

Lectures on
Soil Organic Matter

by

Prof. Dr. W. Flaig*
Visiting Professor

March, April, May 1959

Department of Agronomy
Iowa State College
Ames, Iowa, U. S. A.

Business address:

* Direktor de Institut für Biochemie
des Bodens Forschungsanstalt für
Landwirtschaft, Braunschweig-Volkenrode

Home address:

Braunschweig, Bundessallee 50
Germany

INSTITUT FÜR PFLANZENERNÄHRUNG UND BODENKUNDE
Bundesforschungsanstalt für Landwirtschaft (FAL)
Bundessallee 50 D-3300 Braunschweig

1 634
Bod

21/B 1352

Bundesforschungsanstalt
für Landwirtschaft
Braunschweig-Volkenrode (FAL)
ZENTRALBÜCHEREI

94/660

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In this manuscript are some unpublished results and therefore only for friends and not to use for publication.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

Table of Contents

1. Introduction to the work of the Institute for Soil Biochemistry F.A.L. Braunschweig-Volkenrode	7 pp. & 17 ill.	
2. Historical survey of humus research	8 pp. & biblio.	
3. Humic substances and plant growth in general	25 pp. & biblio.	
4. Considerations in the decomposition of organic matter	23 pp.	
5. The decomposition of known compounds of plant residues and isolation of compounds with definite chemical constitution	19 pp.	
6. Some facts of lignin chemistry	18 pp.	131
7. Chemistry of quinones in so far as they concern chemistry of humic substances	83 pp.	150
8. Enzymes in soil	8 pp.	241
9. Chemistry of fulvic and of humic acids	43 pp. & biblio.	250
10. Experiments of the formation of synthetic humic acids	6 pp.	297
11. Physical chemistry of humic acids	19 pp. & biblio.	303
12. Participation of microorganisms in the formation and decomposition of humic substances	9 pp.	
13. The influence of soil organic matter on plant growth	18 pp.	
14. Physiological effect on plant growth	7 pp. & biblio.	

Chemistry of Fulvic and of Humic Acids

Before discussing the chemistry of fulvic acids and humic acids, there must be made some general considerations. A large number of investigations have been made concerning these problems. But after we know what are the possible reactions of the building blocks of these substances and how further reactions can occur, it is not very difficult to extract all the different papers published till now to find out which facts remain of interest.

By further consideration we know that it will not be possible to find the same humic acids or fulvic acids isolated from different natural sources. This concerns the chemical constitution and more or less also the physical behavior. We can only determine average values.

To study natural humic acids to get ideas about their composition there are three promising ways of procedure:

1. To follow up the way of their formation as much as possible.
 2. To carry out the destruction reactions and reconstruct the constitution by the found compounds.
 3. To build up substances which have chemical and physical properties similar as well as the natural products.
- 1) Chemical investigations about the formation of humic substances out of plant residues.

One of the important works about this point was done at first by Waksman. (Humus, Origin, Chemical Composition, and Importance in Nature. Baltimore, The Williams & Wilkins Company, 1936. The Method of Proximate Analysis and its Application to the Study of Plant Residues, Composts, and Humus Formations. Verh. Kommiss. int. Boden-kundl. Ges. 1939, 3. Kommiss.-Ber. A. 101-119 (1930)). For this purpose, only a repetition shall be given because the method of proximate analysis to the study of plant residues and humus formation is the subject of the lecture for beginners. During our next considerations you must remember and bear in mind especially all the facts about

- a) humus formation as related to soil and climate and the microbial activity caused by these factors,
- b) the different decomposition rate of the compounds which exist in plant and microorganisms,
- c) the reactions of the pure compounds with chemical relationship to the natural especially the oxidation and the possibilities of condensation.

The most important fact of the work of Waksman is the determination of the increase or decrease of the different compounds of plant residues during the decomposition, for instance:

slide 60

Abbau der verschiedenen organischen Stoffgruppen von Maisstroh (ohne Zufugung von Nahrtsalzen).

Organische Stoffgruppen	Nach...Tagen verblieb an Zersetzungsrickstanden									
	Urspr.	27 Tage			68 Tage		205 Tage		405 Tage	
	Masse in g	Ruck- stand in g	in % d. urspr Masse	Ruck- stand in g	in % d. urspr Masse	Ruck- stand in g	in % d. urspr Masse	Ruck- stand in g	in % d. urspr Masse	Ruck- stand in g
Gesamt-Trockenmasse	203,0	129,0	63,5	92,5	45,6	59,5	29,3	41,2	20,3	
Aetherloslicher Anteil in kaltem Wasser losliche organische Bestandteile	3,7	2,5	70	0,9	24	0,5	13	0,14	3,8	
In heissem Wasser los- liche organische Be- standteile	21,5	4,4	20,3	5,3	24,7	1,7	7,8	1,8	8,2	
Hemizellulosen	7,2	3,2	45	2,8	39	2,5	34	3,8	52	
Zellulosen	35,8	21,1	58,9	14,7	41,2	9,1	25,5	4,4	12	
Lignin	60,2	34,0	56,4	20,8	34,6	8,0	13,2	2,0	3,3	
Rohprotein	22,9	23,6	103	18,2	79,4	14,0	60,9	9,8	43	
	4,0	5,6	141	4,3	108	5,4	134	5,3	133	

Waksman gives the analysis of maize straw as follows: He distinguished

1. Part of the plants soluble in cold water; these are mainly sugar- and amino acids.
2. Parts which are soluble in hot water, such as starch, pectins, tannins, and eventually uric acid.
3. By hydrolysis with hot dilute mineral acid and by the determination of the reducing sugars, the hemicelluloses are found.
4. By hydrolysis with cold 80% sulfuric acid and following heating after dilution during several hours the celluloses are determined.
5. By the insolubility in this sulfuric acid the lignins are determined, whereby the content of ash and nitrogen must be borne in mind.
6. The fats and the waxes are extracted with ether.
7. Crude protein is determined.
8. Determination of the alcohol soluble parts.

The crude protein is determined by the determination of nitrogen according to Kjeldahl. The observed content of nitrogen is multiplied by the factor 6.25.

Straw of maize cut in little pieces and inoculated with a soil infusion is incubated under optimal conditions of moisture and temperature of 25° to 27° C. A weak nutrient solution is added with diammonium phosphate. After certain times samples are taken and analyzed. After 14 months the dark colored residues look like humus formed in the soil.

The products consist according to the last column mainly of lignin of the maize straw, of protein, and of other complex nitrogen components. The amount of these increased with the activity of the microorganisms. Furthermore, there was hemicellulose which can be derived from plants as well as microorganisms.

The remainder of the other groups of substances such as cellulose, ether and cold water soluble parts is also present. Waksman explains the relative stability of these substances which are soluble in hot water by the increase of substances newly formed by microorganisms. If one calculates the change of the groups of substances in percent between beginning and end, i.e., related to 203.0 gram of the original amount and to 41.2 gram total remaining after 405 days, respectively, the following changes in percent are given (i.e. 203 gram and 41 gram equals 100 in every case).

The ether soluble part decreases from 1.8 to 0.3 percent.

The cold water soluble organic part decreases also from 10.6 to 4.63 percent.

In contrast the hot water soluble organic parts increase from 3.6 to 9.3 percent.

The hemicellulose decreases from 70.6 to 10.4 percent.

The cellulose decreases from 29.6 to 4.8 percent.

Lignin increases from 11.3 to 24.6 percent. In this group of substances there are also the newly formed humic substances.

The crude protein increases from 2.6 to 13.6 percent. This fact can be explained with a relatively high increase of microorganisms.

In average, the different organic initial materials are decomposed in the following way:

The water soluble parts are at first decomposed by the microorganisms and then the pentosans, the hemicellulose and later on the cellulose.

In spite of this sequence of decomposition and in spite of the higher, absolute initial content of cellulose, this disappears faster and more complete than the hemicellulose. Waksman explained this fact in this way: that the pentosans of the hemicellulose are attacked faster than the cellulose, but other hemicelluloses such as galactosans and mannosans are more resistant against decomposition.

Special kinds of hemicelluloses are revealed by the increasing activity of the microorganisms in form of slimy substances of the bodies of bacteria and in form of mycelia of fungi. Lignin is rather stable and the percent increases more and more during decomposition.

The proteins of the plants decompose relatively fast. The synthesis of the microbial proteins depends on the nitrogen which comes from the proteins of the plants. Generally every decomposition of organic compounds of plant and animal origin is influenced by the following main factors:

1. The chemical composition of the initial material. In the case of plant residues the decomposition depends on the age, kind and former condition of nutrition of the initial plants.
2. The presence of adequate amounts of nitrogen, which is necessary for the decomposition products by the activity of the microorganisms. In the case of plant residues, rich in nitrogen, such as young plants, or leguminous plants, the humification is much faster than in the case of plants poor in nitrogen as straw. The deficiency of nitrogen retards the decomposition.
3. Kind and amount of microorganisms which take part in the decomposition.
4. Some physical conditions during humification. That is mainly the amount of air, oxygen, respectively, the content of water, the temperature, the pH value, and the inorganic compounds of the soil.

Experiments of such kind explain the main facts but many questions are open. With the analysis only 2/3 of the remainder are known, i.e., only 27.25 gram of 41.2 gram total remainder = 66%. It is not known what composition the missing 33% has. Furthermore one must bear in mind that with other plant residues or under other conditions the decomposition follows another way.

Springer uses a modified method. (compare) (Springer, U.: Der heutige Stand der Humus-Untersuchungsmethodik. Z. Pflanzenern., Dung., Bodenkunde 6, 312 (1938); Springer, U.: Über Typen der echten Humusstoffe, ihre Charakterisierung, Trennung und Bestimmung im Boden. Der Forschungsdienst, Sonderheft 7 (1938); Springer, U., und Wagner, A.: Zur Kenntnis der von Pilzen gebildeten huminsäureähnlichen Substanzen unter besonderer Berücksichtigung von *Stachybotris lobulata*. A. Pflanzenbau, Pflanzenschutz 3, 145 (1952); Springer, U.: Farbtiefe und Farbcharakter von Humus-extrakten in ihrer Abhängigkeit von der Alkalikonzentration, zugleich ein Beitrag zur Kenntnis der Humustypen. Z. Pflanzenern., Dung., Bodenkunde, 34, 1 (1934); Lehner, A., und Springer, U.: Über den Stoffabbau und Humusaufbau bei der Zersetzung von Stroh unter dem Einfluss verschiedener N-Formen. Pflanzenbau und Pflanzenschutz 1, 97 (1950); Springer, U., und Lehner, A.: Stoffabbau und Humusaufbau bei der aeroben und anaeroben Zersetzung landwirtschaftlich und forstwirtschaftlich wichtiger organischer Stoffe. I. Z. Pflanzenernähr., Dung., Bodenkunde 58, 193 (1952); Springer, U., und Klee, J.: Die Charakterisierung und Unterscheidung von Waldhumusformen mittels der Natronlauge- und Natriumpyrophosphat-Extraktion und der Stickstoff-Fraktionierung. Z. Pflanzenernähr., Dung., Bodenkunde 80, 109 (1958); Springer, U. u. A. Wagner: Über Komposthuminsäuren aus Zersetzungsrückständen landwirtschaftlich und forstwirtschaftlich wichtiger organischer Stoffe. Z. Pflanzern. Dgg. Bdkde. 64, 259-276 (1954); Springer, U. u. J. Klee: Die Charakterisierung und Unterscheidung von Waldhumusformen mittels der Natronlauge- und Natriumpyrophosphat-Extraktion und der Stickstoff-Fraktionierung. Z. Pflanzenern., Dung., Bodenkunde 80, 2, 109-126 (1958); Springer, U.: Über die Beziehungen zwischen den Veränderungen des Humuszustandes des Bodens und der Art der verabreichten organischen Düngung. (Ergebnisse aus einem Humusdauer-versuch) VI^e Congres International de la Science du Sol. IV, 91-97 (1956) Paris); Springer, U. u. A. Wagner: Zur Beurteilung der organischen Substanz in Humus-düngemitteln. Landw. Forsch. 7, 193-199 (1955)).

In a diagram the decomposition of barnyard manure consisting of a mixture of ryestraw and excrements of cows during two years shall be shown. There are given some special data as they are used by Springer.

S 61

Probe	In % der Trockensubstanz			
	A I frisch	A II ½ Jahr	A III 1 Jahr	A IV 2 Jahre
Asche	10,63	26,81	31,17	33,47
Organ. Subst. (Gluhverl.).....	89,37	73,20	68,83	66,53
C _t	46,05	38,28	35,56	33,14

Probe	In % der organischen Substanz.			
	A I frisch	A II ½ Jahr	A III 1 Jahr	A IV 2 Jahre
Atherloslich	1,57	0,87	0,52	0,57
Alkoholloslich	2,42	4,50	6,59	4,53
Wasserloslich	7,60	19,73	11,99	11,36
Hemizellulose.....	20,90	13,17	12,14	11,84
Salzsaureloslich (D ₁)	29,92	28,65	31,90	31,47
Zellulose	30,22	12,53	4,59	4,20
Schwefelsaureloslich (D ₂)	38,40	17,09	10,80	10,60
Unloslich	18,99	31,14	39,72	40,07
Summe I	81,70	81,94	75,55	72,57
Summe II	98,90	101,98	101,52	98,66
C _t	51,53	52,30	51,66	49,81
Huminstoffe (Z. G.)	12,18	50,55	75,21	84,98
Huminstoffe nach Saurebehandlung (Z.G.).	7,36	30,91	43,60	44,91
Humuskohle	0,75	2,91	2,99	3,66
Laugelocliche Huminstoffe	6,61	28,03	40,61	41,25
Basengebundene Vorstufen	4,82	19,61	31,61	40,07
Uronsaureanhydrid	7,49	12,38	19,78	24,00

VERROTUNG VON MIST

In the course of decomposition the content of ash increases, that of organic substances decreases. The content of total carbon decreased also.

The percentage of the ether soluble, the alcohol and the water soluble substances in the dry weight decreases. Hemizellulose and cellulose decrease, the former not in the same amount. The insoluble part, in this case the lignin fraction increases.

In this table "hydrochloric acid soluble (D₁)" is the amount of cellulose which can be determined in form of reducing sugar after hydrolysis with hydrochloric acid. "Sulfuric acid soluble (D₂)" are those substances which are hydrolyzed with hot sulfuric acid. In this case, there are found higher values and the sum reaches nearly 100.

C_t is a total carbon content of the organic substances. Z.G. (= decomposition degree) is the amount (a measure) of the lumic substances formed, determined with acetyl bromide.

Karrer has worked out a method which allows the separation of the initial plant materials from humic substances in the case of peat. Acetyl bromide dissolves all initial plant materials which are unchanged, while the humic substances remain undissolved. Springer uses this method for determination of humic substances in decomposed plant materials and in soils. After treating the materials with acetyl bromide, humic substances such as humic acids, humins and "humus coal" remain in the residue. The carbon content of the residue is determined and called C_h .

The formula for the decomposition degree is:

$$ZG = \frac{C_h \cdot 100}{C_t}$$

The determination of the humic substances after treating with acid, the decomposition degree after treating with acid, excludes the precursors of humic substances which are combined with calcium ions. Also these kind of substances increase. Furthermore the humic substances which are soluble in alkaline solution or combined with different bases such calcium, etc., increase also. The "uronic acids" determined with 12% hydrochloric acid increase also.

The humus coal is determined by heating with 5% sodium hydroxide during one hour, treatment of the washed and dried residue with acetyl bromide and incinerating the insoluble residue. The amount of carbon is a measure for the humus coal. The alkali soluble humic substances are determined by the difference of the decomposition degree determined before and after treatment with acid minus the amount of humus coal.

The precursors combined with bases can be calculated from the difference of the decomposition degree before and after and treatment with acid; they consist mainly of fulvic and hymatomelanic acids.

We also made some investigations about the decomposition of organic materials, for instance with straw of wheat. (Wolfgang Flaig, Ulrich Schobinger und Hans Deuel: Umwandlung von Lignin in Huminsäuren bei der Verrottung von Weizenstroh, Ber. d. Chem. Ges. in press).

