of well known constitution and behavior of reaction. For a short time we have been working together with the Organic Chemical Institute of the Technical University of Braunschweig to elucidate the chemical constitution of different pigments of streptomycetes. But we are only interested in those strains which are important for our other work, i.e., for the influence of plant metabolism, for the distribution in different cultivated soils and for some other problems.

S. 2 A/2

This scheme is a survey for the alteration and effect of organic matter in soil as far as it gives an idea of our present work. Therefore it is not complete and only some facts are included which are known today. But this survey shows the relationship of the problems which I will discuss.

The residues of plants and animals in the soils consist mainly of carbohydrates and proteins. One of the important substances in the plants is also lignin. The content fluctuates between 10 and 30%.

Proteins and carbohydrates are split up by the enzymes which are in the soil. These enzymes come chiefly from the residues of plants and also from the lower organisms in the soil. If the cellulose is split up more and more, the lignin can be oxidized gradually.

Most of the alterations of the different organic components is caused by the micro-organisms. Some of the products of decomposition or resynthesis are of our special interest, as polyuronides, quinonic metabolism products, amino acids and ammonia fragments or oxidation products of lignin. The humic substances are formed by condensation reactions. In this case we work also with the model substances.

The inorganic components of the soil enter in interaction with the organic ones and form the inorganic-organic complexes of the soil, sometimes called "clay humus complex. These are important for the sorption of ions, aggregation and other physical properties of the soil.

The humic substances themselves can have a direct influence on plant growth. They can be taken up by the plants and cause an alteration of the metabolism of the plants, for instance, acceleration of dehydrogenating processes of the plant metabolism and different other ones.

In the next brief section I will give an introduction to our work which is related to the inorganic part of the soil.

Chiefly we are interested in inorganic soil colloids. During weathering of the minerals and the rocks sesquioxide-hydrates are formed. For instance ferric or aluminum hydroxide are positively charged and flocculate the negatively charged humic acids. When the hydrates are formed, they are amorphous. Gradually they crystallize.

S. 3   6/398

The electron microscopic picture is a sol of aluminumhydroxide, which is three years old. Water has been split out and bayerite has been formed; only a small amount of amorphous material is between the crystals. Amorphous and crystalline particles adsorb different amounts of humic acids. We study in this way the size and shape of inorganic colloids. Perhaps you remember that different kinds of aluminum-oxide with different sorption capacity are produced for chromatography. In this case and also in some cases for the problems of the soil it is necessary to study the hemogenity of the material.

S. 4   6/399

In many cases it is of interest to know the composition of the clay minerals in a soil. I will not go into detail. One of the methods to determine clay minerals is the investigation with the electron microscope. Some clay minerals have a different shape. Kaolinite crystallizes in hexagonal plates, montmorillonite in plates, which can be crumpled, and attapulgite in small needles. The electron microscope is not only useful for the direct observation but also for determinations with electronic difraction.

S. 5   6/401

The OH groups of the clay minerals are important for the interaction with organic substances. It has been possible to identify three OH groups of different energy levels with infra-rédspectography. The three maxima of OH bonds of kaolinte, dickite and halloysite minerals which are not so much different in their structures, have all the same position, but different absorption.

In connection with studies for the special questions of the structure of the clay minerals we investigated other hydroxides as hydrargillite or brucite or other minerals like chrysotile.

On the basis of some of our experiments with soil organic matter, I will tell you how it is possible to come from studies of processes in nature to chemical inferences for the formation of humic substances as well as for their physiological effect on plant growth. At first we tried always to go ahead with the natural substances. If we must stop, we used model subtances. Of course these investigations are not wholly complete.

S. 6 A/13

First I will give you an example for the decomposition of organic material in soil and for the formation of humic substances.

Wheat straw alone has been rotted under conditions which have been as constant as possible in a climate chamber at 27 degrees Centigrade and with a relative air humidity of 90%. A nutrient solution with $NaNO_3$ as N source has been added to the chopped straw.

The organic substance decreases with time. The content of lignin and of the water soluble substances, which contains humic substances, increases in percent of the organic substances. The content of holocellulose decreases rapidly. After 180 days there is no more a noticeable alteration. Later on we will come back to these facts.

S. 7 A/5

Well, we had mentioned that the carbohydrates and the proteins decompose more rapidly than lignin. For this and other reasons we can presume that lignin is important for the formation of humic substances.

The lignin has been isolated. Its elementary composition had been determined. The content of carbon decreases. (410 + N is a special case beyond our consideration.) The content of oxygen decreases at first and then increases again. The most remarkable fact is the alteration of the content of nitrogen and that of methoxyl. The nitrogen content increases with time and the methoxyl content decreases. It seems that there are some chemical reactions.

Phenol-ether is not able to condense with amino compounds. Phenols react very slowly and only under higher temperatures. But quinones can easily react with amino compounds by addition. This table together with the one before confirms that for the formation of humic substances with methoxyl group must be split up, the phenol formed will be oxidized and during the oxidation reaction a condensation with amino compounds will take place. The compounds come from the proteins as ammonia or amino acids.

