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In this manuscript are some unpublished results and therefore only for friends and not to use for publication.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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Participation of Microorganisms in the Formation and Decomposition of Humic Substances

During the lecture it was often mentioned indirectly about the influence of microorganisms on the decomposition and formation of humic substances. At the beginning we discussed the formation of humus depending on the climate. The growth of microorganisms depends also on the climate and therefore, the regularities of the effect of microorganisms on the cycle of humus are given.

We spoke about the influence of the inorganic material of the soil on the formation of humus; we could find a relationship between humus formation and exchange capacity of the inorganic material. These facts can be translated to the effect of the microorganisms during the humus transformation.

In some cases, the physiological activity of phenols or quinones has been mentioned. Also the properties of substances of this kind, which can be intermediate products of the humus formation, must be born in mind.

In one of our last lectures, we have spoken about enzymes in soils. Thereby the conclusion has been made that the amount of enzymes in the soil depends on the living conditions, and therefore, on the amount of microorganisms in the soil.

During our further considerations, all these facts must be born in mind. And if we ask what remains, it can be seen that different biochemical investigations can give further results.

It is a fact that a yearly supply of organic matter to the soil results in a balance of organic carbon in the soil. About the decomposition of proteins and carbohydrates, needs to be spoken no more.

If the decomposition of this part of organic material which is given to the soil is decomposed always to a certain extent, the more or less not available substances for the microorganisms must decompose also. Among this kind of substances there are often mentioned in literature a heterocyclic nitrogen compound. During our discussion about the isolation of well defined compounds, which can be isolated from the soil, we mentioned also different heterocyclic nitrogen compounds.

To find out the possibilities of the decomposition of different types of nitrogen-heterocyclic compounds we made some investigations.

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We worked with proactinomycetes, mycobacteria, streptomycetes and penicillium. The organisms had been isolated from a garden soil with nicotine, pyridine, or humic acids. The work has been done with a hole test. Into the holes there had been put diluted aqueous solution of the different heterocycles as nitrogen- and carbon-source. First diagram shows the results of experiments with Proactinomyces citreus. The heterocycles had been given without sugar. Nicotine (1) can be used. One of its building blocks pyridine (2) will not be decomposed. The hydration product of pyridine, piperidine (3) as well as the partial hydrated derivative, arecolin (4) are decomposed to a certain extent. The growth on the derivatives of pyridine such as nicotinic acid (5) and pyridyl-carbonol (6) is relatively good.

Also the condensation products of pyridine with a benzene ring such as quinolin (7) and quinaldine (8) are not available for the microorganisms as a carbon and nitrogen source. Kynurenic acid (9) is only a little bit available.