The decomposition of plant materials has been indeed investigated by different authors. (Waksman, S. A. u. K. R. N. Iyer: - Soil Sci. 34, 43 (1932); Waksman, S.A. u. K. R. N. Iyer: - Soil Sci. 36, 69 (1933); Waksman, S. A. u. I. J. Hutchings: - Soil Sci. 40, 347 (1935); Broadbent, F. E.: - Proc. Soil Sci. Soc. Amer. 18, 165 (1954); Lehner, A. u. U. Springer: - Pflanzenbau und Pflanzenschutz 1, 97 (1950); Springer, U. u. A. Lehner: - Z. Pflanzenernähr., Dung., Bodenkunde 58, 193 (1952); Springer, U. u. J. Klee: - Z. Pflanzenernähr., Dung., Bodenkunde 80, 109 (1958)). We were mostly interested in the changing of the composition and chemical properties of the lignin fractions. After the mentioned considerations, reaction between lignin or degradation products of lignin, and nitrogenous compounds from the microorganisms, especially proteins and their degradation products form mostly humic substances.

Wheat straw has been cut in little pieces and rotted in a climate chamber at 90% relative humidity and 28° C with addition of nutrient solution with sodium nitrate as nitrogen during 410 days.

S 95

Transformation of a mixture of straw constituents and humic substances during the course of rotting

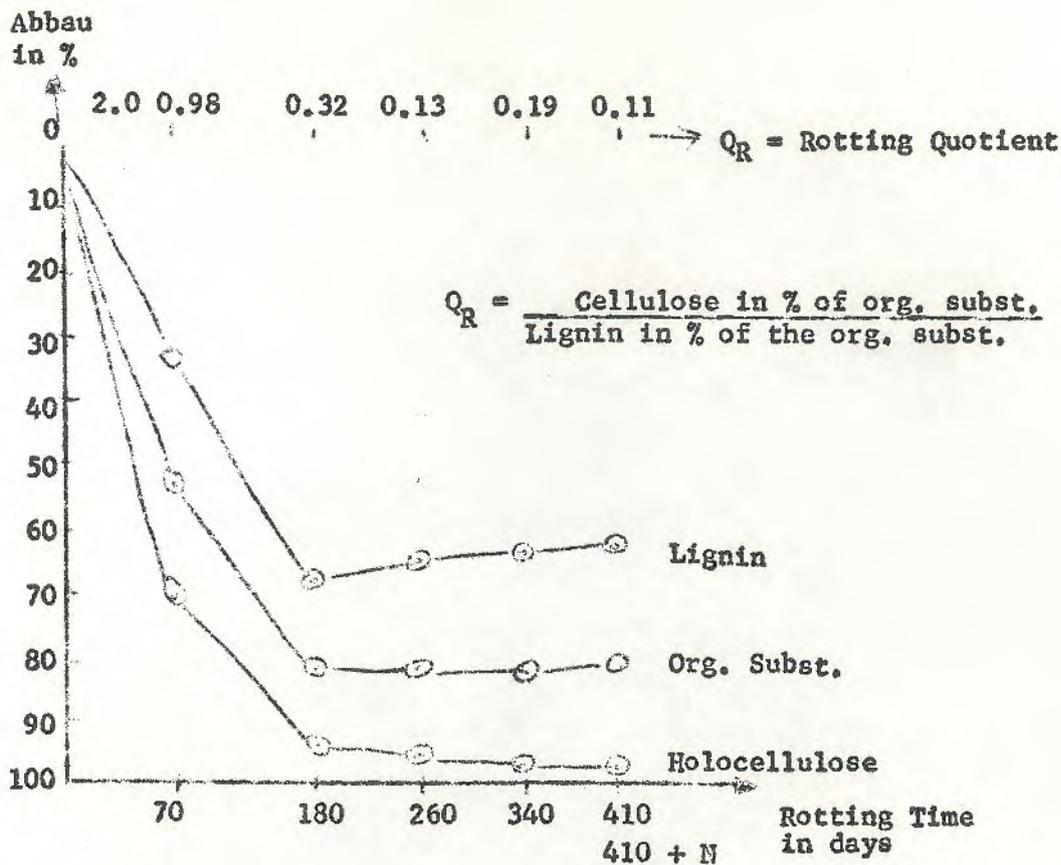
Rotting time in days	Org. Subst. % Abn.	H ₂ SO ₄ Lignin		Cellulose			Humic Acid % Geh.	Fulvic Acid	
		% Abn.	Geh.	Holo- Abn.	% Geh.	- Abn.		% Geh.	% Geh.
0	265g	53g	20	198g	75	106g	40		
70	124g	35g		60g		35g	28	5g	4
180	53	33	28	69	43	67	10	4g	8
	50g	17g		12g		5g		6g	13
260	81	68	34	94	25	95	5	4g	8
	49g	20g		10g		2g		7g	15
340	81	64	38	95	20	98	6	4g	8
	49g	19g		8g		3g		6g	12
410	81	64	38	96	15	97	1	4g	9
	47g	20g		10g		1g		7g	14
	83	63	41	96	20	99			

The numbers show the decrease of the weight of the organic substances in total, the decrease of holo- and α -cellulose. The content of lignin increases. They show further that after 180 days no remarkable changes have taken place.

From these numbers can be seen that the rate of the decomposition of cellulose is much larger than that of lignin. For the determination of cellulose, the values of α -cellulose must be taken, because the determination of holocellulose also includes the polysaccharides such as levans and dextrans formed by microorganisms. (Geoghegan, M. J. u. R. C. Brian; - Nature 158, 837 (1946); Biochem. J. 43, 5 (1948); Swaby, R. J.; - J. Soil Sci. 1, 182 (1949); Quastel, J. H.; - Soil Sci. 73, 419 (1952)). These kinds of polysaccharides decompose more slowly than the β -glucosidic combined molecules of glucose in the cellulose.

In the course of the investigations also the "hot water soluble" parts have been determined. By acidic extraction of these solutions dark brown substances could be isolated which can be called according to definition "humic acids". In the solution acidified with hydrochloric acid remain substances which can be called "fulvic acids".

S 240



By this diagram the different rate of decomposition of lignin, organic substances in total, and holocellulose can be seen. A "rotting quotient" = Q_R can be calculated dividing the amount of cellulose in percent of the total organic substances by the amount of lignin in percent of the total organic substances. If the quotient reaches 0.3 no more remarkable changes in the quantity of organic substances occur.

S 96

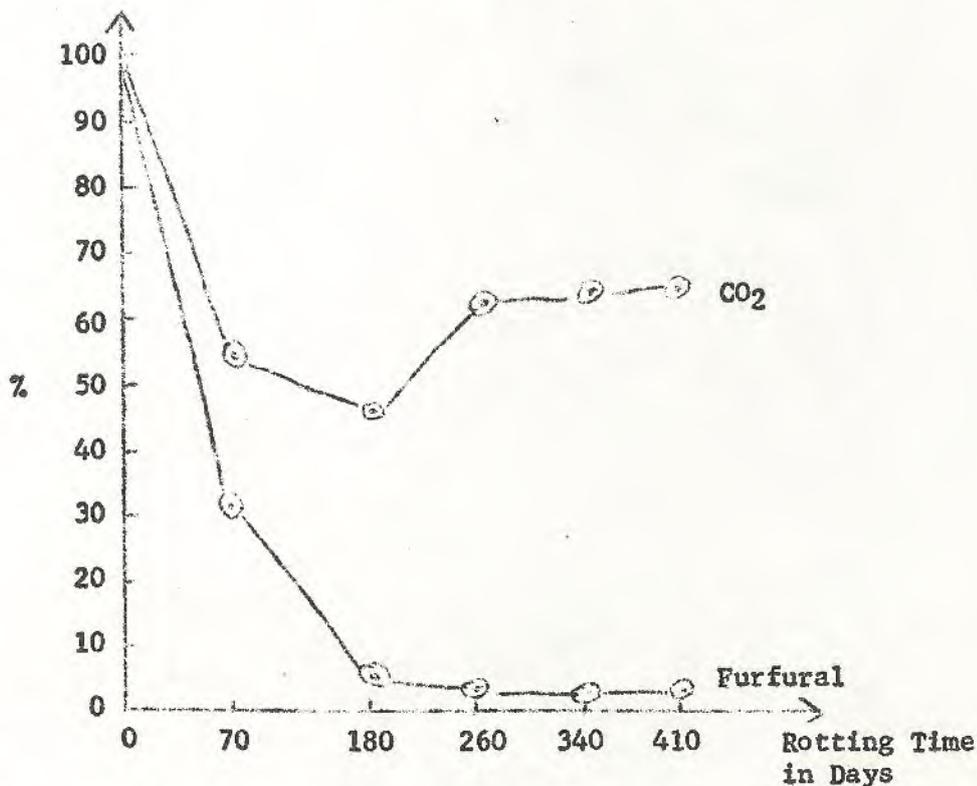
Zusammensetzung der Strohproben in % der org. Substanz

Rottezeit in Tagen	C	H	O	org. N	C:N total
0	46,36	6,47	46,46	0,71	60,2
70	48,34	6,21	43,57	1,88	24,2
180	50,25	6,30	40,08	3,37	12,6
260	49,72	6,01	40,31	3,96	10,6
340	48,24	6,30	41,64	3,82	10,7
410	48,32	5,87	41,52	4,29	9,6
410 + N	45,54	5,58	46,54	4,34	8,8

The increase of carbon content and the decrease of the oxygen content up to 180 days show the rapid decomposition of the cellulose. The following decrease of carbon content and increase of oxygen content show that oxidation reactions must occur. After 180 days of rotting time the cellulose is decomposed up to 75% and more, and more condensation reactions take place between the nitrogen compounds formed by the metabolism of the microorganisms and the oxidation and degradation products of lignin. The steadily increasing content of nitrogen indicates this fact.

S 18

Change in the Furfural and CO_2 values during acid decarboxylation. Content of outgoing material = 100%



Further investigations show that chemical reactions take place after 180 days. By decarboxylation of straw samples rotted different long time with 12% hydrochloric according to a method of Deuel and co-worker, (Dubach, P., G. Zweifel, R. Bach u, H. Deuel: - Z. Pflanzenernahr-, Dung., Bodenkunde, 69, 97 (1955)) and by determination of furfural (Adams, G. A. u. A. E. Castagne: - Cand. J. Ros, Sect. B26, 314 (1948)) it can be shown that the amount of CO_2 formed cannot be only derived from uronic acids but must also be formed by other substances which can be decarboxylated. From this fact, the decrease of the carbon and the increase of the oxygen content of the straw samples after 180 days can be understood.

Substances were isolated with a method for the determination of lignin in plant materials. (Ritter, G. J., R. M. Seborg, U. R. L. Mitchell: - Ind. Ingng. Chem. analyt. Edit. 4, 202 (1932); (vgl. Adams, G. A. u. A. E. Castagne, Canad. J. Res. Sect. B27, 915 (1949)). In this case with sulfuric acid, they are not lignin because they have too high content of nitrogen and a too low content of methoxyl; they are better called lignin-like fractions.

Working with the relatively high concentrated sulfuric acid of 72% for the isolation of the lignin fraction, it is not sure whether condensation reactions can take place. Therefore, in comparison lignin has been isolated with weak acidified dioxane according to the method of Bjorkman. (Bjorkman, A.: Svensk Papperstidn. 59, 477 (1956)).

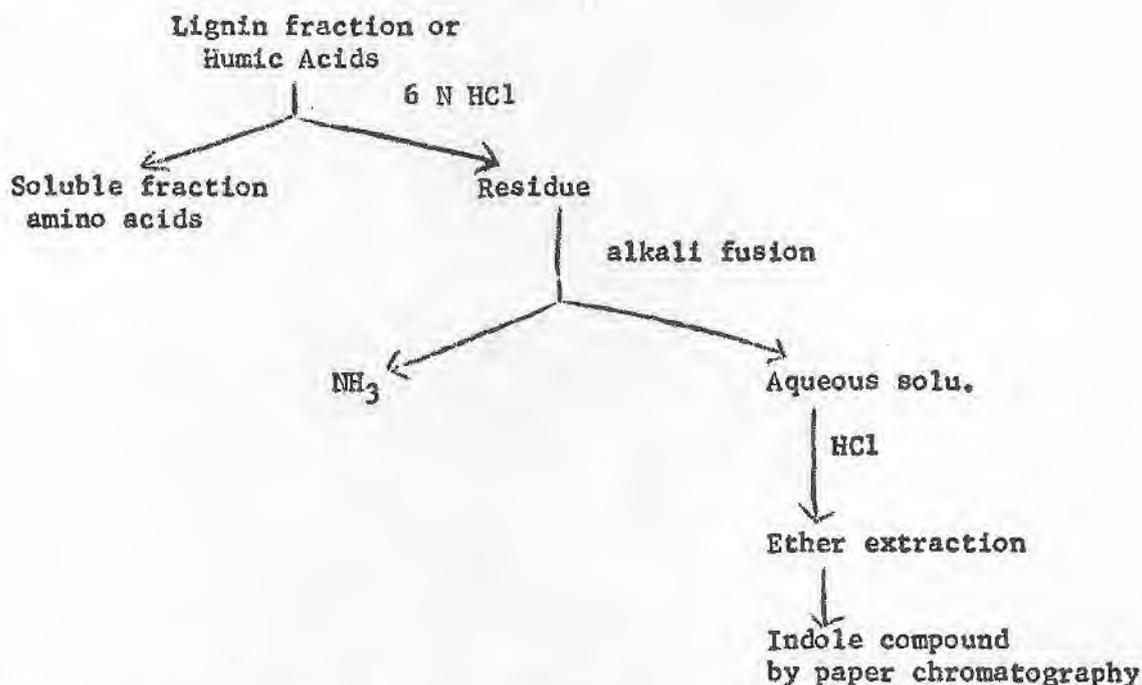
S 14 (See page 4 of section "Chemistry of Quinones")

The elementary analyses of the lignin fraction isolated in both ways show that the changes of the carbon-, oxygen-, nitrogen-, and methoxyl- contents are nearly the same in every case. The parts of lignin isolated according to Bjorkman are mainly lower molecular substances.

The numbers show also clearly that between the decrease of the methoxyl content and the increase of the nitrogen content there must be a chemical relationship. It means that the building in of nitrogenous compounds can take place only after splitting off the phenyl ether.

To get an idea about the transformation of lignin into humic acids the lignin fraction has been investigated. The functions of nitrogen and oxygen are especially interesting in this case.

S 217



For better understanding, I show you a scheme according to which we had analyzed not only the degradation product of lignin but also of humic acids and other humic substances. In this case the isolated sulfuric acid lignin, Bjorkman lignin and also humic acids were heated with 6 N hydrochloric acid. In the soluble fraction are the amino acids. (compare also: Nitrogen transformations during the biological decomposition of straw composted with inorganic nitrogen. J. agric. Sci. 45, 469-475 (1955)). The residue is fused with a mixture of sodium hydroxide and sodium acetate at 250° C under reducing condition. During this operation, ammonia is split off and determined. The fusion is dissolved in water. After acidifying, the nitrogen content of the residue is determined and the acid solution extracted with ether. In the ether extract indol compounds can be identified by paper chromatography. As an example for the determination of the function of nitrogen, the numbers of different analyses are given. The values are nearly the same in the case of sulfuric acid lignin and Bjorkman lignin.