So much for the studies with natural substances. Now I will talk about some model experiments relating to these results.

Lignin, its building blocks and products of fragmentation are partially methylated phenol ethers. Following the work of which I told you and the work of other authors (V. 1 (4) S'.6) with lignin alone it is proved that the methoxyl content decreases during the decomposition of lignin.

S. 8 6/404

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S. 8 6/404

The products of oxidation of lignin are very reactive. The reactions are complicated. This fact would also be confirmed with different model substances.

Therefore, it has been necessary to work with such kind of model substances, whose products of oxidation could be isolated and their chemical constitution known. Well, we studied the oxidation of 2,3-di-tert.butyl-pyrocatechin and 4,6-di-tert. butyl-pyrogallol. These two compounds correspond to the two demethylated main types of the building blocks of lignin, to the guacyl- and to the syringyl-type. In both cases one of the intermediate oxidation products is dimerized 4,6-di-tert. butyl-o-benzoquinone (VI (5) 56). This compound has been isolated and identified. Furthermore, we found interesting reactions of the chemistry of dimerized quinones; some of them are also natural substances.

The next step in oxidation is a cleavage of the ring to 2,4-di-tert. butyl-4-oxalo-crotonic acid. These kinds of acids split off carbon dioxide very easily. By this reaction one part of carbon dioxide is formed during humification. But there are also different other reactions which form CO_2.

These results express that the oxidation of two different types of lignin, the guacyl- and syringyl-type, forms one kind of oxidation product, a hydroxy-o-benzoquinone. This is an important fact of the mechanism of the formation of humic substances from lignin.

We made further experiments with fragments of lignin as protocatechuic and gallic acid. The first compound can be isolated out of the soil.

The oxidation was carried out under conditions like these in the soils; i.e. pH value 6,5 and oxidation with enzymes, with phenol-oxidases. In the presence of proteins or amino acids we got indeed humic acids, which could not be distinguished from these isolated out of soils.

If the experiments with the tert. butyl-substituted phenols correspond to the reactions in nature, we must find as intermediary step also a hydroxy-o-benzoquinone. In the presence of N-methyl-aniline we have been able to isolate the corresponding compound. On the lower part is a scheme which shows the different possibilities of the structural units of humic acids. Some are complete rings; others are rings which are cleaved. There exist different connections for the nitrogen, some of which are in heterocyclic rings.

Summarizing, it can be noticed that the formation of humic substances depends upon the demethylation of phenol ethers, generally by micro-organisms splitting off the side chains, oxidation of the polyphenols to a hydroxy-o-benzo-quinones and its condensation with amino compounds. The demethylation of the ethers of di-tert. butyl-substituted phenols has until now not been possible. But we succeeded in demethylating acetic-anasidine with phenol oxidases of mushroom in the presence of hydrogenated triphosphopyridine-nucleotide.

To get such results it has been necessary to study more intensively the chemistry of quinones. In addition to other chemical experiments we made also physical-chemical investigations, such as systematic investigations of the ultra-violet spectra. (R/S. 63; 2/S104)

S. 9 2/132

 8

For instance the spectra of the methyl-substituted p-benzoquinones show some regularities for the position of the second maximum between 300 and 340 mμ. The second maximum posses no additive shift with the number of the methyl groups like the first maximum. In comparison with the fragments of lignin, phenols in the planes and quinonic metabolism products of micro-organisms we investigated all possible different combinations of monohydroxymethyl-, monomethoxylmethyl- and methoxyl-substituted and some dimerized benzoquinones. With the same quinones we investigated, the infra-red spectra (unpublished) and the half step potentials (Koelke). Hereby it could be noticed also some regularities.

I believe that these measurements are also necessary in connection with the testing of these substances for their physiological effects on plant growth. For a long time the farmers had had the experience that organic fertilizing had an effect on the yield. Not all of this effect depends upon the correction of the physical conditions of the soil. A part of this must be a direct effect on the metabolism of the plants.

S. 10 8/868

Lignin fragments as protocatechuic acid influence the speed of germination of rye (8/V26 S.3), up to 14%. The effect depends upon the concentration of protocatechuic acids.

S. 11 8/524

A model substance as thymohydroquinone accelerates also the germination and increases the dry weight of rye seedlings (8/V23 S.7).

This substance has been added together with nutrient solutions to sand cultures in the mentioned concentrations. It must be especially noticed that the effect of natural substances like fractions of the humus as well as the model substances can be only observed in nearly all cases in the presence of sufficient inorganic nutrients. Deficiency of inorganic nutrients causes an additional decreasing of the yield. Later on we will discuss more about this point and talk about the reasons for this fact.

Furthermore, it is to remark that the effect depends upon the concentrations of the substances used. High concentrations are toxic, low concentrations have no effect. But the interval between toxic and ineffective is much larger than in the case of inorganic fertilizer. It is two up to three power of ten. That is also an important fact and a remarkable difference between the effect of organic and inorganic substances on plant growth.