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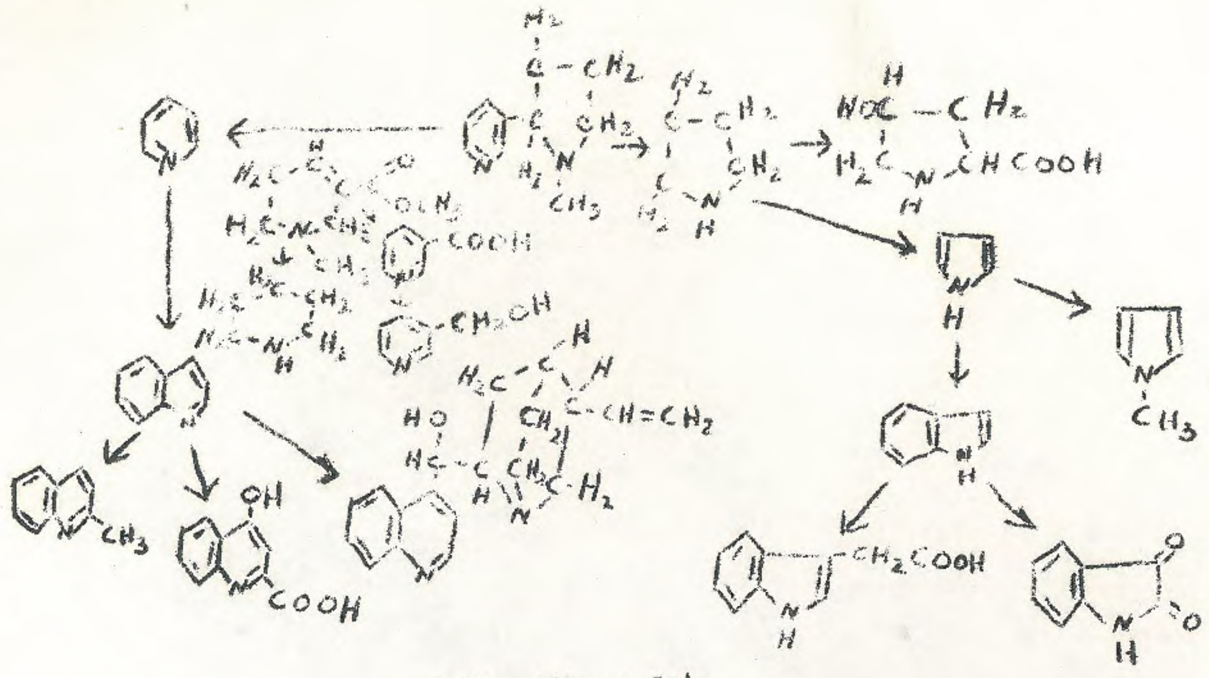
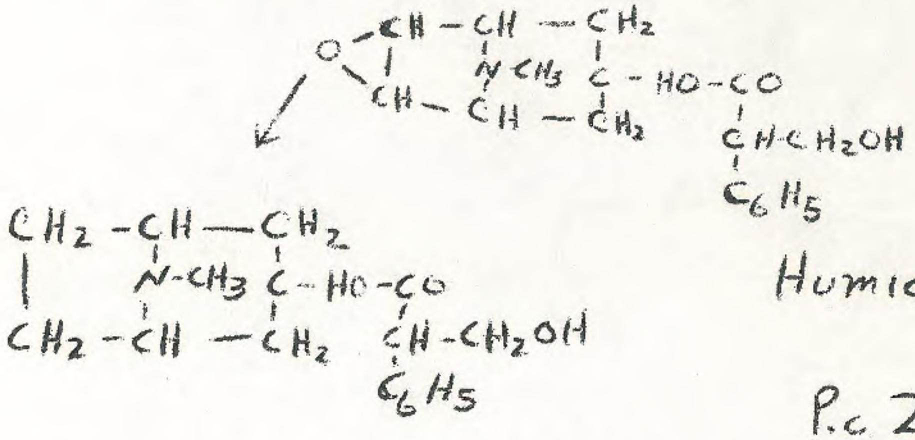
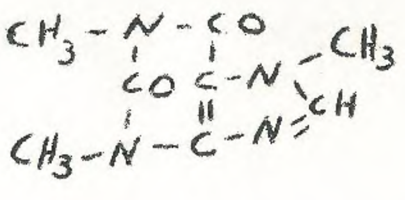




Humic Acid

P.c 2

ohne Glukose



Wachstum von Proactinomyces citr. auf Heterocyclen ohne Glucose

The compounds of the group of quinine such as quinine (10), quinidine (11) and cinchonidine (12) which consists of two ring systems from which one is comparable with piperidine and the other with quinoline. The growth of micro-organisms is not very good.

In another group of substances, which are comparable with piperidine is that of atropin, scopolamine (13), atropine (14). In this case also the piperidine, pyrolidine system is present. The availability of this compound is not very high.