S 15

N-Gehalte der Schwefelsaurelignine und deren Hydrolysate

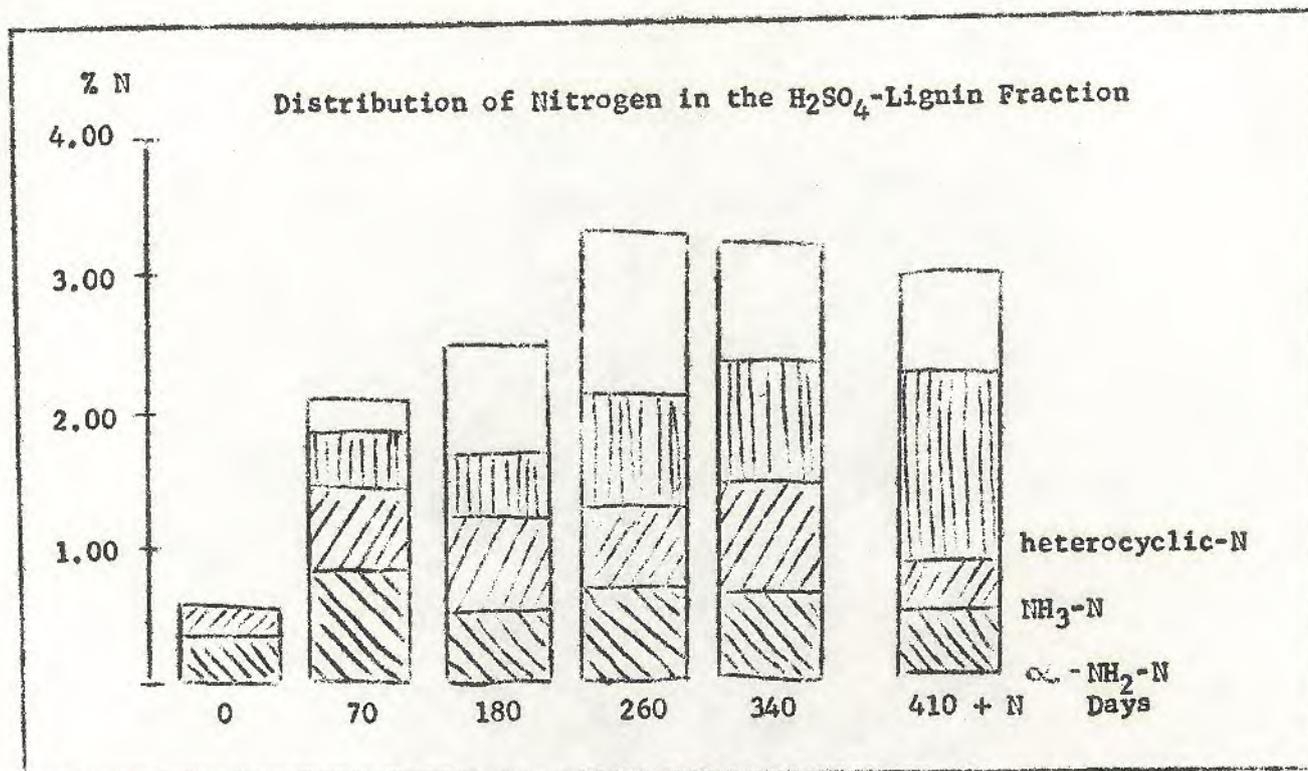
- Aminostickstoff						
	Rottezeit in Tagen	%*N vor der Hydrol.	in %	im Hydrolysat in % des ges org N vor der Hydrol	%*N im Hydrol- ruckst.	NH ₃ -N*% Rest-N* in %
H ₂ SO ₄ -Lignin	0	0,55	0,32	58,1	0,22	0
	70	2,09	0,82	39,2	0,97	0,39
	180	2,46	0,52	21,1	1,08	0,46
	260	3,26	0,68	20,8	1,38	0,82
	340	3,20	0,65	20,3	1,67	0,85
	410 + N	2,97	0,52	17,5	1,77	1,40

* (N in % der org. Substanz, bestimmt nach Dumas)

At first the values before hydrolysis are given. They show the mentioned increase. Next, hydrolysis with 6 N HCl acid under the usual conditions had been made for the determination of α -amino nitrogen. The percentage of α -amino nitrogen decreases soon to 20% after 180 days and remains there. In the residue of the hydrolysis, there had been determined with a new method a further part of the nitrogen. By fusion with a mixture of sodium hydroxide and sodium acetate under reducing conditions at 250° C, ammonium nitrogen is split off. (Flaig, W. u. Th. Breyhan: - Z. Pflanzenernähr., Dung., Bodenkunde 75, 132 (1956)). The amount of this ammonia nitrogen increases with time. According to model investigations, it seems that this nitrogen belongs to amino acids or parts of peptides which cannot be hydrolyzed under the usual conditions with 6 N HCl acid. It is interesting to remark that under the conditions of the fusion, phenylglycine doesn't form indigo.

In the residue of the fusion a further part of nitrogen can be determined. By paper chromatography and comparison with heterocyclic compounds, it could be ascertained that nearly the total amount is heterocyclic combined nitrogen. A part of this nitrogen is of indole type (are indole compounds). This fact is interesting in so far as not only in this isolated lignin fraction but also in humic acids, this type of compounds could be identified. I remember that it has never been possible to isolate indole compounds, such as tryptophane, by hydrolysis of soils.

S 99



In this slide, the nitrogen distribution in columns is shown. The case 410 + N we will exclude, because it is a special one. It is to observe that the heterocyclic bound nitrogen increases with the time of rotting. Also the unknown part increases with rotting. The kind of combination of this part of nitrogen is not known yet. Only some investigations have been made. This kind of nitrogen is in the hydrolyzate, in which the α -amino-nitrogen is determined. Possibly this part of nitrogen is derived from amino-sugars. By its increase with time of rotting, it can be assumed that with increasing time of rotting changes of the parts of peptides, amino-acids can occur.

After recognizing that a part of the nitrogen can be split off as α -amino nitrogen, the investigation of the residue of hydrolysis must give better ideas about the changes of lignin during rotting. Furthermore, it can be said that the analyses show that the amino-acids or the parts of peptides are condensed with the changed lignin during the course of rotting.

S 16 (see next page)

All the hydrolyzed sulfuric acid lignin-, Bjorkman lignin-fractions and "humic acids" have been investigated. By elementary analysis the changes of the compounds can be recognized best. The data of sulfuric acid lignin and humic acids before and after hydrolysis are given.

S 16

Elementarzusammensetzung der H_2SO_4 -Lignine und Huminsäuren vor und nach der Hydrolyse

Rottezeit in Tagen	H_2SO_4 -Lignin							
	C %		H %		O %		N %	
	vor	nach	vor	nach	vor	nach	vor	nach
0	58,59	63,39	5,41	31,37	30,98	0,54	0,22	
70	58,78	60,15	5,68	29,48	33,20	2,01	0,97	
180	58,67	60,40	5,66	29,15	32,86	2,37	1,08	
260	58,00	58,86	5,61	29,93	34,15	3,14	1,38	
340	54,97	56,66	5,62	32,09	36,05	3,08	1,67	
410	54,29			32,88		2,97		

"Huminsäuren" aus Heisswasserextrakt

260	53,74	57,53	4,58	37,63	36,94	3,44	0,95
340	52,92	56,70	4,56	38,93	37,78	2,94	0,96

The content of carbon and that of oxygen increase in each case after hydrolysis, i.e., the compounds after hydrolysis are richer in carbon and oxygen than before. This means that compounds have been split off with a relatively high oxygen content. In average the oxygen content of amino-acids can be assumed as 30%.

In the residue after hydrolysis, the content of carbon decreases and the content of oxygen increases with time of incubation. This means that during the course of transformation of lignin compounds richer in oxygen are formed. It will be shown that also an increase of acid groups occurs.

After our considerations about model substances and possibilities of building-in nitrogen no further details need be added. All the mentioned possibilities can happen.

Now the functions of the oxygen must be discussed. In this case more details can be given. But in the case of all reactions which I mention the restrictions must be considered, which I have shown to you with the model substances.

For the investigations of the function of oxygen, the changes of the acid groups will be discussed first. Broadbent (Soil Science Soc. Amer. Proc. 18, 165 (1954)) has shown that during rotting of oat straw samples the content of carboxyl groups/ m.e. per 100 gm., the exchange capacity m.e. per 100 gm., the neutral equivalent in m.e. per 100 gm., and the exchangeable H^+ in m.e. per 100 gm., increases.

Days incubated	COOH m.e. per 100 gm.	Exchange capacity m.e. per 100 gm.	Neut. eq. m.e. per 100 gm.	Exch. H ⁺ m.e. per 100 gm.
0	31	28	16	7
88	72	48	12	14
180	90	61	14	14
355	107	70	13	18
452	107	72	9	28

For this table, the methods used by Broadbent must be mentioned:

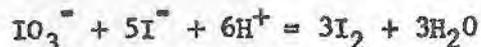
1) Carboxyl was determined by Fischer esterification of samples in methanol saturated dry hydrogen chloride, followed by methoxyl determination. The esterification was carried out in the cold to minimize transmethylation of carboxyl groups exposed by hydrolysis of ester or lactone linkages. An equilibrium methoxyl content was usually obtained after one or two treatments in the cold. By heating the mixture of methanol-HCl and the samples, much higher values were obtained. I remember that I have shown you with model substances that in the case of hydroxybenzoquinones a methoxyl group can be introduced by splitting off an aliphatic group by treatment with methanol-HCl.

2) Exchange capacity. The cation exchange capacity was determined by leaching with barium acetate. The adsorbed barium ions were displaced with hydrochloride-acid and determined.

Broadbent assumed that trans-esterification might have occurred even in the cold, because the values for the carboxyl groups are higher than those for cation-exchange capacity.

3) The neutralization equivalent was determined by suspending small samples of organic materials in a known volume of standard carbonate-free alkali for a period of 24 hours. Afterwards the excess of alkali was titrated potentiometrically.

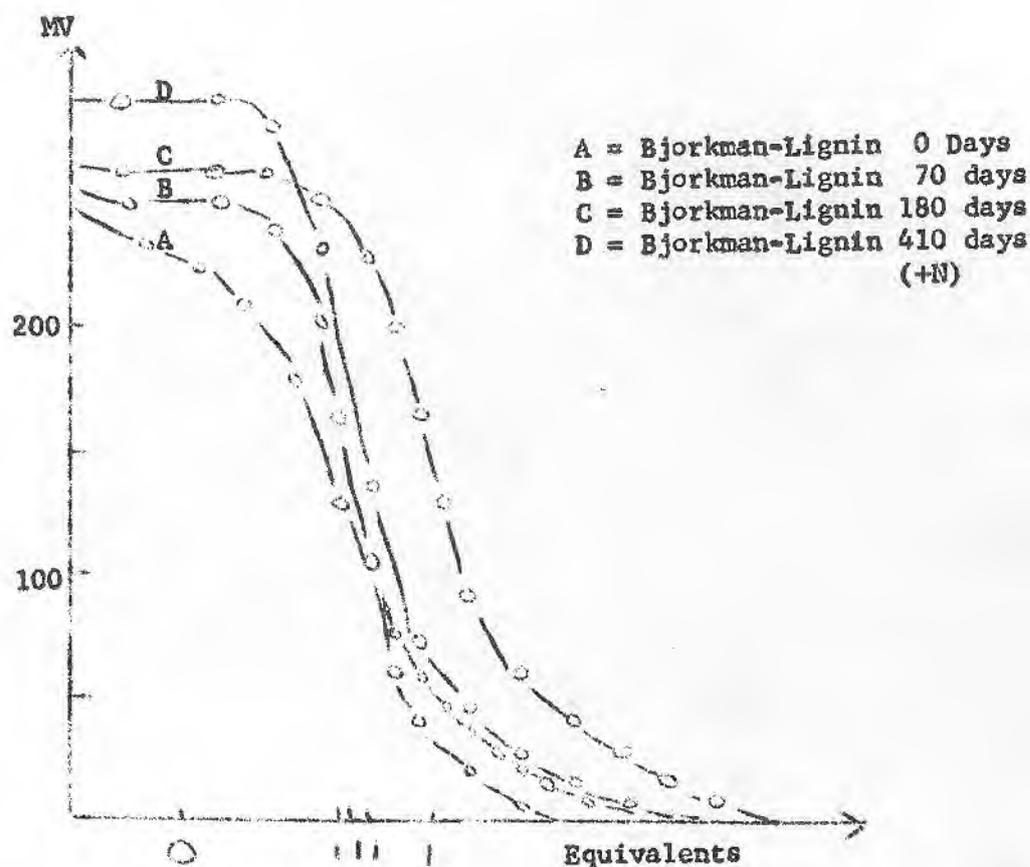
4) The exchangeable hydrogen ions were determined by an iodometric titration. The principle of the method is that hydrogen ion from the sample causes the liberation of an equivalent amount of iodine according to the reaction.



The liberated iodine has been titrated with thiosulfate.

We titrated potentiometrically the different samples of lignin fractions according to the method of Brockmann and Meyer (Brockmann, H. u. E. Meyer: Chem. Ber. 86, 1514 (1953)).

S 17



The samples had been dissolved in ethylenediamine and titrated with ethanolamine. With the aid of this method, weak acid hydroxyl groups can be determined, even in the presence of carboxyl groups.

The course of the titration curve indicates that at the beginning of the decomposition mainly phenolic hydroxyl groups are present. The content of strong acid groups increases with time of rotting. This increasing amount of strong acid groups may be almost carboxyl groups, it may be that these are also relatively acid hydroxyl groups of hydroxy-quinones.

Due to the different titration curves the following equivalent weights can be determined.

Bjorkman-Lignin, fresh:	560 ± 13
Bjorkman-Lignin, 70 days rotted:	577 ± 11
Bjorkman-Lignin, 180 days rotted:	429 ± 7
Bjorkman-Lignin, 340 days rotted:	412 ± 9

On the basis of a molecular weight of 180 to 190 for one phenyl-propane unit, there is one phenolic-hydroxyl group for three units of fresh Bjorkman-lignin, this means 0.33 phenolic-hydroxy groups per unit. This result agrees with that of Freudenberg in the case of spruce-lignin. (K. Freudenberg, *Angew. Chem.* 68, 84 (1956)).

Brauns found that mainly phenolic hydroxyl groups are determined by methylation with diazo-methane in ether. In dioxane also the enolized carbonyl groups are included.

S 103

Methylation of Lignin-fractions According to Bjorkman

Lignin from fresh straw	16.73% OCH ₃
Lignin from fresh straw methylated	21.76% OCH ₃
Lignin rotted 70 days	10.84% OCH ₃
Lignin rotted 70 days methylated	13.97% OCH ₃

We wanted to identify the existence of free hydroxyl groups and methylated Bjorkman lignin with diazomethane in acetone with addition of a small amount of water in the cold for 12 hours. The methylated preparations showed nearly a white color. The numbers show that in spite of the splitting-off of the methylether during rotting the number of the free hydroxyl groups does not increase. This fact indicates that the formed hydroxyl groups are very reactive and are transformed very fast.

The investigations of the decarboxylation of lignin fractions with 12% hydrochloride acid should give an indication of the way the oxidation took place.

S 104

Decarboxylation of Lignin-fractions According to Bjorkman

Lignin from fresh straw	0.47% CO ₂
Lignin from straw rotted 70 days	0.64% CO ₂
Lignin from straw rotted 180 days	1.34% CO ₂

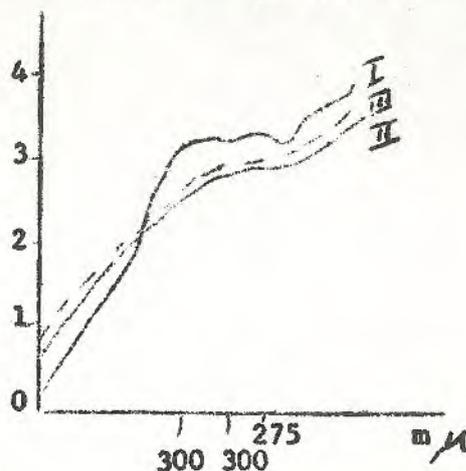
With increasing time of rotting, the amount of formed carbon dioxide increases. According to our former experiences with straw samples, the formed carbon dioxide is originated from oxidized lignin components.

For the determination of carbonyl groups in lignin and the lignin fractions, a method according to Traynard and Eymery has been used. (Ph. Traynard and A. Eymery, *Holzforschung* 10, 6 (1956)). The content of carbonyl groups has been determined by the increase of nitrogen after the reaction with 2,4-dinitrophenylhydrazine.

	carbonyl content in %
Bjorkman lignin, fresh	0,36
Bjorkman lignin, 70 days	0,47
Bjorkman lignin, 410 + N	0,59

The carbonyl content of the fresh Bjorkman lignin may be partly responsible for the color reaction caused by the cinnamic aldehyde group. The increase of carbonyl oxygen during rotting may be connected with the oxidative processes. During rotting, CO-groups can be formed in the side chains, or by the oxidation of phenolic OH-groups to quinones.