A third difference is the fact that the usual inorganic substances, the macronutrients have only an effect on plant growth in relatively large quantities and organic substances in very small amounts comparably with the effect of trace elements. Therefore, the organic substances are no foodstuff for the plants, they are to some extent catalysts in the plant metabolism as likewise the growth-promoting substances. Indeed they alter the plant metabolism.

S. 12 8/869

Not only the primary growth is increased, also the growth during the entire life of the plant. In some cases the yield of grains is increased. The figure is an example for pot experiments (8/V8 S.3).

The increasing of the yield cannot always be measured. The effect of this kind of substances depends from climatic conditions. For this purpose we also made field experiments and could show that one of the reasons for the increased yield is the higher resistance against wilting.

If we observe such results, an alteration in the metabolism of the plants must have taken place.

S. 13   8/551

The metabolism of the plants is regulated by enzymes. In varition of the former experiments I show a figure illustrating how the activities of different enzymes are altered in the presence of humic substances (A/S 102 S.33).

The activity of aldolase in the sprouts of rye seedlings is increased, the activity of amylase decreased in the presence of humic substances in the nutrient solution. The activity of saccharase in the roots is decreased.

All these enzymes are involved in sugar metabolism. We could, therefore, find that the content of reducing sugars increases depending on the concentration of thymohydroquinone and depending on the concentration of humic substances according to other authors.

The sugar metabolism is related to the respiration. Model substances as well as fraction of humic substances increase in certain concentrations the respiration.

S. 14   8/509

We made measurements in the Warburg apparatus with yeast. The consumption of oxygen depends upon the concentration of thymohydroquinone as a model substance. The oxygen consumption in μl/g dryweight yeast/hr. is significantly (8/V21/ abb 4/7).

S. 15   F.448

Christewa made an interesting experiment with Na-humate. Barley plants have been grown on the one hand in water, on the other in Na-humate solution. In the Warburg apparatus the leaves of the treated plants took up 100% more oxygen in 60 minutes than the untreated plants. If one infiltrates the two kinds of plants grown in water and grown in Na-humate solution, then the uptake of oxygen is nearly the same after 60 minutes.

But compared with the first oxygen uptake now the uptake of oxygen is increased 352% in the case of the plants, which have grown in water and only just 100% in the case of the plants, which have been cultivated in Na-humate solution. This experiment shows very clearly that the increased oxygen uptake is caused by the Na-humate.

In connection with the respiration is also the citric acid cycle.

S. 16   8/ 528   (V23/Tab. 5/5)

To enlarge our knowledge of the effect of thymohydroquinone on plant growth we determined different acids of the citric acid cycle as pyruvic, α-ketoglutaric-, citric, malic- and fumaric-acid. In this figure a variation of the experiments is made. Beans have been cultivated 19 days in nutrient solution (Knop) when put in nutrient solution with a concentration 1.5 x 10^{-3} M. of thymohydroquinone, and analyzed. The content of the reducing sugars, pyruvic-, α ketoglutavic-, citric-acid are increased. The content of malic and fumaric acid are decreased.

The increased content of pyruvic acid can be explained by acceleration of the sugar metabolism or an inhibition of its decarboxylation.

The change of the content of the other acids may be explained by an inhibition of the α-ketoglutavic acid oxidase or of the succinodehydrogenase. The effect of malonic acid has been compared with this of thymohydroquinone. Other authors (8/V23/Lit.9 S4) described that malonic acid inhibits also the succinic acid and the malic-acid-dehydrogenase. In contrast to thymohydroquinone malonic acid diminishes the content of reducing sugars and citric acid, and increases the content of malic-acid.

S.17 8/531

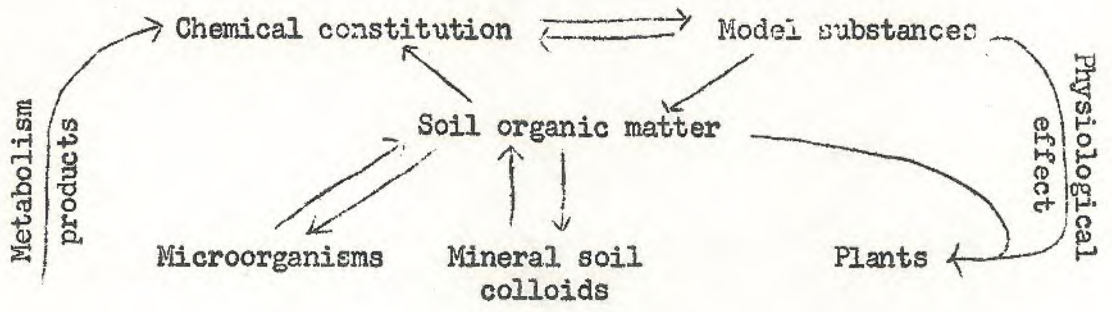
Further experiments have let us presume that these different substances have an effect on central processes as the phosphorylation. Therefore, we investigated the influence of thymohydroquinone on phosphorylation. The decrease of the content of inorganic phosphorus under aerobic and anaerobic conditions is a measure of the phosphorylation. In every case the phosphorylation is decreased by thymohydroquinone. I mentioned before that thymohydroquinone increases the respiration in a concentration of 10^{-5} M. The figure shows that the phosphorylation is also decreased. These two facts give us an idea that thymohydroquinone has a small uncoupling effect on oxidative phosphorylation increases plant growth.