The other part of the molecule of nicotine corresponds to pyrrolidine (15) which allows a relatively good development of the microorganisms. Hydroxyproline (16) gives only a weak growth. Pyrrol (17) and its derivatives such as N-methyl-pyrrol (18) are not decomposed by the microorganisms. Neither indole (19) nor isatine (20) are decomposed. Indole-3-acetic acid (21) is slightly available. Caffeine (22) effects a good growth. On humic acid as nitrogen- and carbon-source no development of microorganisms could be observed.

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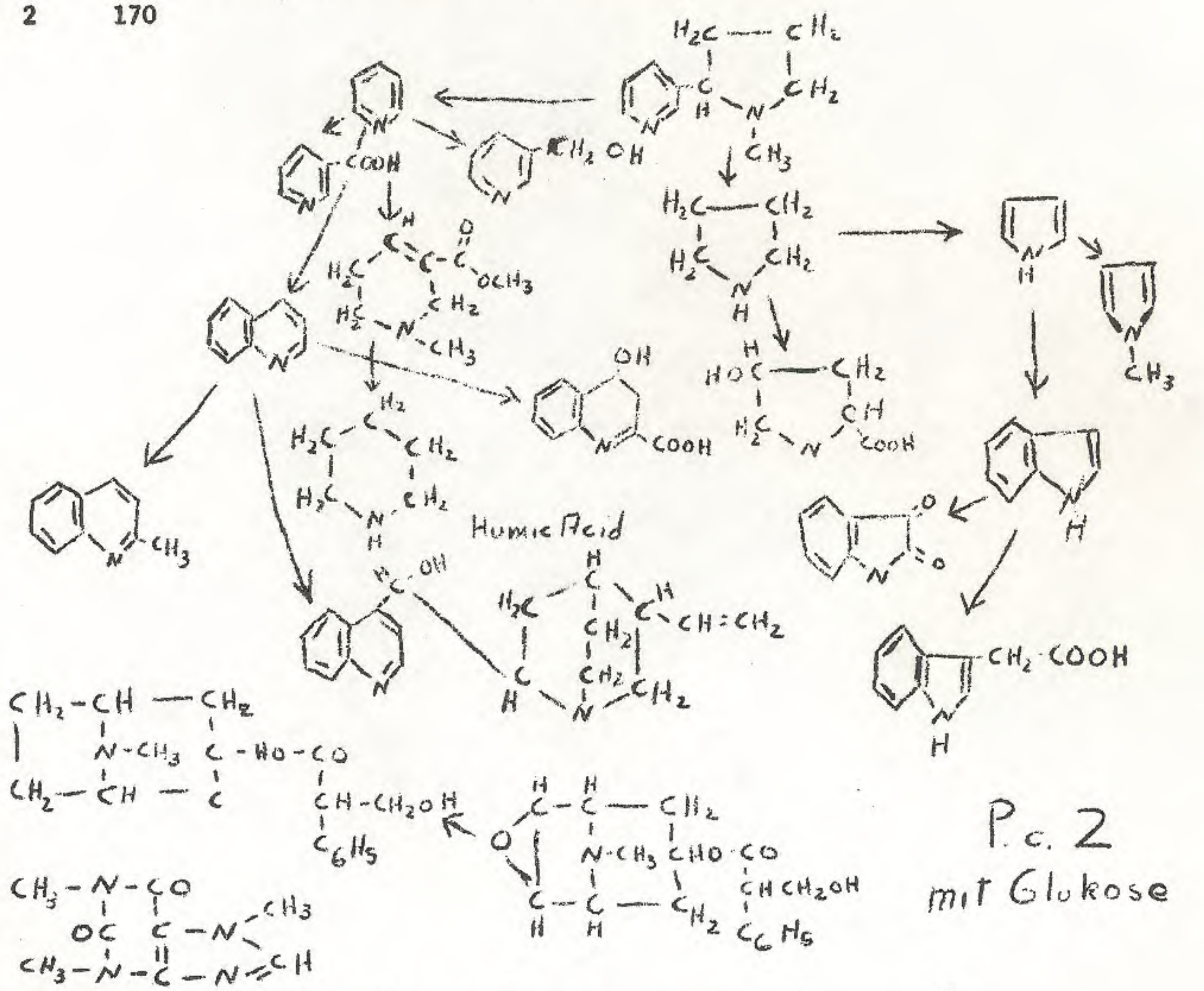
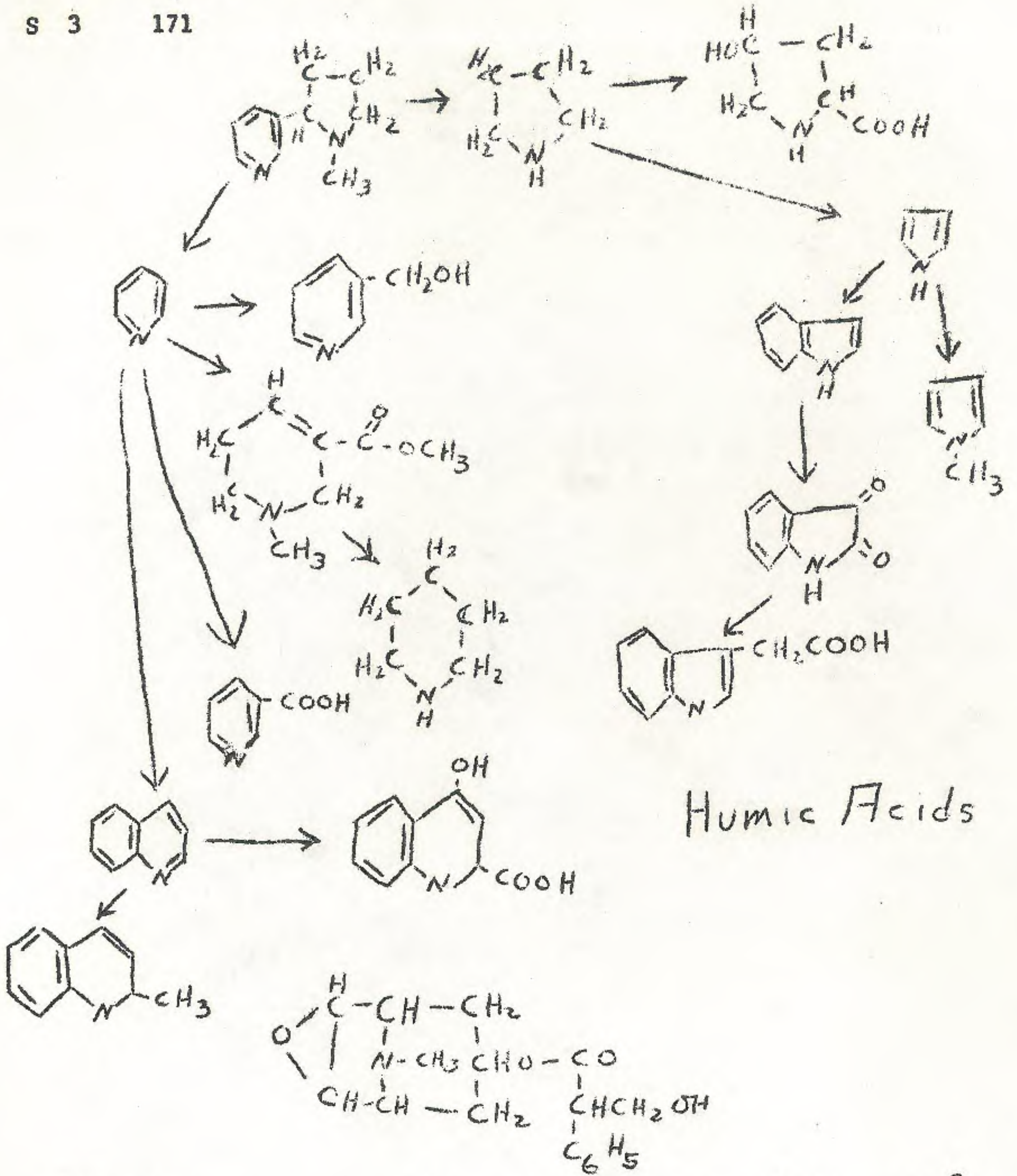