In the course of our considerations about the transformations of lignin in humic substances, some spectrophotometric data shall be given. Lignin has a maximum in the range between 275 - 285 m μ , which belongs to the benzene ring substituted with oxygen, and an absorption between 300 and 350 m μ caused by the presence of chromophoric groups in the side chain such as carbonyl groups or conjugated double bonds. The spectra of humic acids usually show no maximum and are not relatively characteristic. Scheffer and Welte investigated on the one hand different lignins and on the other hand humic acids by UV-spectra and suggest a structural relationship. (*Z. Pflanzenernahr., Dung., Bodenkunde* 48, 250 (1950)).

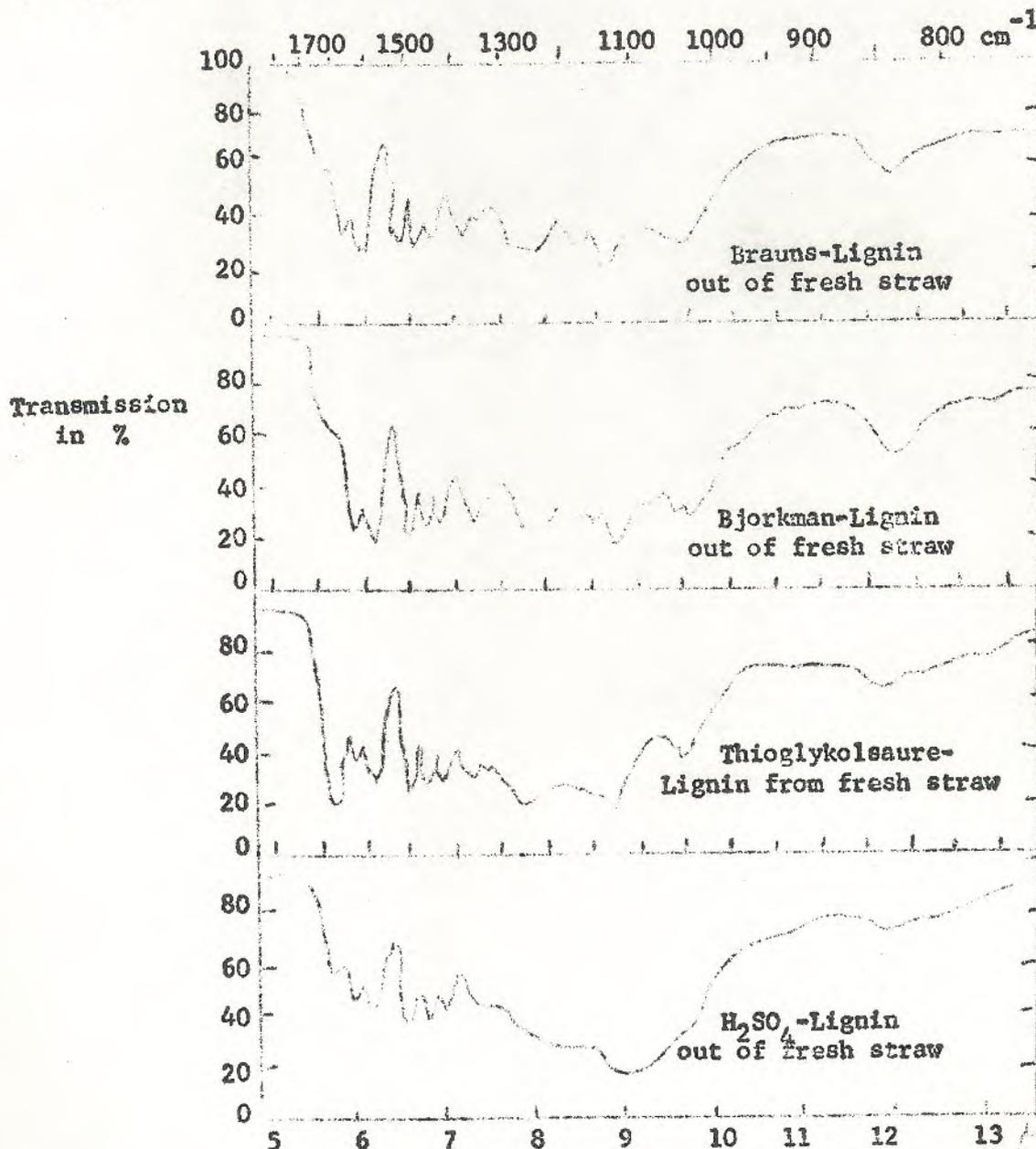


- I Bjorkman-lignin fresh
- II Bjorkman-lignin 410 days rotted
- III Humic acids from straw (410 days rotted)

The curves of the spectra show that in the case of Bjorkman lignin, 410 days rotted, the absorption which concerns the groups in the side chains disappears, in the case of humic acids, isolated from straw, 410 days rotted, the curve becomes more and more non-characteristic.

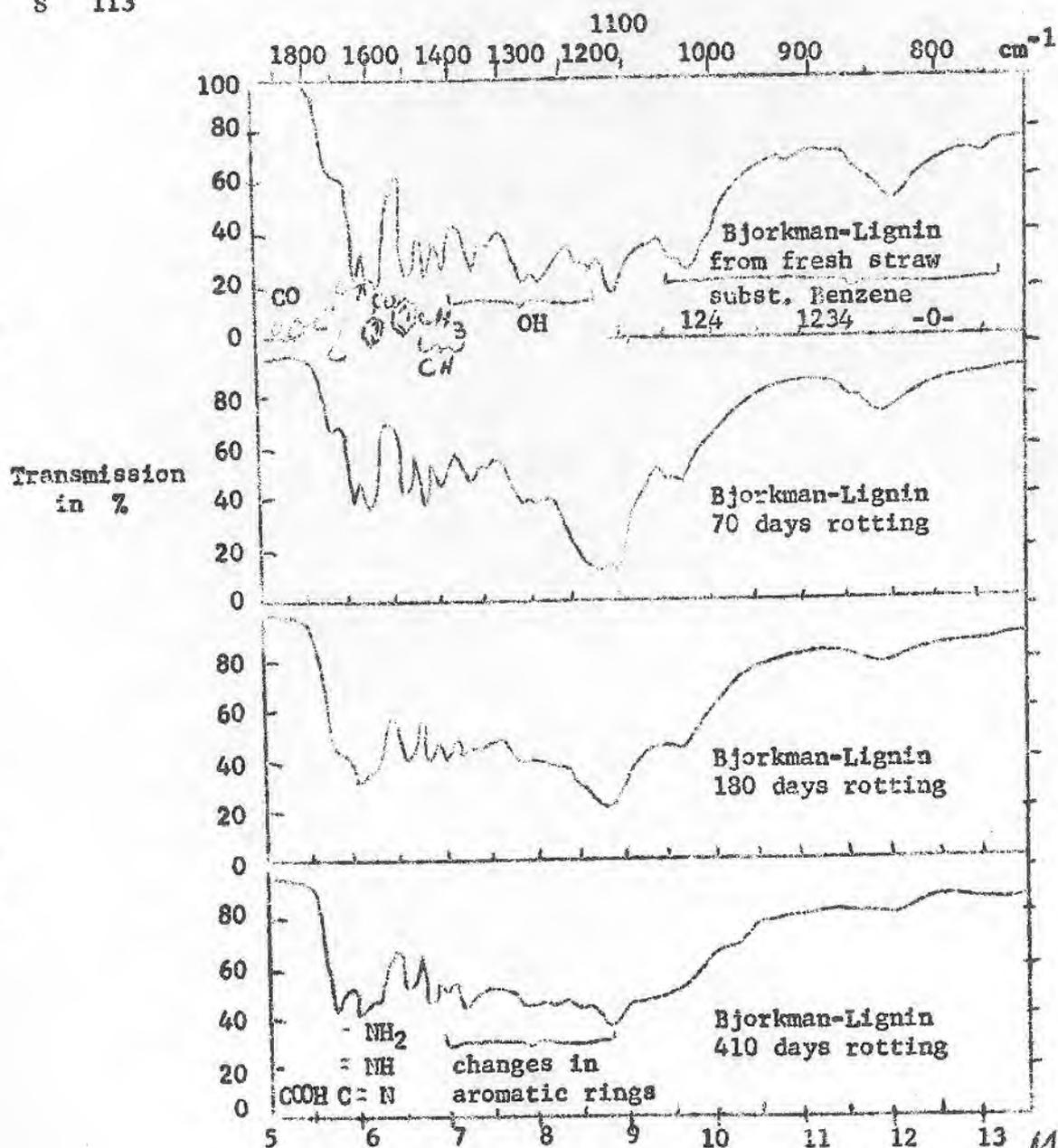
In some cases the infrared-spectra are more characteristic than the UV-spectra. Therefore, we investigated the transformation of lignin in humic substances also with IR-spectra. Some spectra are known in literature (I. A. Breger, *Fuel* **30**, 204 (1951); M. Ceh und D. Radzi, *Fuel* **35**, 77 (1956); R. M. Eloffson, *Can. J. Chem.* **35**, 926 (1957); K. Kumada and K. Aizoaw, *Soil and Plant Wood (Tokyo)* **3**, 152 (1958) ref. in *Chem. Abstr.* **52**, 13165c (1958); compare also M. M. Kononowa "Die Humusstoffe des Bodens:", VEB Deutscher Verlag der Wissenschaften, Berlin, 1958, S. 61; Infrared Spectra of coalification series from cellulose and lignin to anthracite. *Nature* **182**, (4638), 785 (1958)).

S 114



In this slide, the bands of lignin isolated by different methods are given. Between Brauns lignin and Bjorkman lignin are nearly no differences. In the case of thioglycolic acid-lignin and sulfuric acid-lignin, not only the different influences of the extraction agent can be seen but also that some changes in the lignin must have occurred. These results have been the motivation to use Bjorkman lignin for further studies with IR.

S 113



The next slide shows the changes of the spectra of Bjorkman lignin during rotting.

The different peaks mean:

In the range of 5.5μ is in the case of unrotted lignins a shoulder. In this range are the characteristic C=O bonds of aldehydes, ketones and esters and carbonic acids. During rotting, the intensity of this bond increases up to the spectra of humic acids. The bond at 6.1μ belongs to the valence-vibration of C=C-double bond, which is in conjugation with C=O-group presumably.

The bonds in the range of 6.25μ and 6.67μ are presumably ω -vibrations of aromatic systems. According to Smith (Smith, D. C. G.: - Nature 176, 267 (1955)) the bonds in the range of 5.8μ till to 6.25μ are also found in the case of model substances, such as ferulic, 3,4-dimethoxy-cinnamic aldehyde and vanillin.

The bonds in the range of 6.85μ are asymmetric CH-deformation vibrations and in the range of 7.3μ symmetric CH-deformation vibrations of CH_2 groups. The intensity decreases in each case during rotting. The intensity of the bond of the symmetric CH-deformation vibrations is according to Freudenberg a measure of the number of CH-groups. (Freudenberg, K., H. Dieckrich und W. Siebert: - Chem. Ber. 84, 961 (1951)).

The bonds in the range of 7.0μ to 7.1μ are presumably C-O-valency and OH-deformation vibrations of phenols, respectively. According to comparative investigations they can be probably those of hydroxy-quinones. Also in this case, a decrease of intensity of absorption can be noticed during rotting.

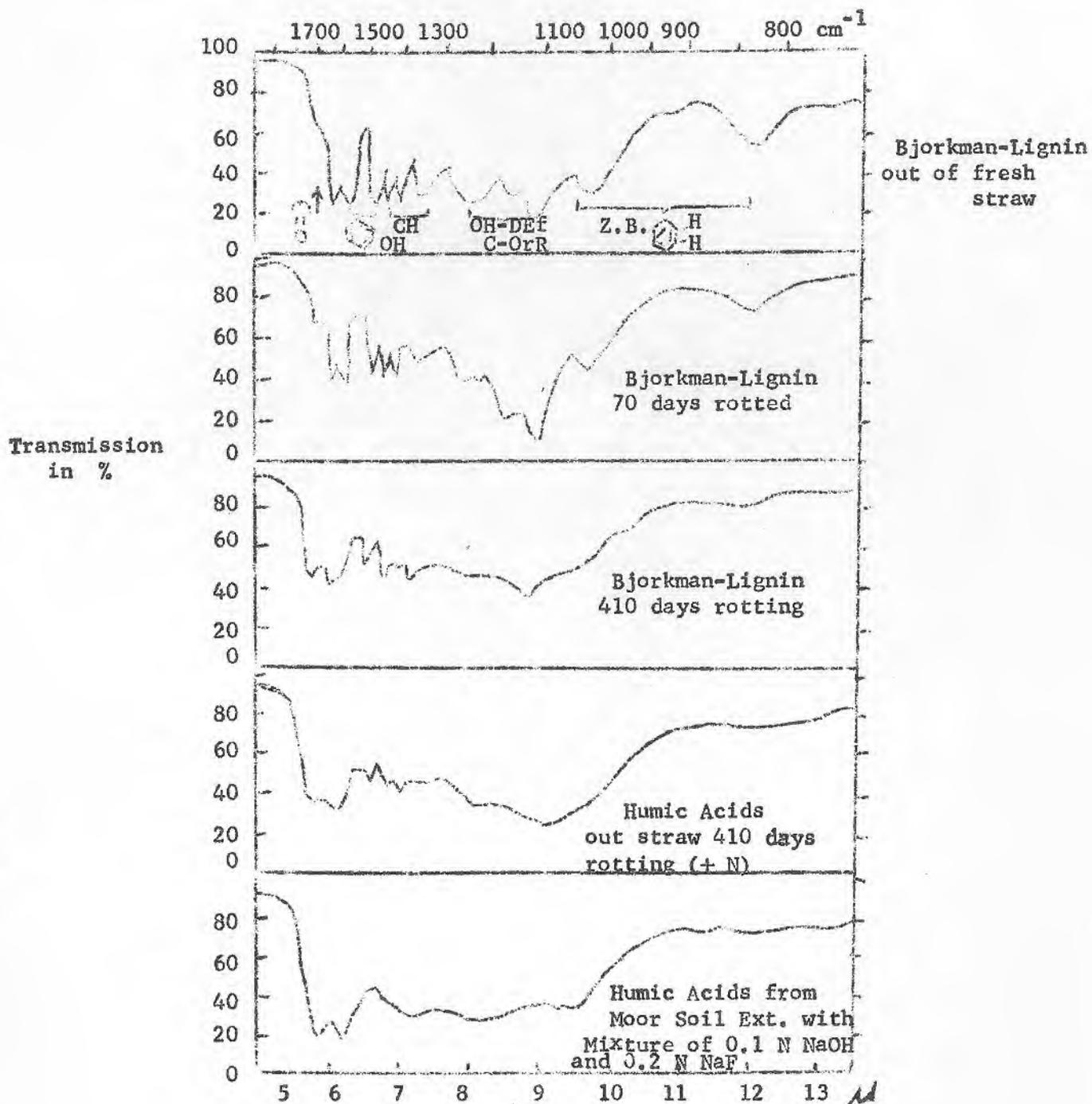
The bonds in the range of 7.9μ to 8.9μ are OH-deformation vibrations of phenols or alcohols, and C-O-valency vibrations of aryl- or alkyl ethers, respectively. The intensity of absorption decreases during rotting, furthermore, these bonds become uncharacteristic.

The bonds in the range of 9.6μ to 9.7μ and 10.2μ are presumably in connection with a bond in the range of 11.9μ which belongs to a benzene ring with 2 adjacent hydrogen atoms, the vibrations of aromatic systems. They decrease during rotting.

The increasing broadening of the bands between 6.1μ and 6.25μ during rotting can be explained with the building in of nitrogenous compounds in the molecule of lignin. This is especially noticeable in the case of lignin fractions rotted 180 and 410 days.

In this range is the C-N-valence vibrations of heterocyclic aromatics, NH-deformation vibrations of primary and secondary amines and NH_2 -deformation vibrations of primary amides.