Also in this case we compared thymohydroquinone with substances of known effects, 2,4-dinitrophenol uncouples also the oxidative phosphorylation. Further experiments resulted in interesting relations between the degree of oxidative phosphorylation depending on the concentration and the physiological effect of plant metabolism. Thymohydroquinone, other quinones, β indole acetic acid, 2,4-dichlorophenoxy acetic acid are included in these investigations. Perhaps we will succeed in future to find explanations for the mechanism of growth promoting substances and special compounds of humus. On the one hand it would be no longer be necessary to talk about the dubious "pure humus effect," which is well known to the farmers on the other hand, there may be new possibilities for agriculture.

We made field experiments during the last six years with model substances especially with thymohydroquinone. Generally we can confirm the results of basic work. But there are some other new factors, which will be considered. The effect of the model substances depends upon the climatic conditions as one of the factors and probably in a different way from the inorganic fertilizers.

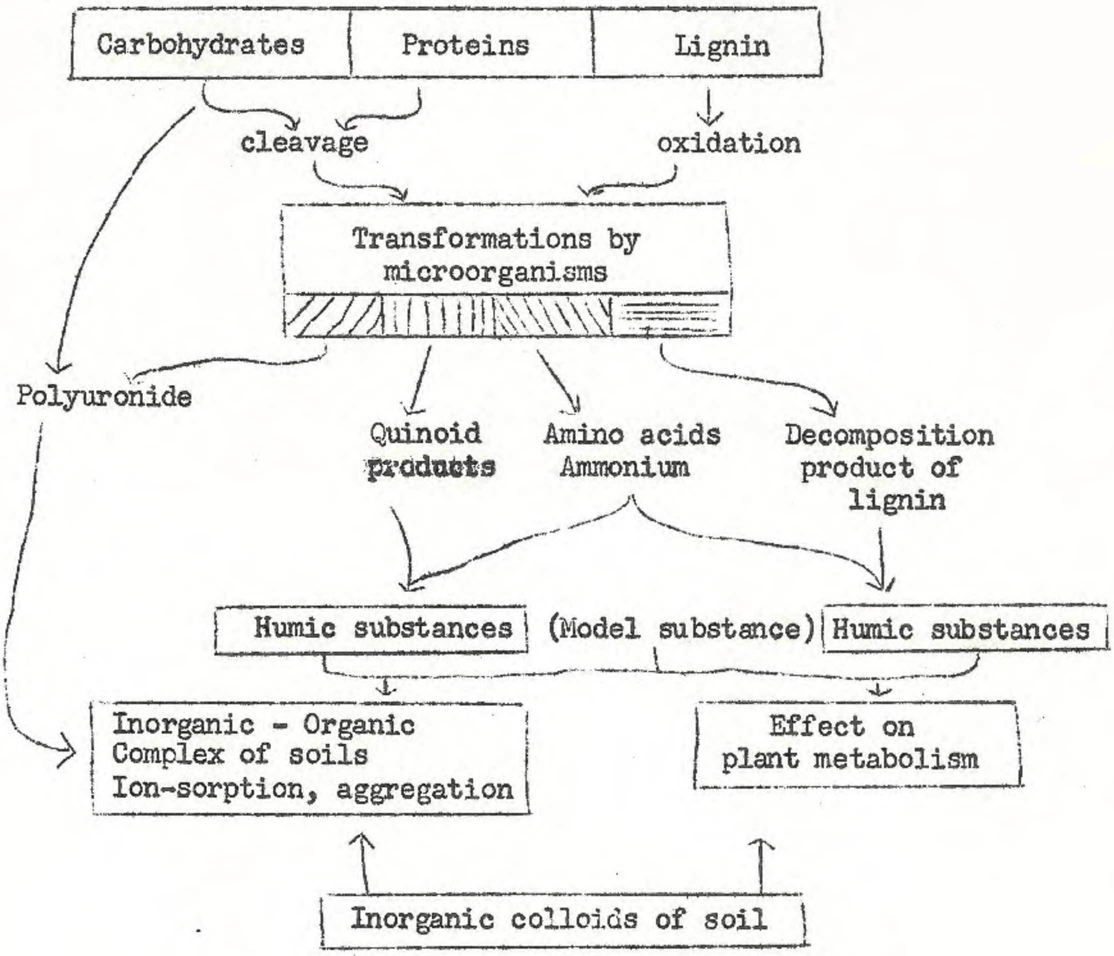
#1

Institute for Soil Biochemistry

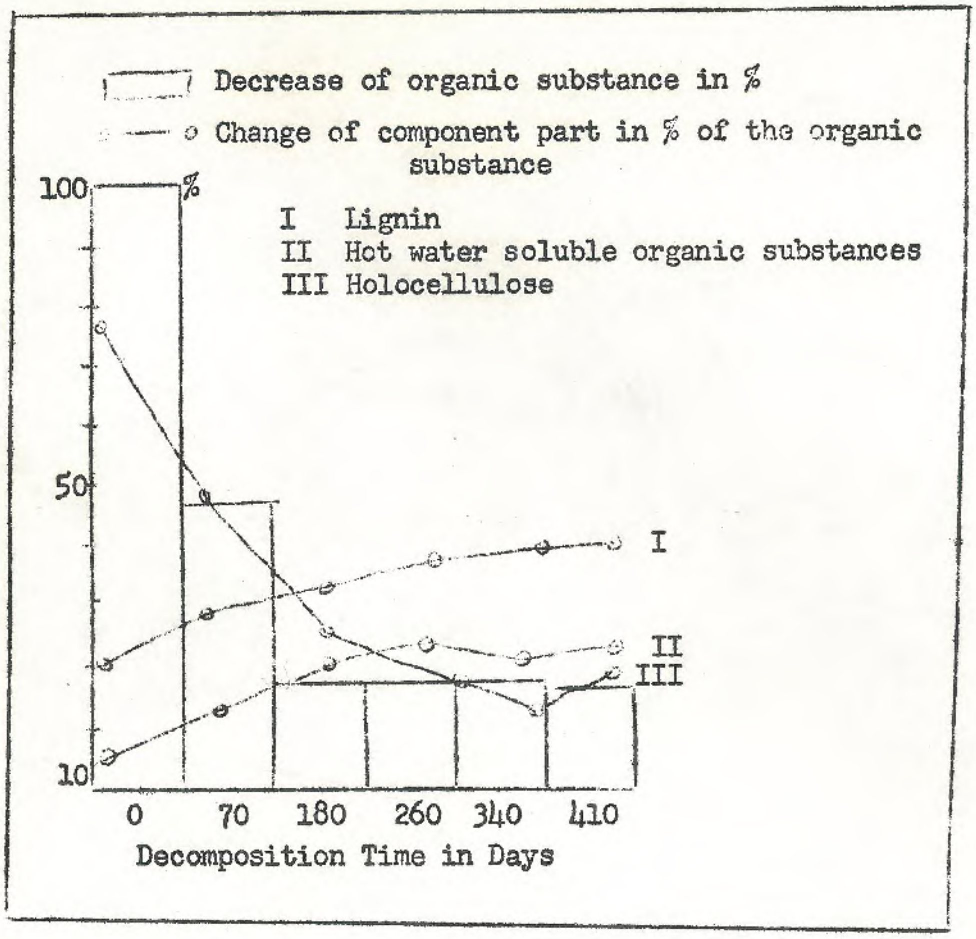


#2

Transformation and Action of Organic Materials in the Soil

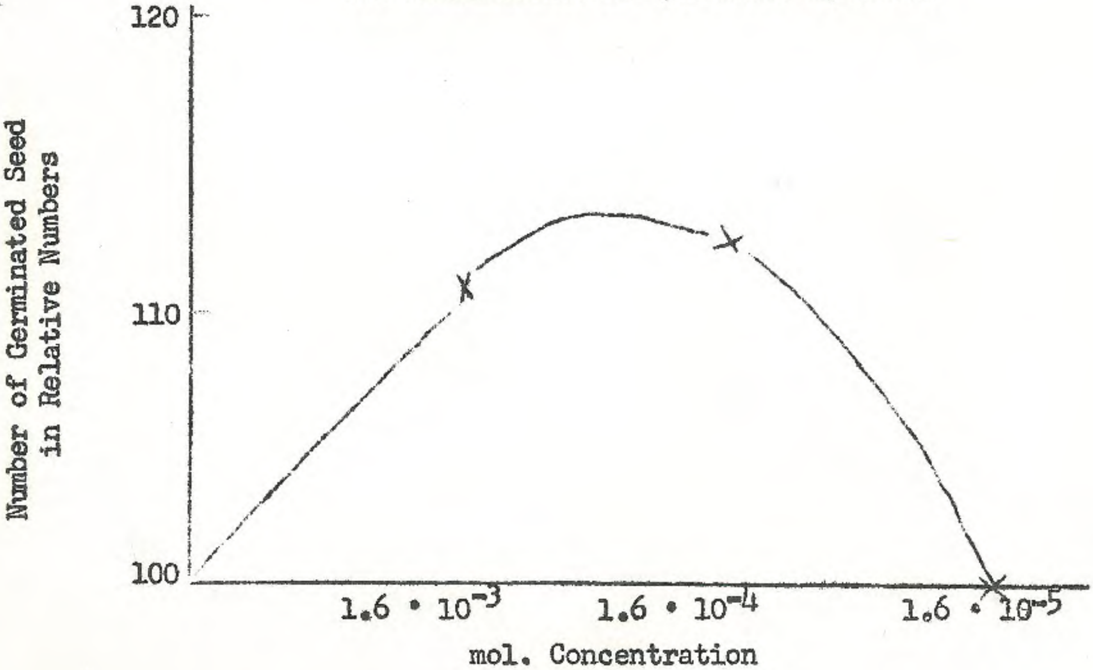


#6



#10

Influence of Protocatechvic Acid on
the Germination of Rye after 14 hours



#7

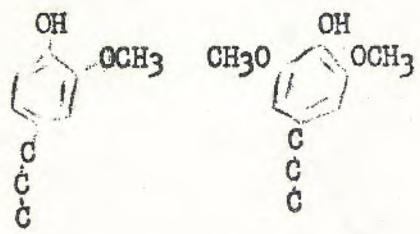
Elemental Analysis of Sulfuric Acid Lignin Isolated after Various Time Periods (calculated on ash free basis)