Wachstum von Proactinomyces citr. auf Heterocyclen mit Glucose

In this case the different heterocyclic compounds have been given only as nitrogen source. Glucose had been added as a carbon source. In general the growth of proactinomycetes has increases and the substances come more to the middle of the table. This means that most compounds can serve as nitrogen source, but not as carbon source.

S 3 171



Wachstum von Mycobakterien spez. auf Heterocyclen ohne Glucose

In this case has been used nearly the same substances, but another microorganism has been cultured, a myobacteria spec. In this case also, the hydrated rings with 6 or 5 atoms are more available for the microorganisms.

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Substanz		ohne glukose	mit glukose
Nikotin	0.2%	-	/
Pyrrolidin	0.2%	-	-
Pyrrol	0.1%	/	+
	0.2%	+	+
	0.3%	+	+
N-methylpyrrol	0.1%	/	/
	0.2%	+	+
	0.3%	/	+
Indol	0.2%	-	-
Isatin	0.5%	/	+
	0.1%	/	+
	0.2%	/	/
Pyridin	0.1%	-	-
	0.2%	/	/
	0.4%	/	/
Piperidin	0.1%	/	-
	0.2%	-	-
	0.4%	-	-
Chinolin	0.1%	-	-
	0.2%	-	-
Coffein	0.05%	/	+
	0.1%	/	+
	0.2%	/	+
Huminsaure	0.03%	+	++
	0.06%	+	++

Wachstum von Penicillium Stamm H 2 auf Heterocyclen

In another case the growth of different microorganisms, of penicillium has been investigated. These strains which grow on humic acid preparations seem to use dehydrated 5-ring compounds, as pyrrogallol and its derivatives.

In the case of streptomycetes the growth had not been influenced very much by addition of heterocycles to the nutrient solution and they cannot be used as carbon or nitrogen source.

The availability of purified humic acids as nitrogen- or carbon-source has been investigated in the case of different strains of penicillium which have been isolated from peat. It could be shown that the humic acids could not serve as carbon source whether unsterilized or sterilized.

The experiments show that humic acids can be used by this fungus only in a small amount as nitrogen source. During these experiments, it could be excluded that the nitrogen of the air could be obtained.

Further investigations showed, that the weight of mycellium alone is no significant measure for the availability of nitrogen of humic acids. The nitrogen content of the mycellium must be determined because the nitrogen content of the mycellium is different according to the nitrogen nutrition of the fungi.

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In this slide, the growth of different definite strains of penicillium are shown, influenced by different amounts of humic acid.

The low availability of the nitrogen of the humic acids caused a state of hunger. This could be observed by a submersed growth and the formation of fat in the cells. An adaption to the humic acids as a nitrogen source could not be found.

The growth of fungi on humic acids as nitrogen source, when sterilized in an autoclave, has been better. A possible hydrolytic splitting up of the polypeptides in the molecule of humic acids is assumed. Therefore, the amino nitrogen can be taken up.

A stimulation effect on the growth of microorganisms in the same concentration as had been used as nutrient could not be observed. In low concentrations they have a certain stimulation effect on the growth. The apparent increase of growth, determined by higher weights of mycellium, is caused by the fact that the humic acids are absorbed on the mycellium.

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By addition of humic acids to a nutrient solution according to Czapek, the development of the colonies of Penicillium chrysogenum is decreased in higher concentration but an increasing of the formation of penicillin could be observed.