S 112

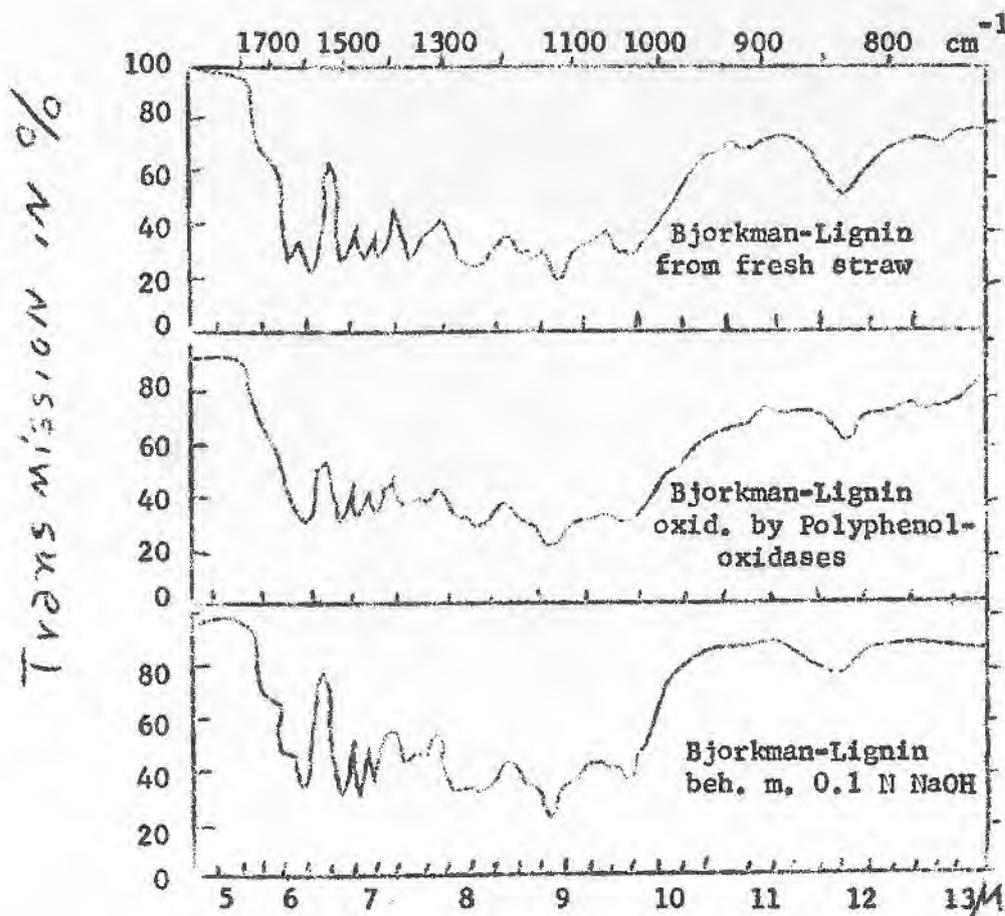


In this slide, the infrared spectra of Bjorkman lignin isolated from fresh straw, isolated from straw, rotted 70 days and 410 days, and humic acids isolated from straw, rotted 410 days and humic acids isolated from peat soil are shown.

Generally it can be said in accordance with other authors (Cah, M., u. D. Hadzi.: Fuel 35, 77 (1956) - Breger, I. A.: - Fuel 30, 204 (1951); Kononowa, M. M.: - Rapports VIe. Congr. Internat. Sci. du Sol, Chemie du Sol, S. 5, Akad. d. Wiss. UdSSR Moskau (1956); Kononowa, M. M. u. N.P. Beltschikowa: - Rapports. VIe. Congr. Internat. Sci. du Sol, Paris B. 557 (1956)) that the infrared spectra of humic acids have only few and not sharp bands even in the cases where they are isolated from different soils.

The mentioned spectra show that the "incomplete" humic acid isolated from straw have a large similarity with the rotted lignin fractions. In the soil, there are further changes of the humic acids than those that occur during a rotting time of 410 days in the model experiment. A gradual transformation of lignin into humic acids is evident by the spectra as well as by the chemical investigations.

S 239



In the next slide some characteristic changes of lignin with different chemical agents or enzymes are shown, which are interesting for the chemistry of humic acids.

With the oxidation of lignin with phenol oxidases, the bonds in the range of 6.1 μ decreases and also a change in the range of 7.4 μ takes place. It is interesting to remark that the same change occurs by treatment of Bjorkman lignin with 0.1 N sodium hydroxide during 35 hours at room temperature.

2) To carry out the destruction reactions and reconstruct the constitution by the found compounds.

By the last consideration we come more and more to the chemistry of humic acids. It has been necessary to mention the transformation of lignin into humic substances, the final product of this process. The special difficulties in the case of humic acids result from the fact that they consist not of one monomeric compound which polymerizes or polycondenses but of several. Furthermore, they are tridimensional components. By the fact that they are mixed polymerisates or a mixed polycondensates the situation gets still more complicated. One of the simplest reactions shall be mentioned. Natural and also synthetic humic acids reduce an ammoniacal solution of silver nitrate forming very stable silver sols, both of them acting as a protective colloid at the same time. Fehling's solution is also reduced. Natural, as well as synthetic, humic acids are able to couple diazo compounds forming dark colored red brown dyestuffs. Furthermore, they have properties of tannins. Gelatin solutions are precipitated by solution of humic acids. This influence is chiefly due to the phenolic hydroxyl groups. (Jodl, R.: Brennstoff-Chemie 20 (1939) 87; Z. wiss. Photogr. Photophysik, Photochem. 37 (1938) 111).

Also the chlorinated compounds of synthetic and natural humic acids are very much alike in their behavior in both cases, also the final constitution is not yet known.

Fischer and H. Schrader obtained hydroxy-benzene carbonic acids and benzene polycarbonic acids under the rough oxidation conditions in alkaline solution in an autoclave at 150 to 180° C under pressure. They concluded, that humic acids are formed from lignin, because lignin gives similar results. (Fischer, F. und E. Schrader: Brennstoff-Chemie 3 (1922) 65-80).

According to Willstatter and Cramer carbonyl groups can be indicated by means of phenyl-hydrazin-carbonate. The evolution of nitrogen from this reaction is characteristic for compounds containing quinoid groups. (Willstatter, G. und K. Cramer: Ber. deutsch. Chem. Ges. 43 (1906) 2976).

Zetzsche and Reinhart succeeded in reducing humic acids by means of sodium amalgam. During this reduction a number of color gradients is observed. In the beginning the solutions clear up, then they are intensively green, then red, orange, and finally they are lemon yellow. The reduction products are extremely easily oxidized and stable derivatives can be formed only by complete methylation or by acetylation. By shaking with air the color of the starting solution is regained. Because of these facts humic acids have redox properties. (Zetzsche, F. und H. Reinhart: Brennstoff-Chemie 20 (1939) 84).

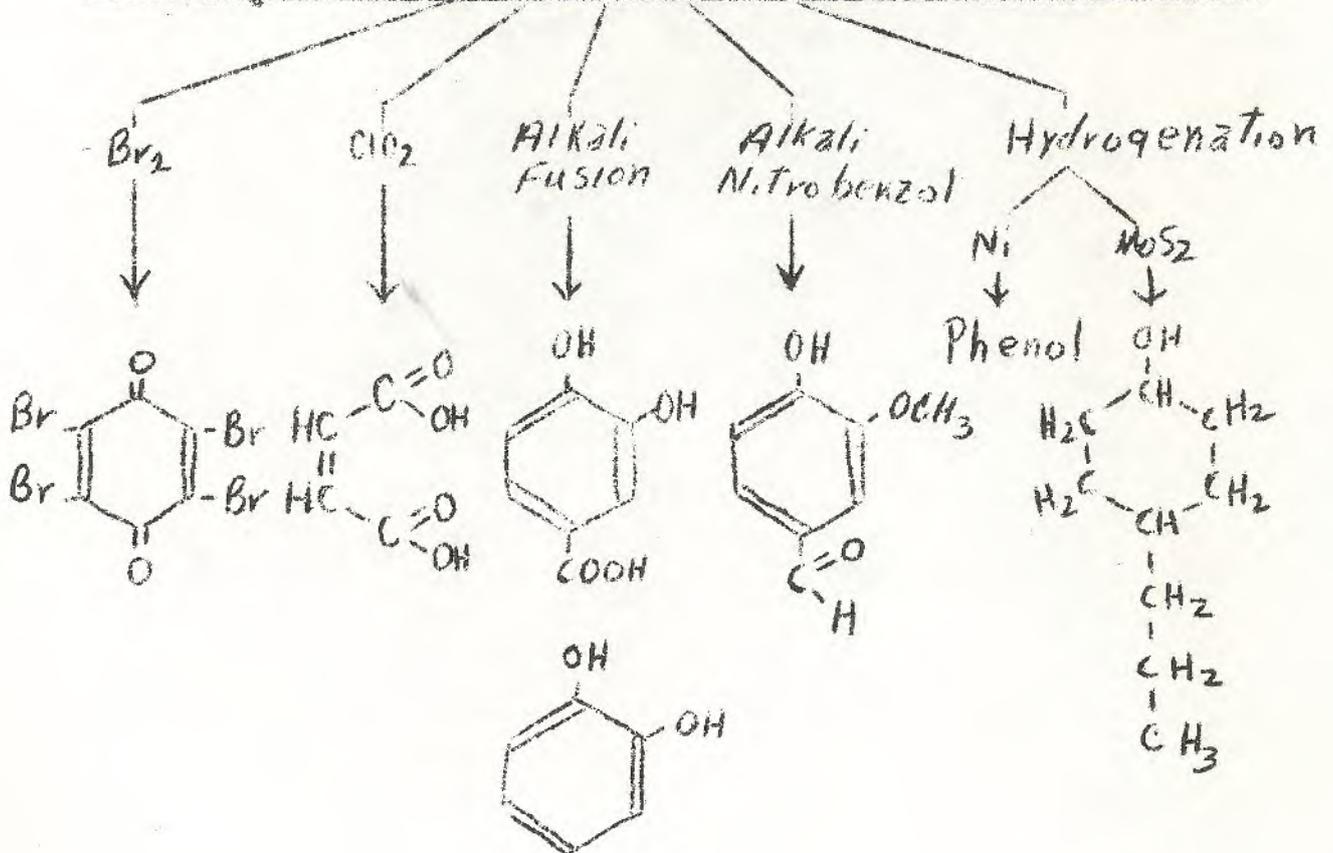
Bone, Horton and Ward investigated fractions of humin which are insoluble in benzene, by treating with alkaline permanganate solution. They could isolate 50 and even more percent of benzene carbonic acids. These high yields of benzene carbonic acids allow the supposition that the ring structure of benzene already must be represented in humic acids.

Some other authors suppose phenolic hydroxyl groups as well as carboxylic groups in the molecule of humic acids. (Stadnikoff, G., S. Ssyskow und A. Uschakowa: Kolloid-Zeitschrift 71 (1935) 206 - Fuchs, W. und O. Horn: Brennstoff-Chemie 11 (1930) 372 - Fuchs, W. und W. Stengel: Brennstoff - Chemie 10 (1929) 303-307). Fuchs determined the carboxyl group by methylation with absolute methyl alcohol in the presence of hydrochloric acid and the phenolic hydroxy groups by further methylation of the ester formed by the first mentioned reactions with diazomethane. Stadnikoff determined the hydrogen of the carboxyl groups by the exchange in neutral reactions with calcium acetate, the phenolic groups in alkaline reaction with sodium hydroxide. Similar investigations were made by Chainski (Chainski, I. A.: Untersuchung uber die Prozesse der Humatbildung mit elektrometrischen Methoden. Kolloid-J. 2, Heft 1, 1936). It had also been possible to distinguish between the carboxyl groups and the phenolic hydroxyl groups by differentiated saponification of the ester- and the ether-methoxyl.

In the next slide there are mentioned the isolated oxidation products.

S 22

Clearance Products of Natural Humic Acids



The oxidation of humic acids with bromine results in tetrabromo-quinone. (Feustel, I. C. und Beyer, H. C.: Soil Sci. 42, 11 (1936). Schmidt and Atterer obtained maleic acid when they oxidized peat and synthetic humic acids by means of chlorine dioxide, up to 10% of the initial material. By reason of detailed model experiments with quinone or furfural, these results point out that by this specific oxidation defined structural elements must be in the humic acids, which consist of a chain of 4 carbon atoms as C-C=C-C and C=C-C=C (Schmidt, E. and Atterer, M., Ber. dtsh. chem. Ges. 60, 1671 (1927)).

By means of alkaline fusion, protocatechuic acid and pyrocatechol could be isolated. (Dragunow, S. S., Schelochowtsewa, N. N., u. Streikowa, E. I.: Pedologija 1948, 409 (Russ.), Pedologija 1950, 151 (Russ)).

The oxidation in alkaline solution with nitrobenzene resulted in a low yield especially vanillin, furthermore syringaldehyde and p-hydroxybenzaldehyde (Bremner, J. M.: Z. Pflanzenernahr. Dung. und Bodenkunde, 69, 32 (1955), (Stach, H.: Das Braunkohlenarchiv, Heft 40, 1 (1933)).

By the hydration with a nickel catalyst under pressure, phenols can be isolated. (Kucharenko, T. A., und Ssawelkew, A.: Ber. A kad. Wiss. UdSSR 76, 77 (1951)).

Propyl-cyclohexanol-derivatives are formed by the hydration with molybdenum-sulfite (Ganz, E.: Ann. chim. 19, 202 (1944)).

Fuchs and Stengel succeeded by oxidation of humic acids from brown coals with diluted nitric acid (1:1) at 90° C during 1½ hours to isolate nitro-phenols and mixtures of crystallized benzene-carbonic acids. The yield of crystallized products has been 10%. (Fuchs, W.: Die Chemie des Lignins, Springer-Verlag, Berlin (1926); Die Chemie der Kohle. Springer-Verlag, Berlin (1931), - u. W. Stengel. Zur Kenntnis der Hydroxyl- und Karboxylgruppen der Huminsauren, Brennstoffchemie 10, 303 (1929), Liebigs Ann. Chem. 478, 267 (1930)).

Kucharenko found ether-linkages in humic acids of mineral coal by splitting off with liquid sodium metal in liquid ammonia. (Kuchanenko, T. A., T. Wweden-skaja u. W. Belgowa: Wechselwirkung der Huminsauren aus Steinkohlen mit metallischem Natrium in flussigem Ammoniak. Berichte d. Akad. d. Wiss. d. UdSSR 86, Nr. 3, 601 (1952)).

Schmuk could identify indole, skatole and derivatives of pyrrol by fusion with alkali. Besides these heterocyclic compounds, he found also protocatechuic acid and phenol. (Schmuk, A. A.: Zur Chemie der organischen Stoffe des Bodens. Arbeiten des Kubaner Landw. Instituts 1, Heft 2, 1924)).

By hydrolysis of humic acids it has been possible to identify amino acids. Not all nitrogen could be hydrolyzed; some of this remains in the residue of hydrolysis.

S 235a (See page 13a in section "The decomposition of known compounds of plant residue, etc.)

S 235b

Gesamt-N, Amino-N und Humin-N in Huminsäuren

Autor	Material	Gesamt-N	Amino-N	Humin-N
			in % von	Gesamt - N
Sowden (1949)	Huminsäure aus kanadischer Schwarzerde	3,5-3,8	16	etwa 50
Laatsch (1951)	Cellulose-Huminsäure	8,1	20	68
	Weisstorf-Huminsäure	1,9	nicht bestimmbar	nicht bestimmt
	NH ₃ -Chinon-Huminsäure	0,95	desgl.	85
	Pepton-Chinon-Huminsäure	5,6	28,5	64
Laatsch (1952)	Pilz-Huminsäure (<i>Spicaria elegans</i>)	8,47	60	5
Parker (1952)	Verschiedene Huminsäurefraktionen aus verschiedenen Böden	3,2-6,2	12-39	26-57
Wittich (1952)	Huminsäure aus verschiedenen Böden und Humusaufträgen	2,37-5,42	39-64,2	7,5-22,5
Sowden (1953)	Huminsäuren aus Böden	-	20-30	34
Schlichting (1953b)	Huminsäuren aus:			
	Fehmarn-Braunerde A	3,78	36,5	37
	Heidemoor-Podsol A _h	1,87	18,0	69,1
	Heidemoor-Podsol B _{he}	1,61	24,1	53,8
Okuda (1954)	Huminsäuren aus Böden	keine Mengenangaben		
Bremner (1955)	Braunhuminsäure	4,01	37,5	30,9
	Grauhuminsäure	2,29	10,5	76,4
	(aus "Hildesheimer Schwarzerde")			

In this slide a summary is given about the nitrogen content of different kinds of humic acids and the distribution of amino-nitrogen and humin-nitrogen. The most extensive investigations about the amino acids in humic acid was made by Bremner. (Compare: Bremner, J. M. Studies on Soil Humic Acids. I. The chemical nature of humic nitrogen. *J. Agric. Sci.* **46**, 247-256 (1955); J. M. Bremner: Recent work on soil organic matter at Rothamsted; *Z. Pflanzernähr. Düng. u. Bodenk.* **69**, 32 (1955); Bremner, J. M. Studies on Soil Humic Acids. II. Observations on the Estimation of Free Amino Groups. Reactions of Humic Acids and Lignin Preparations

with Nitrous Acid, Agric. Sci. 49, 352-360 (1957)). He found nearly the same amino acids which have been mentioned when talking about amino acid content of soil organic matter.