Rotting Time in Days	% C	% H	% O	% N	% S	% OCH_3
0	58.59	5.60	31.37	0.54	3.9	15.33
70	58.78	5.93	29.48	2.01	3.8	11.14
180	58.67	5.91	29.15	2.37	3.9	9.06
260	58.00	5.83	29.93	3.14	3.9	7.84
340	54.97	5.96	32.09	3.08	3.9	8.57
410	54.29	5.86	32.88	2.97	4.0	7.32
410 + N	56.71	5.58	30.66	2.85	4.2	7.16

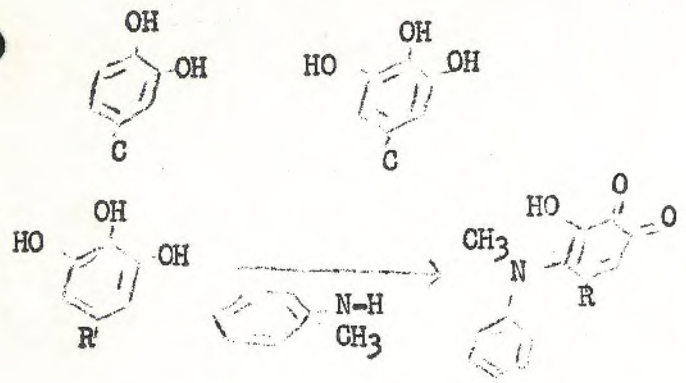
#11 Influence of Thymohydroquinone on the Germination and Growth of Cereals (Relative Values)

Concentration Thymohydroquinone	Number of Germinated Seed after 16 hours		Sprout Dry Weight after 14 days	
	Wheat	Rye	Wheat	Rye
0	100	100	100	100
1.5×10^{-3}m	0	0	57	0
1.5×10^{-4}m	100	89	104	114
1.5×10^{-5}m	123	115	110	128
1.5×10^{-6}m	106	114	104	121

Building Blocks of Lignin



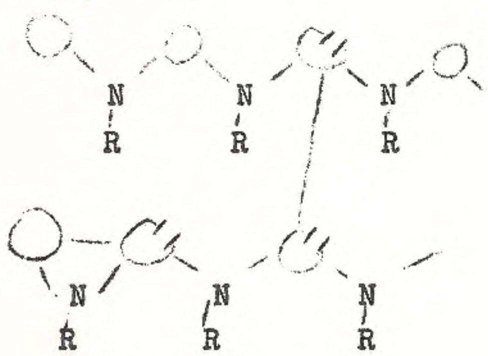
Through Microorganisms
Demethylation and Degradation
of the sidechain