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Einfluss von Huminsauren auf die antibiotische Aktivitat von Pen. chrysogenum (Hemmzonen in Millimeter)

Bakterienkultur	Pilzatamm	Ohne Humin-saure	0.1% Humin-saure	1.0% Humin-saure
Bac. mycoides	2300/1	1.0	3.0	3.0
	2	1.0	1.5	3.0
	3	1.0	2.0	3.0
	4	0.5	2.0	3.0
	5	1.5	2.5	3.0
	6	1.0	2.0	2.5
	7	2.0	2.0	2.5
Bac. subtilis	2300/1	1.0	1.5	2.0
	2	0.5	1.5	1.0
	3	-	1.0	2.0
	4	0.5	1.0	2.5
	5	0.5	1.0	2.0
	6	-	1.0	2.0
	7	-	1.0	2.5
Staph. aureus	2300/1	1.0	8.0	1.0
	2	5.0	10.0	10.5
	3	2.5	3.0	3.0
	4	3.0	4.5	3.0
	5	3.0	4.5	3.5
	6	3.0	4.5	2.0
	7	6.5	7.5	4.0

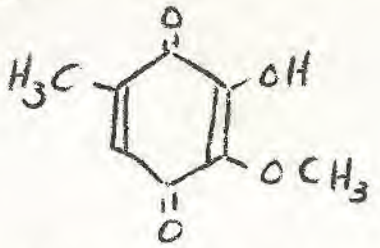
In some cases the antibiotic activity is more than 3-fold. According to the investigations of other authors the inhibition of the velocity of growing increased the formation of penicillin. (Foster, J. W.: Biochemical Activities of Fungi. New York: Academic Press, Inc. Publishers, 1949).

In the next section we will speak about the formation of humic acids in culture media. This fact has been observed by many investigators. (Laatsch, W., Bauer, I., und Bieneck, G.: Die Bildungs-weisen der Huminsauren. Landwirtsch. Forschung 2, 38 (1950) - Laatsch, W., Hoops, L., und Bauer, I.: Uber Huminsauren mit Aminostickstoff. Z. Pflanzenernahr., Dung., Bodenkunde 53, 20 (1951) - Pochon, I., und Wang, T. L.: Bacteriologie, Fromation de substances humiques par les Azotobacter a partir des noyaux benzeniques. C. R. Acad. Sci. 230, 151 (1950) - Scheffer, F., Plotho, O. V., und Welte, E.: Untersuchungen uber die Bildung von Humusstoffen durch Actinomyceten. Landw. Forschung 1, 81 (1950) - Scheffer, F: Humus und dessen Bildung durch die Mikroorganismen. Trans. VI. Intern. Congr. Microbiol. 6, 265 (1953) - Plotho, O. V.: Die Humusbildung der Mikroorganismen. Z. Pflanzenernahr., Dung., Bodenkunde 51, 212 (1950) - Flaig, W., Kuster, E., Segler-Holzweissig, G., und Beutelspacher, H.: Zur Kenntnis der Huminsauren. V. Uber die Bildung von huminsaureahnlichen Stoffen aus Streptomyceten-Kulturen. Z. Pflanzenernahr Dung., Bodenkunde 57, 42 (1952) - Kuster, E.: Umwandlung von Mikroorganismen-Farbstoffen in Humusstoffe. Z. Pflanzenernahr., Dung., Bodenkunde 57, 51 (1952).

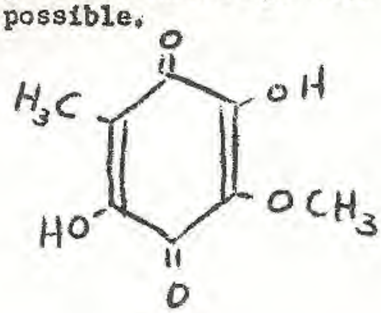
At first we made investigations with such kind of microorganisms, from which the metabolism products have been known and have been quinones. We investigated the formation of the brown dark products out of the known quinones, which are formed as metabolism products. (Kuster, E.: Mikrobielle Polyphenoloxydasen und Humusbildung Zbl. Bakt. I. Orig. 160, 207 (1953)).