Only in humic acids, which can be isolated from older cultures of streptomycetes, have a higher content of tyrosine. (Bremner, J. M., W. Flaig, and E. Kuster: Zur Kenntnis der Huminsäuren. IX. Der Gehalt an Aminosäuren in Streptomyceten-Huminsäuren. Z. Pflanzenernähr., Dung., Bodenkunde 71, 58-63 (1955)).

In connection with these investigations Bremner determined the differences of composition of humic acids isolated on the one hand with alkali and on the other with pyrophosphate solution as an extraction agent. The alkali extracted humic acids, had a higher total nitrogen content and a higher proportion of acid soluble nitrogen and α -amino nitrogen. He investigated 9 different soils and found that 30 to 50% of the nitrogen in the alkali extracted preparations and 20 to 35% of nitrogen in the pyrophosphate-extracted preparations was liberated in the form of amino acids by acid hydrolysis. At the same time, he determined by means of paper chromatography also the content of amino sugar nitrogen using the method of Tracey and found a content of 3 to 8%. A considerable amount of nitrogen in the humic acids examined was not dissolved by hydrolysis with acid or alkali. The amount has been between 20 and 60%.

By the use of the mentioned method, fusion with a mixture of sodium hydroxide and sodium acetate, we could show that a part of this not hydrolysable nitrogen in the residue of the hydrolysis is heterocyclic bound nitrogen, some of this is combined in indole derivatives.

One of the main tasks is to find methods to divide the humic acids which consist of a mixture of relatively similar groups of substances. Some work about this problem shall be mentioned.

The procedures applied so far to isolate the humic acids of soil always result in a mixture of related substances. Therefore every possibility to separate the humic acid fraction that can be isolated from soil, into components of relatively greater uniformity has to be considered as means of progress in investigating this group of natural elements.

Observations on the separation of the humic acids by sodium hydroxide and sodium chloride have already been made at an earlier date by U. Springer (1) and A. Th. Tjulin (2). It also has been stated by other authors (3, 4, 5, 6, 7, 8, 9, and 10) that the humic acids have to consist of different fractions. We, however, have followed this distribution farther on, and have considered it as a main task to characterize and compare the substances thus separated as thoroughly as possible regarding their physical and some chemical characteristics. Such investigations on humic acid fractions from the same soil have till now only been described with the exception of the nitrogen determinations by L. Meyer (11, 12) and the definition of the sorption capacity and the ash contents by A. Th. Tjulin. (2)

The humic acids of a black soil were isolated and by fractionated precipitation with sodium hydroxide or in nearly neutral medium with sodium chloride were divided into two components. These components in their characteristics greatly correspond with what in present literature is known as "Gray" and "brown" humic acids (1). For this reason the preliminary names chosen for these two components were "G-fraction" and "B-fraction"

As extraction method sodium hydroxide in weak concentration (approximately 0.3%) was chosen so as to treat the humic acids as carefully as possible. To avoid repetition, refer to the following bibliographical data (5, 6, 8, 13, to 36) concerning the problem of extraction of humic acids from the soil. After investigations of F. Scheffer and E. Welte (34, 37, 38) and in accordance with U. Springer (1), no differences in the absorbing spectrum of neutral and weakly alkali isolated preparations can be found. A quantitative extraction of humic acids was not intended.

Extraction of humic acids.

80 kgs of black soil (Acker-Schoepenstedt) (pH KCl 7.10; pH H₂O 7.33; 0.154% Total-N; 4.2% lost by combustion 1.6% carbon (chromic sulphuric acid method) were screened air dry to 2mm and added with approximately 300 liters of tap water and 8 liters of concentrated hydrochloric acid and repeatedly stirred in the cold. After standing over night the liquid was decanted, the soil was first washed with tap water and then two times with 100 liters of softened water each.

The soil pretreated in this manner, then was added with approximately 200 liters of softened water and 2 liters of 50% sodium hydroxide (total concentration of sodium-hydroxide = 0.3%) and again thoroughly and repeatedly stirred in the cold. After setting through the night the dark brown alkaline solution was removed.

In order to separate inorganic admixtures the whole solution was put through the Padberg-centrifuge (continuous cylinder). The humic acids were precipitated with hydrochloric acid and centrifuged. For further cleaning the solution was reprecipitated three more times. Every time, inorganic impurities were separated from the alkaline solution by centrifuging (in the Stockcentrifuge).

The acid centrifuged humic acids were frozen in dry ice in V2A-steel cups. After thawing, the now granulated humic acids were separated from thaw water on a suction filter and washed with distilled water, until, after the destruction of the organic substance with hydrogen peroxide, no more chlorine ions were found.

The product was dialysed in a Brinzing dialyser against slowly flowing distilled water and was stirred, at first without electric current for some days, after that with maximum 50mA, until the passage of current sank down below 10mA at 110 Volts. While dialysing, yellow to red coloured substances segregated through the membrane, electro-dialysing into the anode area exclusively, while the cathode area stayed colorless.

The dialysed solution then was concentrated at 14 mm Hg on a water bath at 30°C at the most and mainly under exclusion of oxygen.

Immediately afterwards, the flask was connected with the high vacuum apparatus for freeze-drying. The evaporation energy was given by irradiating the flask from the outside with an incadescent lamp (60 Watt). In this way, humic acids in lamelliform shape and with moisture contents of 2 to 3% are produced.

The ash content amounted to 14.7%.

From the product from the described isolating and cleaning process neither ether soluble nor alcohol soluble substances were dissolved.

Some authors (10, 39, 40) in the past gave the information that humic acids can change themselves irreversibly when drying. This could not be observed with our products dehydrated in all cases in the vacuum of 0,01 mm at room temperature or by freeze drying.

100 mg of the electro-dialysed humic acid product dried in high vacuum at room temperature were dissolved without residue in 100 ml.

0,01 n NaOH	pH 9,5
0,01% Na ₂ CO ₃	pH 6,2
0,1% NaF	pH 6,2

In 100 ml of the concentration each time less a decimal power, the same amount could no more be dissolved without residue.

Fractionating

The division of the humic acid fraction into a precipitable G-fraction and a non-precipitable B-fraction was done in two ways.

a) With 2n sodium-hydroxide

A quantity of moist humic acids conforming to about 65 g of dry substance (ash content 9.6%) which had been reprecipitated three times with 0.1 percent sodium fluoride, frozen out and washed in the suction filter after thawing until they were free of fluoride, was dispersed in approximately 0.01 N sodium hydroxide. The solution of about 4 liters was increased to a total concentration of 2N (=8%) by the addition of sodium hydroxide. Immediately afterwards the solution was hurled through the Stock-centrifuge for 60 minutes, and the dark brown solution (B-fraction) was separated from the sediment.

The sediment (G-fraction) was stirred with distilled water and dissolved again. The solution still reacting alkaline but weaker was again hurled for 60 minutes, whereby only a very small, inorganic sediment segregated. After this the solution was acidified with hydrochloric acid.

The brown solution, in which the B-fraction was, was acidified with hydrochloric acid. The acid precipitation solution of the B-fraction, containing a large amount of sodium chloride, was colored red yellow; the precipitation solution of the G-fraction, however, was colorless, and according to definition, did not contain any fulvic acids.

Both the sediments (G- and B-fraction) of the humic acid were separately from each other dissolved again in weak sodium hydroxide. The G-fraction solution was treated with sodium hydroxide for the second time, that is, reprecipitated in alkaline solution. The main quantity of the humic acids was precipitated again. The sediment was added to water; the weakly alkaline aqueous solution was centrifuged for 60 minutes in order to sediment some more small quantities of inorganic by-products. Then the product was acidified with hydrochloric acid, whereupon the black-grey humic acids of the G-fraction were deposited.

The G-fraction sediment was frozen out and produced after thawing a phase separation into colorless water and granulated humic acids. These were electro-dialysed; the solution in the outer areas stayed colorless.

More sodium hydroxide was not added to the solution of the B-fraction up to a total concentration of 2N in order to avoid the damage eventually to be expected with this concentrated alkali. The neutral solution was again acidified; the B-humic acids were segregated from the red-yellow fulvic acid solution. The sediment of the B-fraction was washed out by centrifuging and then frozen out in dry ice. Since the gel was reformed by thawing, this fraction was immediately electro-dialysed, whereupon yellow colored substances formed in the anode area. pH after completion of the dialysis: G-fraction 3.4 and B-fraction 3.2. The concentration and the freeze-drying were done as with the whole product. When concentrated, the B-fractions foamed considerably more than the G-fractions. The G-fraction possessed an ash content of 14.6%, the B-fraction one of 3.4%.

The precipitation of the G-fraction cannot depend on the OH-ion concentration, but only on the concentration of the Na-ions, as the same fractioning in B- and G-fraction is also possible with NaCl in neutral medium.

b) with 2N NaCl

A quantity of humic black earth equivalent to about 8 g of dry substance precipitated and burst with frost as done with a) was dispersed in water and regulated with sodium hydroxide to pH 7 to 8. To the solution now consisting of approximately 1 liter was added sodium chloride up to a total concentration of (12%). After that it was centrifuged for 60 minutes, whereby the G-fraction sedimented as dark brown solution. The pH decreased about 0.5 units through the addition of table salt.

The solution (B-fraction) was poured off and acidified; the humic acids sedimented from red-yellow fulvic solution.

The sediment of the sodium-chloride-precipitate (G-fraction) was dissolved with distilled water, the watery solution was centrifuged, whereby only a small amount of inorganic sediment formed, and was also acidified. The supernatant solution was also completely colorless.

In order to complete the separation the precipitation was repeated three more times. At the third sodium chloride precipitation the G-fraction practically falls out completely, while no more sediment can be centrifuged out of the B-fraction. Finally the acid sediments of both fractions were electro-dialysed, whereby colored particles of the B-fraction migrated into the anode space, while the G-fraction did not yield any colored particles to the outer solution. While being concentrated in the water jet vacuum at 30° C, at the most, the B-fraction foamed, however, not the G-fraction. Following this the product was dried in the high-vacuum. Nearly the same operation for separation has been made with sodium chloride instead of sodium hydroxyde. As a dry preparation, the B-fraction is a dull brown powder, the G-fraction a greyish black gleaming powder. The ash contents of the G-fraction is 10.18%, the one of the B-fraction 3.42%.

Working with a weakly alkaline to almost neutral medium and at room temperature, makes a chemical reaction of the preparation nearly impossible. Involved is only a division of a mixture into the two different components contained in that mixture. By the means of paper electrophoresis it can be demonstrated that both components exist in juxtaposition. By repeated separation both fractions can be cleared of contamination of the other components.

The experiment to apply this kind of fractionating to other findings of humic acids, too, was performed by us only in some small evaluations in the manner of samples at random. The isolation of these two types (G-fraction and B-fraction) was also successful from the A-horizon of a podzol. Two products in about the same quantity each were received, which correspond to the two fractions of the black earth and which were partly included in further experiments.

The comparison of results of fractionating does not allow for an exact statement as to the actual relation in quantities, since losses are unavoidable. However, it could be noted that the relation of 1 to 1 cannot deviate too much, although in the black earth the G-fraction and in the podzol the B-fraction predominate.

In this paper, the preparations separated with sodium hydroxide were provided with the subscript a = alkaline, the ones separated with sodium chloride were provided with the subscript n = neutral:

G_a = G-fraction precipitated with 2 N NaOH

G_n = G-fraction precipitated with 2 N NaCl at pH 7-8

B_a = B-fraction, not precipitable with 2 N NaOH

B_n = B-fraction, not precipitable with 2 N NaCl at pH 7-8

Some characteristic differences between the two humic acid components already appear at the fractionating. The G-fraction is more apt to flocculate. The differences in depth of color and shade of color in the solutions as well as in the separations finds its objective expression in the absorption curves.

Again the striking difference in the color of the hydrochloride solutions should be pointed out. The yellow coloring always occurs when humic acids are flocculated from any solution by acid. Even products dialysed over a long time and then dried demonstrates the yellow acid solution when separated from a solution. Then it is more surprising that the G-fraction does not show any characteristic coloring of its acid separating solution. This can be taken for a characteristic feature of this fraction. The separating solutions of the B-fraction, however, are colored yellow to yellow-red.

U. Springer and A. Wagner (29) also observed that the weakly alkaline solution of their grey-humic acids resulted in a pellucid solution on acid-separation. The same happened with their fungus humic acids while their brown humic acids and their quinone-humic acids resulted in yellow acid solutions, that is fulvic acids. At an earlier date K. Simon (5, 41) had stated that "humate solutions from coals get completely colorless after separation, whereas soil extracts always give reddish residue solutions."

According to the observations made at acid separation no colored substances in the outer solution can be seen for all G-products dialysed. Dialysing the whole preparation, and particularly the B-preparation in the anode dialysate, always resulted in outer solutions colored yellow to yellow-red.

An essential point in the preparation is the freezing out of the gels, (42,43). The G-fraction frozen-out and rethawed can be washed free of ions on the suction filter. The B-fraction easily redisperses after freezing out and therefore cannot be washed. This different reaction might well be connected with the opinion presented by F. Scheffer (44) that grey humic acids have to be considered as weakly hydrophilic, the brown humic acids, however, as strongly hydrophilic.

Under the electromicroscope the products do not differ from the ones described at an earlier date by W. Flaig and H. Beutelspacher (45, 46). One recognizes that at the same pH the G-fraction forms denser aggregates than the B-fraction, which is a consequence of its being more capable to flocculate.

Insoluble in acetylbromide were referred to ash-containing dry substance:

90% of the total preparation of black soil

92% of the G-fraction

84% of the B-fraction

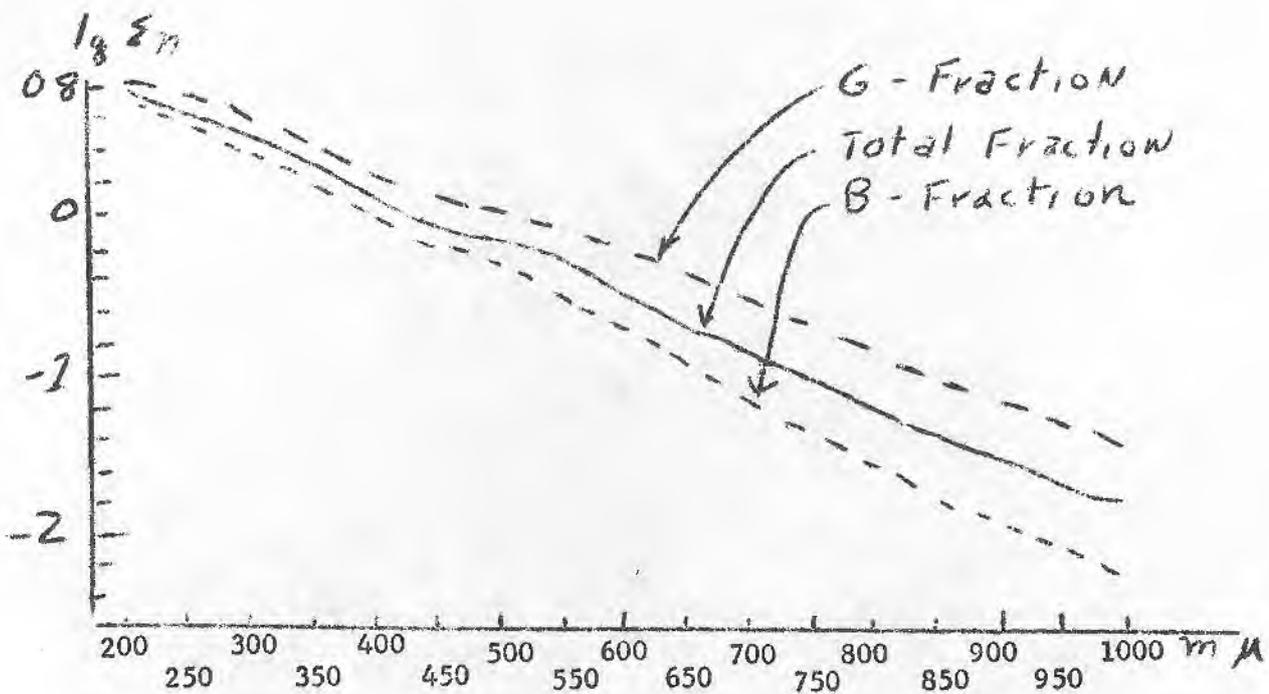
The ash content after the treatment with acetylbromide was around 1% or below. The organic admixture of the G-fraction as well as of the total preparation therefore proved to be practically insoluble in acetylbromide. Of the organic admixture of the B-fraction, about 13% were soluble in acetylbromide.