R = COOH for example

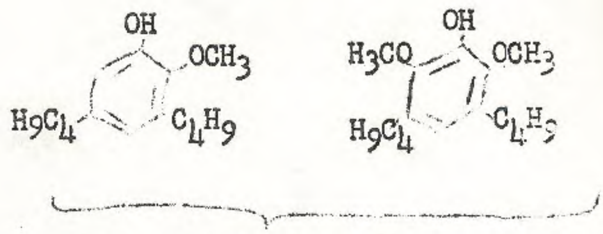
protein

Humic Acid

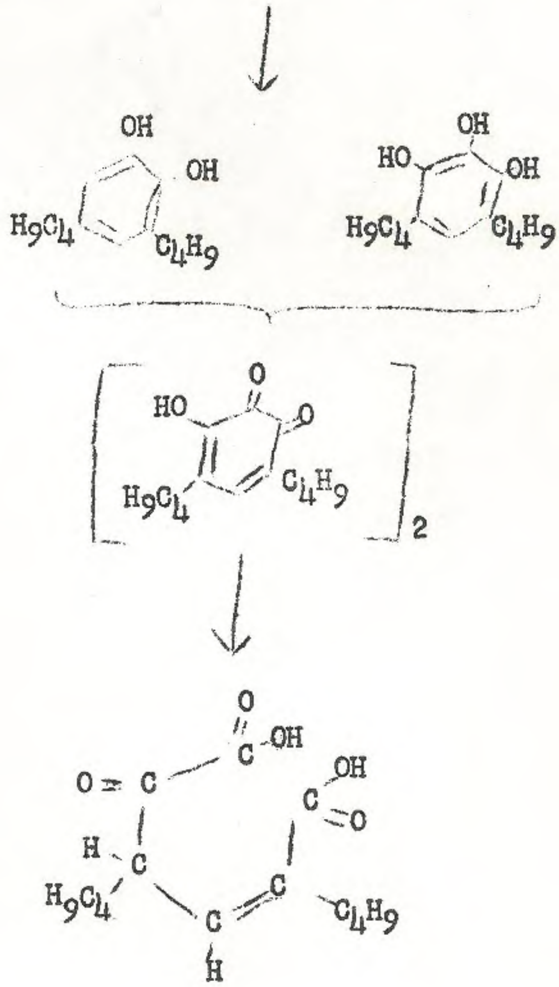


R = H Amino acid and Protein

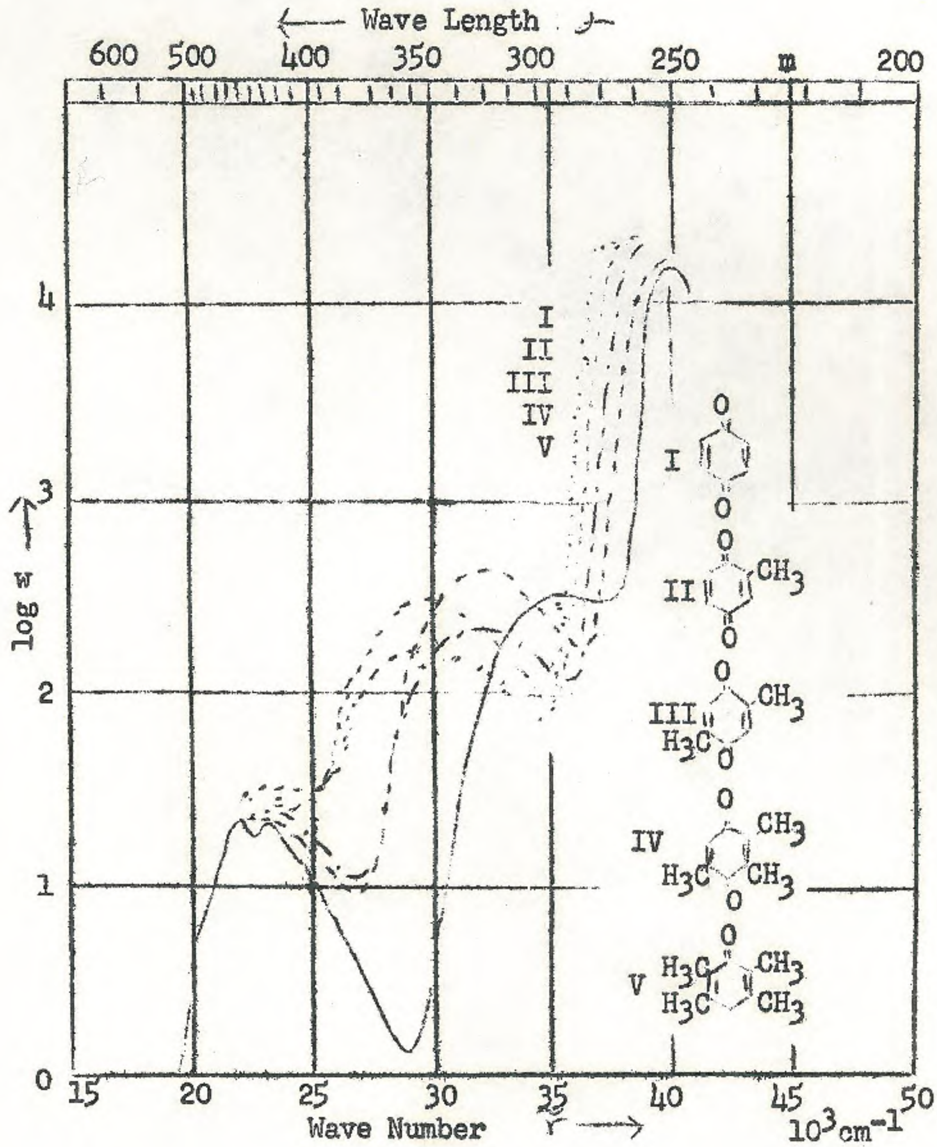
Model Reaction



Till now still not demonstrated

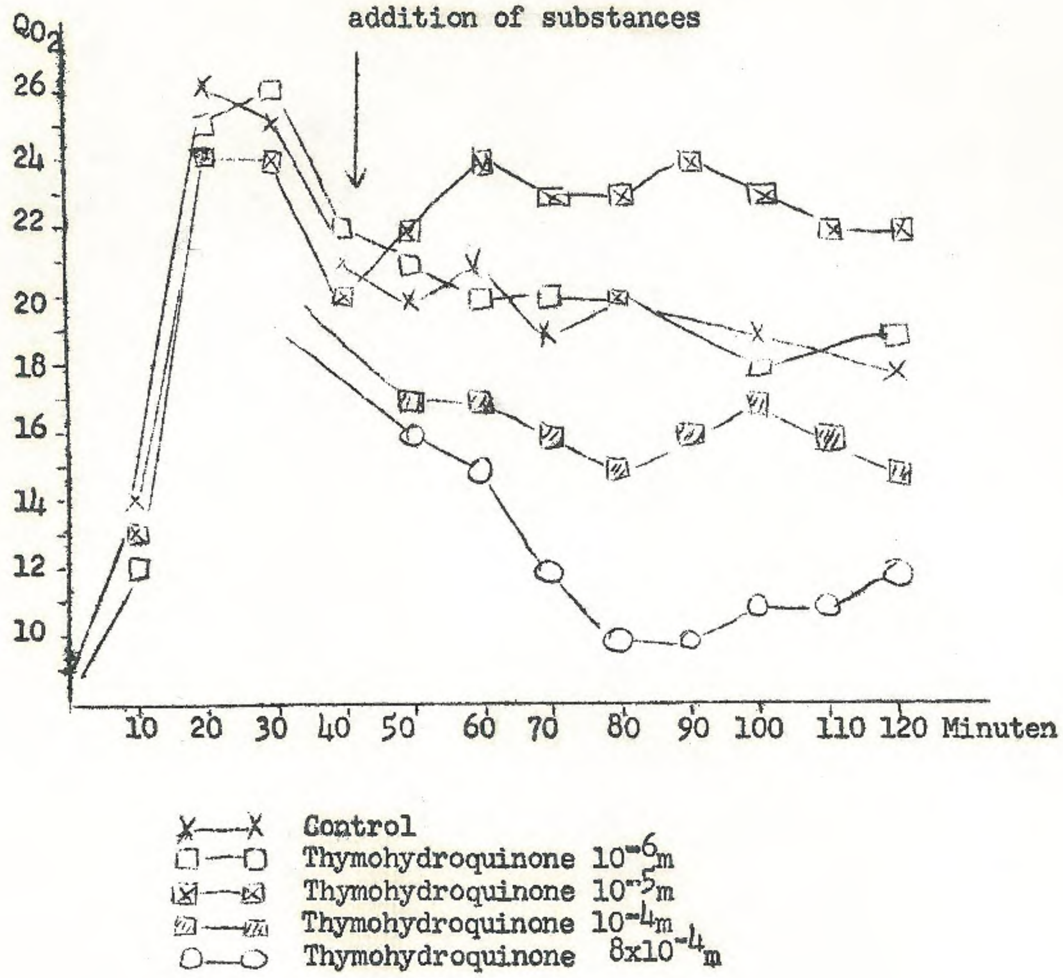


#9


Wave Length
600
500
400
350
300
250
mμ
200
I
II
III
IV
V
log w
4
3
2
1
0
15
20
25
30
35
40
45
50
Wave Number
ν
10³cm⁻¹
CH3
H3C
O

#14

Influence of Thymohydroquinone on Oxygen Uptake of Yeast
(measured in a Warburg)



#12

Influence of Protocatechuic Acid on the Yield of
Summer Rye in Mitscherlich Pots

Substance	Grain Yield		Stover Yield		Root Yield	
10^{-5} Mole	grams	rel	grams	rel	grams	rel
Untreated	24.4±0.74	100	49.3	100	4.42	100
2	25.5±0.54	105	53.1	108	4.65	105
6	27.2±0.48	111	51.4	104	4.48	101
12	26.9±0.75	110	55.6	113	4.38	99

#15

Uptake of Inorganic P under Aerobic and Anaerobic
Conditions in 1% Glucose Solution

		Mg P - Used/g yeast	
		aerobic in 10 min.	anaerobic in 20 min.