S 8 864 (Slide cannot be reproduced on stencil)

During these investigations we determined the phenol oxidases with the tannin reactions according to Bavendamm. (Bavendamm, W.: Uber das Vorkommen und den Nachweis von Oxydasen bei holzzerstorenden Pflanzen. Z. Pflanzenkrankheiten u. Pflanzenschutz 38, 257 (1928)).



In the case of Aspergillus fumigatus, which produces fumigatin as metabolism product, the formation of humus-like substances has been found. The tannin reaction has been positive. According to our knowledge with model substances the formation of condensation products is possible.



In the case of Penicillium spinulosum which produces spinulosin as a metabolism product, neither the formation of humus-like substances could be observed nor the tannin reaction has been possible. Spinulosin as a 2,5-di-hydroxy-p-benzoquinone is very stable and gives no condensation product.

We have made similar observations with different species of streptomycetes. (Kuster, E.: Humusbildung und Phenoloxydasen bei Streptomycetin. Z. Pflanzenernahr., Dung., Bodenkunde 69, 137 (1955)).

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For instance, on doing systematic work on comparatively comprehensive assortment of streptomycetes we could establish that some strains colored glucose-peptone-agar not at all, or only a little. But the same strains produce an intensively dark color in glycerine- glycine- solution. From this reason using tyrosine agar we tested different amino acids as well as different sugars as additional nitrogen- and carbon-sources. Almost every strain needs a certain combination of nitrogen- and carbon-sources to produce an intensive staining on tyrosine agar. Other combination only showed a little stain or it did not occur at all in spite of the presence of tyrosine.

The more intensive the stain was, the higher was the activity of phenol oxidase. Formation of brown colored humic acid-like substances is dependent on the intensity of activity of phenoloxydase.

This fact lets us conclude that humic substances are formed also in this case during one or the other step by an aromatic compound. The aromatic compounds may originate from aromatic amino acids such as tyrosine, phenylalanine or tryptophane, which are needed by microorganisms to form organic peptides.

A further possibility is the formation of aromatic compounds out of carbohydrates or the degradation product. For this hypothesis, S. A. Brown has given reasons on shikimic acid and Grisebach on the condensation of acetyl groups from acetyl-coenzyme A. (Brown, S. A., und Neish, A. C.: Shikimic acid as a precursor in Lignin biosynthesis. Nature 175, 688 (1955) - Grisebach, H.: Zur Biogenese des Cyanidins. I. Versuche mit Acetat- (1-^{14}C) und (2-^{14} C). Z. Naturforsch. 12b, 227 (1957)).

Following this experiment and the experience with model substances it is convincing that the formation of humic acid needs at least in the first steps aromatic compounds. These considerations must be supported by further experiments. This is one of the trials to find out if all possibilities of reactions take place which we discussed talking about degradation products of lignin, phenols or quinones in plants and microorganisms and the corresponding model substances.

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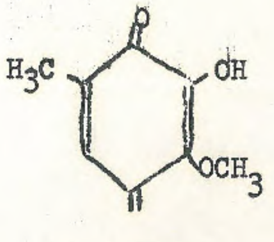
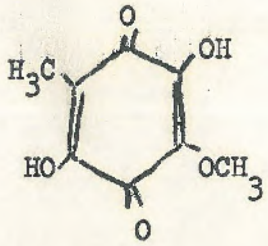


Fumigatin



Spinulosin

Stramm	C-N Kombination	E Farbung 500 m/	Phenoloxydasen-Aktivität Filtrat	Mysel	Ges Aktivitat
G59	Glucose-Tryptopan	1.05	84.5	154.3	238.8
	Maltose-Tryptopan	0.37	-	70.7	70.7
K1034	Mannit-Lysin	0.222	53.6	nicht	gem. 53.6
	Mannit-Phenylalamin	0.075	13.0	"	" 13.0