Dried sodium humate preparation soluble in water could be produced by separation with sodium hydroxide of neutralized solutions of the total humic acids from black soil of the G- and B-fraction with acetone. For some investigations it is valuable if the samples of humic acid preparations can immediately be dissolved in water.

1) Absorption of light. The following figure shows that the curves of total humic acids are in the middle between those of G- and B-fraction and that the curves of the G-fraction of chernozem and the G-fraction of podzol are the same in a space from 350 m μ to 900 m μ . There are only small deviations which are within the limit of error. The elementary analysis of the G-fractions from the two different soils are also nearly similar.

The measurements have been made in such a way that the amount weighed has been calculated from the equivalent weight. For comparison distilled water was used. Because the relation with the molecular weight in these cases is not possible, the humic acids had been calculated uniformly for the normal concentration of 100 micrograms of organic substance. According to the references out of literature, (1, 34, 37, 38, 47, 48) the curves do not show maxima. Similar to the curves of grey humic acids given in the literature and those of brown humic acids, those of the B-fractions are steeper than those of the G-fraction. In the case of the humic acids of chernozem the absorption of the total preparation is also measured before the fractionation. The curve of the total preparate lies in the middle.

S 156



Humic Acids From Chernozem

Springer has introduced the color quotient

$$A_{4/6} = \frac{\text{Extinction at } 472 \text{ m } \mu}{\text{Extinction at } 66 \text{ m } \mu}$$

According to our measurements we introduce the color quotient

$$\frac{E_{475 \text{ m } \mu}}{E_{675 \text{ m } \mu}}$$

S 157

The G humic acids of chernozem and those of podzol have practically the same color quotient. The color quotient of the humic acids of the B-fraction of podzol is a little higher than the one of chernozem. In accordance with older measurements of grey humic acids and brown humic acids the color quotient of the G-fraction is lower than that of the B-fraction, but in every case it is higher than assumed by E. Welte and Springer for grey humic acids.

The light absorption has also been measured for preparations from chernozem. After hydrolysis with HCl the course of the curve and therefore the color quotient, is not changed. In the case of the G-fraction the location of the curve has not changed, either. The curve of the total preparation and the one of the B-fraction

are a little higher after hydrolysis. The transmission of light is also smaller in both cases in the solution of the residues of hydrolysis, even at the same amount weighed, than in the solution of the organic preparations. The intensity of color is therefore greater during the hydrolysis; especially in the case of the B-fraction yellow-colored substances similar to fulvic acids are split off. On reprecipitating the residues of hydrolysis of the B-fraction, there are no more substances similar to fulvic acids.

2) Conductometric Titration. With conductometric titration we found that the total preparation of black soil and the single fractions have the same behavior. The equivalent weight is calculated at about 270, the sorption capacity at 375 meq/100 g. After salt hydrochloric acid hydrolysis, the degree of acidity decreased uniformly. Calculation resulted in an equivalent weight of about 300 and a sorption capacity of 330 meq/100 g. The degree of acidity of both podzol fractions was very different compared to each other and also against the black soil humic acids. For the podzol-G_n-fraction resulted an equivalent weight of about 225, for the B_n-fraction one of about 140 and a sorption capacity of 450, respectively, 700 meq/100g.

3) Paper Electrophoresis. H. Beutelspacher (45) found when determining the migration rate of humic acids in the electric field that two layers occurred in the anodic space with natural and synthetic humic acids, of which one migrated faster. Therefore, it was of interest for us to investigate the total humic acids as well as both fractions by way of paper electrophoresis.

The electrophoresis was done in the apparatus by Grassmann and Hannig. As a solvent they used veronal buffer pH 8.6. In this the humic acids were dissolved to 0.4% (total preparation) or to 0.2% (B- and G-fraction). 0.3 ml of this solution were put on a start line which was a little displaced (or shifted) in direction of the cathode. The rate of running was 1 and 2 hours, respectively, at 110 volt direct current.

In the electropherogram of the total preparation of black soil humic acids, there appear two fractions very clearly: One slow moving of black color, and one moving faster of brown color. As electrophoresis of the single fractions shows, these correspond to the substances of different migration rate which are visible in the total preparation. Through this is proved by means of a method which is completely different than the one of precipitation, that in the humic acid fraction of chernozem there must be two different components which in themselves are relatively uniform, and that these components can be separated by the described fractionating method by means of sodium chloride. Hence, we do not have secondary formations with the B-fraction and G-fraction, but they prove to be performed in the electropherogram and in the starting preparation (total prepareate). More than these two fractions do not occur in the electropherogram of the total prepareate at a pH 8.6. We leave it to later investigations to determine the influence of inorganic constituents on fractionating by means of precipitation and on separation by electrophoresis.

Simultaneously with us, also E. Welte found paper electropherogram of humic acids of different origin. Also he describes the separation of humic acids in two or more fractions under influence of the electric field, as well as the different color character of the fractions. Contrary to this, W. Laatsch (43) could not succeed with a separation in fractions, neither with nitrogen-rich fungi humic acids nor with nitrogen-poor white peat by means of electrophoresis with the apparatus of M. Macheboeuf (49). The preparations investigated by him do not contain inorganic substances.

4) Viscosity. As already found by W. Flaig and H. Beutelspacher (46) with measurements of the viscosity of solutions of natural humic acids and of humic acid model substances, the humic acids are sphero-colloids. This result is proved by the following curves. The measurements were done with the viscosimeter of Wi. Ostwald at a temperature of $20.00^{\circ} \pm 0.05$. By application of water-soluble sodium humates, the concentration range could be enlarged. The solutions react almost neutrally (pH 6.6 - 7.4). The viscosity number η spec/C is constant and is for the B-fraction 0.0088 and for the G-fraction 0.0071. From the ratio of the found to the theoretical viscosity number, one can conclude according to H. Staudinger (50) in sphero colloids the volume strain and hence the hydration of the colloids. The volume strain of the B-fraction is 3.5; the one of the G-fraction is 2.8. Hence, the B-fraction is stronger hydrated than the G-fraction, a result which agrees with the earlier discussed observations.

The surface tension of the same sodium-humate solutions on which the viscosity was determined, was measured with a stalagnometer (drop-counting method). All solutions of the G-fraction showed the same drop number like the double distilled water which was used as solvent - according to this, the G-humic acids do not lower the surface tension of water until concentration of 2%. Contrary to this, the 3.2% solution of the B-fraction showed about 18% decreased surface tension compared to distilled water. We would like to remind you again that the B-fraction foams more with evaporation.

5) Ash-Contents. According to the analysis of ash (total preparation black soil, non-dialyzed), it consists of about 70% SiO_2 , 20% Al_2O_3 and 10% Fe_2O_3 . Slight traces of Mg. and Ca. could be found. Phosphate and sulfate could not be found in the ash, and also not alkalis.

With the fractionation, also the inorganic part distributes in characteristic way between the two components. The G-fraction contains about three times as much ash as the B-fraction. When boiling the fractionated preparations with hydrochloric acid, results indicate that all the existing silicate is in the G-fraction. (The ash of the hydrolysis-residue of the G-fraction is completely white and consists of silicic acid and aluminum oxide.) The B-fraction is silicate-free, its total ash contents dissolve with hydrochloric acid.

Also the ash of podsol-B-fraction solves completely when heated (boiled) with hydrochloric acid, hence, does not contain silicate. However, the ash of the podsol-G-fraction contains again silicic acid. Also in another preparation, which was isolated from clay-rich black soil of Harsum fraction B does not contain silicic acid.

The ash of the G-fraction shows strong interferences with electron diffraction : after wet combustion with 15% hydrogen peroxide. We did not yet succeed to classify it to a certain clay mineral. For this, further investigations are necessary. The ash of the B-fractions shows no interferences in the diffraction diagram.

III. Chemical Investigations

1. Elementary Analysis

The results of the elementary analysis are summarized in the following table:

	%C	%H	%O	%N	Sum	%OCH ₃	%Ash	Color of the Ash
G-fraction	62,61	2,74	33,23	2,27	100,85	0,14	14,76	carmine red
G _a				(2,53)				
Black soil	61,60	2,79	32,42	2,83	99,64	0,78	10,18	carmine red
G _n								
G-fraction	61,82	2,96	33,93	2,18	100,89	0,00	5,36	carmine red
Podsol								
B-fraction	57,62	3,97	33,01	4,08	98,68	1,41	3,39	black-brown
B _a				(3,79)				
Black Soil	56,97	4,25	33,66	4,19	99,07	1,60	3,42	black-brown
B _n								
B-fraction	59,40	3,07	35,34	1,89	101,10	0,27	2,38	grey
Podsol								
Black Soil								
Total	57,78	3,25	34,32	3,90	99,52	0,95	9,60	carmine red

The nitrogen values (in brackets) were found on a later sample of the same material.

We can say that the way of fractionating (alkaline way with 2n NaOH or neutral way with 2n NaCl) had no considerable influence on the elementary composition. Especially, the oxygen content in preparations which were exposed to stronger alkaline influence was not higher than in those which were never exposed to higher NaOH-concentration than 0.3%. If, then "changes" in the sense of different authors (1, 6, 15, 16, 17, 22, 28, 30) should occur by means of alkaline reaction in the presence of oxygen, they either do not concern the elementary composition or they occur already with concentration of 0.3% during extraction. Also with all other investigations, there could be no difference found between the preparations of the same fraction but different way of fractionating (G_a and G_n on one hand, and B_a and B_n on the other hand. Hence, from this result it cannot be supported that the^a separation into G-humic-acids and B-humic acids with NaCl is more "careful" than the precipitation with 2n NaOH. Nevertheless, it will be of advantage, if one can obtain the same effect with a mild rather than with an aggressive reagent, and one then will prefer, especially with natural substances, the milder conditions.

Comparison of the two types shows that the G-humic acids contain more carbon and less hydrogen than the B-humic acids. Comparative elementary analysis of both humic acid types have not been published so far. If one would take conclusions from the elementary analyses of humic acids of different origin which are given in the literature, one could assume higher carbon content in brown humic acids than in grey humic acids, since peat humic acids mostly contain more carbon than soil humic acids. Recently, U. Springer has indicated this. (29).

The higher carbon content of the isolated G-fractions compared to the B-fractions from the same soil (black soil and podsol) has to be considered in connection with the extremely low hydrogen content. Such low hydrogen contents are, as far as we know, until now not given for humic acids. Among 75 elementary analyses of humic acids of different origin, which we compiled, one cannot find a one value for hydrogen below 3% and only 10 hydrogen values below 4%. Of these, six were found with Kassel Brown and the two lowest in black soil of the Magdeburger Borde (51).

However, it has to be emphasized that we have only very few elementary analyses of soil humic acids. In most cases only the carbon was determined by wet combustion with chromic sulphuric acid and the nitrogen by means of Kjeldahl method.

Further investigations on separated preparations of both types of soil humic acids are necessary, before we can say if the amount and the ratio of carbon and hydrogen, like it is found here in the G-fraction of two different soils, can be regarded as characteristic for grey humic acids. The grey humic acids from marshy soil by U. Springer (29) do not fit in here - they have a carbon content of 54.52%, the hydrogen content was not determined.

The carbon- and hydrogen values of the B-fraction correspond approximately to the values given for humic acids, though the hydrogen content, especially with podsol-B, is pretty low.

Concerning oxygen content, which was determined separately, the two fractions of black soil practically do not show any difference. How far this agreement extends also to the functions of oxygen, has to be found by further investigations. So far, we could only find that the degree of acidity, that is the equivalent weight resulting from the conductometric titration, is the same for B-humic acids and G-humic acids from black soil.

The oxygen content of the podsol-G-fraction corresponds approximately to the black soil humic acids, the podsol-B-fraction, however, has a higher oxygen content. Both preparations are more acid than the black soil humic acids.

The nitrogen values of black-soil humic acids agree with the only thus far known comparison numbers of L. Meyer (12), however, are extended more and contradict with these the older assumptions about the nitrogen content of grey humic acids and brown humic acids.

It seems as if a higher nitrogen content, contrary to the original assumption of U. Springer (1) and after him of different other authors, is not an important characteristic of the grey humic acids. The nitrogen values of both fractions of podsol behave to each other reversely; the difference here is, however, slight but the higher value is due to the G-fraction.

Besides, it has to be considered that the analysis numbers do not say anything about the bonding form. As it will be shown later, part of the nitrogen is dissolved during hydrolysis, from the B-fraction of black soil so much that the nitrogen content of both fractions is practically equal after hydrolysis.

The sum of the values of carbon, hydrogen, oxygen and nitrogen, calculated on ash-free dry substance, shows that other elements cannot be there is considerable amount.

A safe possibility to separate "genuine" humic acids from lignin does not exist so far; hence, we also do not know if humic acids can contain methoxyl or if the methoxy content indicates that there are impurities with lignin. The presence of 10% lignin with 15% methoxyl content in a humic acid preparation would result for this a methoxyl content of 1.5%. There exists no proof for or against the presumption that the B-preparate of black soil was mixed with 10% lignin. From this, it might become clear that also the separated components of the humic acids of black soil and podsol do not present a uniform compound in themselves.

The preparations further were examined for uronic acids.

From these investigations one could estimate a total uronic acid content of the humic acids as less than 1%. According to this, probably the methoxyl content in humic acids is not due to the polyuronic acids.

2. Reaction with dinitro-fluorobenzene, model experiments

The acid properties of humic acids are generally due to carboxyl groups and acid hydroxyl groups. Different methods (16, 17, 39, 52-56) have been applied to distinguish carboxyl groups and phenolic hydroxyl groups from each other.

All these differential methods are based on certain assumptions about reactivity of functional groups in a certain kind of bonding. But model experiments show again and again that the same assumptions can only be applied on complicatedly built or different organic compounds with great restriction.

With these considerations, the experiment has been done to determine the phenolic hydroxyl groups in the humic acid preparates by means of dinitro-fluorobenzene. (DNFB) (57-59)

<u>% N Black Soil</u>	<u>Total</u>	<u>G-fraction</u>	<u>B-fraction</u>
Initial material	3.84	2.40	3.94
After treatment with DNFB	4.01	2.41	5.07
Difference	0.17	0.01	1.13

As one sees, only the nitrogen content of the B-fraction increased. By means of calculation, under the condition that dinitro-fluoro-benzene reacts with phenolic hydroxyl with separation of fluorohydrogen, a phenolic hydroxyl content of 0.65% results; this is a phenolic OH- group on a molecule of the approximate size of 2500.