Control		1.28	1.15
Thymohydroquinone	10^{-3}m	0.90	0.85
	10^{-4}m	1.03	1.02
	10^{-5}m	1.12	1.01
2-4 Dinitrophenol	10^{-3}m	0.65	0.69
	10^{-4}m	0.85	0.80

#16

Plants Grown in Water Culture
19 day old plants, placed in test solution for 3-4 days. Analysis of the leaf; Relative values calculated on the basis of 100 g of dry substance

Solution	Free Reducing Sugar	Total Hydrazones	Pyruvic Acid	Keto-glutaric acid
Knop	100	100	100	100
Knop + 1.5 x 10^{-3}m Thymohydroquinone	183	131	146	145
Knop + 1.5 x 10^{-3}m Malonic Acid	73	122	144	140

	Citric Acid	Malic Acid	Fumaric Acid 23 day old plants
Knop	100	100	100
Knop + 1.5 x 10^{-3}m Thymohydroquinone	110	92	44
Knop + 1.5 x 10^{-3}m Malonic Acid	73	129	8 Malonic Acid 1.5 x 10^{-2}m

#17

Influence of Sodium Humate on the Oxygen Uptake by Leaves of Barley
By L. A. Christewa

	Oxygen Absorbed in 60 minutes	Oxygen absorbed in 60 min. after addition of Na-Humate	Increase of uptake in %
Water	0.16 ml.	0.68	+352
Na-Humate	0.32 ml.	0.64	+100