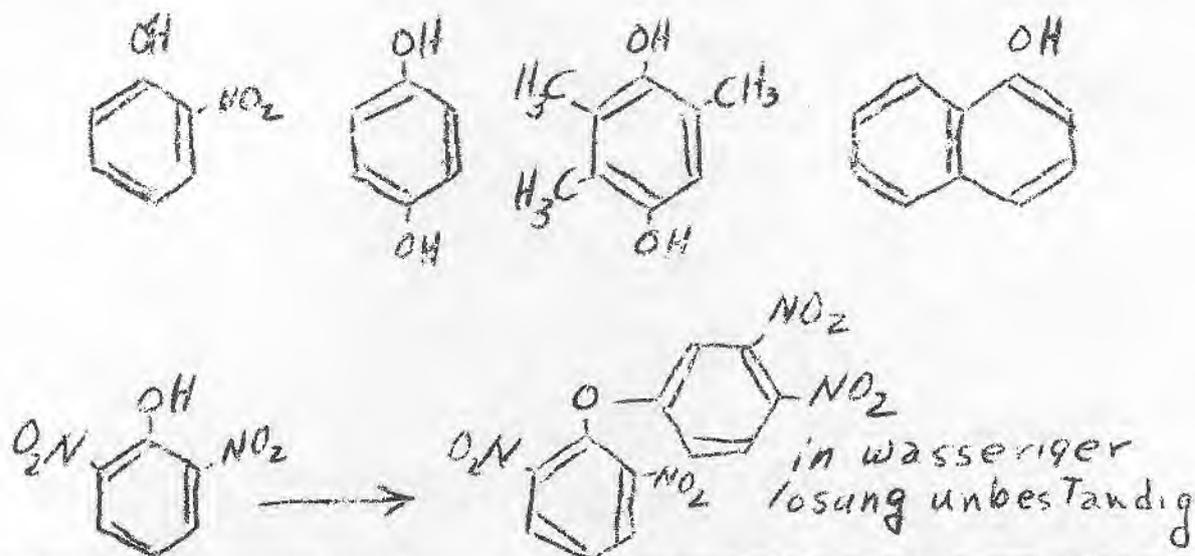
The assumption of such a low content of hydroxyl groups contradicts strongly all assumptions that one can make about the chemical structure of humic acids. Therefore, it was examined in model experiments which hydroxyl groups can be etherified with DNFB under the conditions that have been used for humic acid fractions.

Examined were:

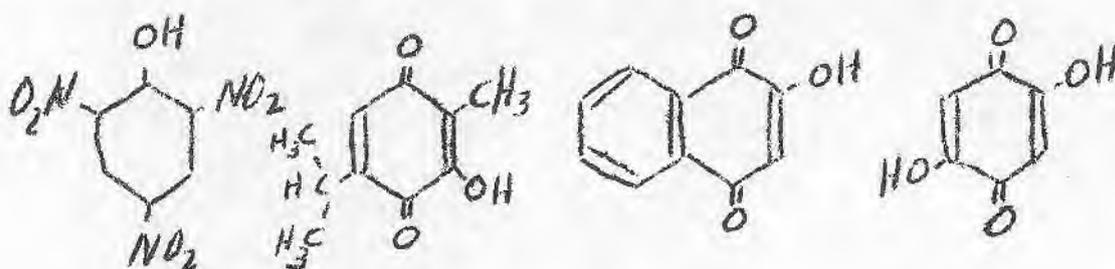
- a) hydroquinone
- b) α -naphthol
- c) o-nitrophenol
- d) trimethyl-hydroquinone
- e) 2,6-dinitrophenol
- f) picric acid
- g) 3-oxy-thymoquinone
- h) 2-oxy-naphthoquinone-1,4
- i) 2,5-dioxyquinone

S 283

Bilden 2,4-Dinitrophenyl ether



Redoxen nicht unter den Bedingungen der Verätherung von Humic acids



The model experiments show that strongly acid phenolic hydroxyl groups cannot react with DNFB. This is also valid for the oxy-groups of quinones which are also very acid. Results from this show that the presence of phenolic OH-groups cannot be excluded for characterization of humic acids because humic acids do not react at all or only slightly with DNFB. If, in fact, the humic acids should contain quinoid structure elements, then one has to consider that the acid character of humic acids can possibly be derived from hydroxyl groups of quinones, since these are strongly acid; hence, there is no forcing reason to postulate carboxyl groups for the acid nature of humic acids.

3. Decomposition with Hydroxylamine and Phenylhydrazine

Furthermore, it was tried to prove carbonyl-groups and quinone groups (60-63) in humic acids and possibly to determine them quantitatively.

When trying to determine the "carbonyl number" according to the instructions, some disturbance occurred due to reductivity of humic acids. It seemed to us to be more useful to obtain the decomposition products of the humic acids prepared with these two reagents and to characterize them by means of nitrogen analyses.

S 161
S 162

During decomposition with hydroxylamine, one nitrogen atom per decomposed carbonyl group occurs, whereas during reaction with phenylhydrazine two nitrogen atoms per decomposed carbonyl group are added. From the nitrogen increasing results for both fractions and with both reagents indicated a carbonyl content of about 2.7%. Hence, one carbonyl group comes on one molecule of size 1000, which hardly agrees with the hypothetical assumption of the quinoid structure of humic acids. The conformity of the values of the carbonyl group determination with hydroxylamine and phenylhydrazine is surprising; however, it should not be used for wider conclusions. Because here we have the same question as with dinitrofluorobenzene; under which conditions, in neighborhood of what groups, do carbonyl groups react with hydroxylamine and phenylhydrazine and when not. How easily quinones react with hydroxylamine differs a lot (64). From the "carbonyl number" the actual content of quinone groups in humic acids cannot be concluded.

The former results indicate how critically all reactions between humic acids and organic group reagents have to be considered. Only by cautious comparison of all results from reactions of the humic acids themselves, which one can do with different methods by decomposition reactions and by investigations on model substances, one can hope to bring the problem of the chemical structure of humic acids nearer to its solution.

It can be assumed, however, that the determination of the chemical constitution of the two separated fractions of the natural humic acids can be done more successfully than in such investigations if only a mixture of the two types is available. Our results indicate that they somehow differ in their chemical structure and that they have to be separated from each other.

4. Hydrochloric Acid Hydrolysis

S 163

Elementary composition of the hydrolysis-residues in comparison with the initial preparates and hydrolysis

	%C	%H	%O	%N	sum	% ash
Black soil total preparation	57,8	3,52	34,3	3,9	99,5	9,6
Hydrolysis residue	63,2	2,7	31,9	2,0	99,9	3,3
Hydrolysate (calculated)	44,2	5,5	40,2	8,7	98,5	6,3
Black soil G-fraction	62,6	2,7	33,2	2,3	100,9	14,8
Hydrolysis residue	64,2	2,1	32,3	2,0	100,6	7,1
Hydrolysate (calculated)	53,5	6,9	35,0	3,9	99,3	7,7
Black soil B-fraction	57,6	4,0	33,0	4,1	98,7	3,4
Hydrolysis residue	62,11	3,1	32,6	2,1	99,9	0,0
Hydrolysate (calculated)	51,9	5,2	33,8	6,9	98,4	3,4

In the table the elementary composition of hydrolysis residues is given in comparison with the elementary composition of the initial material. The elementary composition of the hydrolyzed part was calculated from this as difference. It has to be noticed, that these values do not need to agree directly with that which is contained in hydrochloric solution as organic substance, i.e., a small part of the carbon escapes as gaseous carbon dioxide.

The hydrolysis residue compared to the initial material contains more carbon and less of all other elements, the parts which are in the hydrolysates are hence richer in oxygen, hydrogen and nitrogen. The hydrolysis residues of both humic acids types are less different in their elementary composition than their initial preparator themselves.

Also the nitrogen content is in agreement. As mentioned before, also after hydrolysis the B-fraction does not release fulvic acid-like substances with acid precipitation. If one especially compares the analysis numbers of the hydrolysis residue of the B-fraction with the non-hydrolyzed G-fraction, then these values are very similar. By this, one could assume that the differences between types are mostly based in the parts which are in the hydrolysates. Contrary to this, however, the color quotients of the hydrolysis residues are different in the same way as the color quotients of the non-hydrolyzed preparations.

Especially in the B-humic acids, a great part of the nitrogen (70%) can be split off by hydrochloric hydrolysis. Contrarily, the G-humic acids contain 76% of their nitrogen content in such firm bond that it cannot be hydrolyzed by 24 hours of boiling. If one assumes that the non-hydrolyzable nitrogen is bound heterocyclicly, then it could be assumed that both fractions contain this in the same amount. The higher nitrogen content of the B-fraction of the black soil hence originally was bound in side chains. That, however, both types also have to differ in the non-hydrolyzable part, can be taken from the fact that the hydrolysis residues show different light adsorption behavior.

What percentage of the content of single elements in humic acid preparations remains after hydrolysis and what percentage dissolves, is shown in the following table.

S 164

Calculated ratio of the dissolved to the undissolved part of the single elements with hydrolysis (Element in the initial substance = 100)

	% C	% H	% O	% N	% ash
Total preparate					
residue	78,1	55,4	66,5	36,6	23,1
hydrolysate	21,9	44,6	33,5	63,4	76,9
G-fraction					
residue	88,2	64,1	85,0	76,2	37,6
hydrolysate	11,8	35,9	15,0	23,8	62,4
B-fraction					
residue	62,0	45,0	56,9	29,2	0,0
hydrolysate	38,0	55,0	43,1	70,8	100,0

This shows that the G-humic acids are less changed by hydrolysis than the B-humic acids which can be seen also from the less substance loss of the G-humic acids during hydrolysis.

The hydrolysates were investigated paper chromatographically after removing the hydrochloric acid and the inorganic part. With this, a different composition of the two hydrolysates occurred clearly.

Summary

The humic acid fraction of two soils is divided into two fractions for comparison of their material properties. With reference to the symbols used in literature we mark the precipitable fraction as G-(grey-humic-acid) fraction and the one which remains in suspension as B-(brown-humic-acid) fraction.

I. Investigations in Humic Acids of Black Soil

1. From a black soil (Ackerboden) the humic acid fraction was isolated and in two different ways, once with 2n sodium hydroxide, and once with 2n sodium chloride with almost neutral reaction, divided into two components which exist in the initial mixture in almost same amount.
2. The paper electrophoresis shows that the G-fraction and the B-fraction correspond to the two components appearing in the electropherogram of the total preparation with different migration rate.
3. The preparation of water-soluble sodium humates as dry preparation is described. With the sodium humate of the B-fraction a 4%, with the one of the G-fraction a 2% aqueous solution can be prepared.
4. Measurements of the viscosity show that the B-fraction is more strongly hydrated than the G-fraction. The B-fraction decreases in higher concentrations the surface tension of the water.
5. In hydrochloric hydrolysis 40% of the organic substance of the B-fraction and 12% of the G-fraction is dissolved. In the elementary composition, the hydrolysis residues do not differ much, the nitrogen content is in approximate conformity.
6. The preparations were reacted with dinitrofluorobenzene with the intention to determine phenolic hydroxyl groups. Only the B-fraction could be etherified slightly. Model experiments with acid phenols and oxyquinones showed that strongly acid hydroxyl groups do not react at the ring with dinitrofluorobenzene. Furthermore, it was tried to prove carbonyl- and quinone groups with hydroxylamine and phenylhydrazine. For both preparations with both reagents there resulted the same carbonyl content which was about 2.7%. The possible difficulties of the reaction are indicated.

Furthermore, from the sodium hydroxide-extract of a podsol-A horizon, G-fraction and B-fraction were separated with sodium chloride.

II. Comparison of the G- and B-humic acids from black soil and from podsol

1. The G-humic acids contain or form in all cases no fulvic-acid parts. Their acid precipitation solutions as well as their anode dialysates are not colored. Contrary to this, the corresponding solutions of the B-fraction, as with humic acids in general, are yellow to red-yellow colored.

2. The light absorption of the G-fraction is stronger than the one of the B-fraction. The differences in the slope of the curves correspond to those which were found earlier for grey humic-acids and brown humic-acids. The course of the curves is not changed by hydrochloric acid hydrolysis.
3. From the conductometric titration, the same equivalent weight of about 270 results for the G-fraction and the B-fraction of the black soil. The G-fraction of the podsol is slightly, the one of the podsol fraction is considerably stronger acid.
4. The elementary analyses show that the G-fractions have higher carbon content and lower hydrogen content than the B-fractions. The oxygen content of both fractions of black soil is in conformity with each other and is also approximately in conformity with the one of the podsol-G-fraction; the podsol-B-fraction contains more oxygen. In black soil, the B-fraction has higher nitrogen content than the G-fraction, with podsol it is reversed. The methoxy content is slight.
5. The ash content of G-humic acids is in all cases higher than the one of B-humic acids. Only the G-fractions contain silicate in the ash. The ash part of the B-fractions is dissolved completely when heated with hydrochloric acid.

1. Springer, U.: Der heutige Stand der Humus-Untersuchungsmethodik. Z. Pflanzenern., Dung., Bodenkunde, 6, 312 (1938).
2. Tjulín, A. F.: Herkunft, Struktur und Eigenschaften organomineralischer Bodenkolloide. Z. Pflanzenern., Dung., Bodenkunde 21/22, 544 (1940).
3. Gortner, R. A.: The organic matter of the soil. Soil Sci. 2, 395 und 593 (1916).
4. Piekenbrock, F.: Lichtabsorption und Teilchengröße in Humuskolloiden, Kolloid-Z. 102/103, 146 (1943).
5. Simon, K.: Über die unterschiedlichen Eigenarten extrahierbarer Humussubstanzen. Z. Pflanzenern., Dung., Bodenkunde, 34, 144 (1934).
6. Schlichting, E.: Zur Kenntnis des Heidehumus. I. Fraktionierung und Untersuchung des ganzen Humuskörpers. Z. Pflanzenern., Dung., Bodenkunde 61, 1 (1953).
7. Springer, U.: Über Typen der echten Humusstoffe, ihre Charakterisierung, Trennung und Bestimmung in Boden. Der Forschungsdienst, Sondeheft 7 (1938).
8. Duchaufour, P.: Attempt to fractionate the different humic complexes in soils. C. R. Acad. Sci. 238, 836. (1954). Ref.: Chem. Abstr. 48, 6630. (1954).
9. Natkina, A. J.: Investigations on the composition and properties of humic acids of Chernosem and Podsolis soils. Trans. Dokutchaev Soil Inst. USSR 23, 9. (1940). Ref.: Chem. Abstr. 35, 5178. (1941).
10. Oden, S.: Die Huminsäuren. Kolloid-Beih. 11, 75. (1921).
11. Meyer, L.: Ton-Humus-Komplexe als Träger der Bodenfruchtbarkeit und als Bodenverbesserungsmittel. Der Forschungsdienst 11, 344. (1941).
12. Meyer, L.: Bestimmung der Grau- und Braunhuminsäuren. Der Forschungsdienst, Sonderheft 17, 36. (1941).
13. Bremner, I. M.: Some observations on the oxidation of soil organic matter in the presence of alkali. J. Soil Sci. 1, 198. (1949).
14. Bremner, I. M., und Lees, H.: Studies on the soil organic matter, part II: The extraction of organic matter from soil by neutral reagents. J. Agric. Sci. 39, 274. (1949).
15. Chaminade, C. R.: Sur une méthode de dosage de la fraction humifiée de la matière organique des sols. C. R. Acad. Agric. 32, 131. (1946).
16. Dragunov, S. S., Schelochowtzewa, N. N., und Strelkowa, E. I.: Vergleichende Untersuchungen von Huminsäuren und Torf. Pedology 1948, 409.
17. Dragunow, S. S., Schelochowtzewa, N. N., und Strelkowa, E. I.: Untersuchungen über die chemische Natur der Huminsäuren. Pedology 1950, 151.
18. Duff, R. B.: The occurrence of methylated carbo-hydrates and rhamnose as components of soil polysaccharides. J. Sci. Food Agric. 3, 140. (1952).
19. Fischer, G.: Die Säuren und Kolloide des Humus. Kuhn-Archiv 4, 1 (1914).

20. Forsyth, W. G. G.: The characterization of the humic complexes of soil organic matter. *J. Agric. Sci.* 37, 132. (1947).
21. Ganz, E.: Herstellung enstaschter und von Stickstoff und Schwefel freier Huminstoffen. *C. R. Acad. Sci.* 216, 122. (1943). *Ref. Chem. Zentralbl.* 1944, II, 21.
22. Kondratjew, I. W., und Ssacharow, A. P.: Untersuchung der Huminsäuren. Zur Frage der Ausscheidungsmethoden und der Charakteristic von Huminsäuren (Russisch). *Ref: Chem. Zentralbl.* 1940, II, 3481.
23. Nöhling, K., und Schiemann, R.: Untersuchungen zum Humusproblem. 2. Teil: Beiträge zur Kenntnis der Huminsäuren. *Z. Pflanzenern., Dung., Bodenkunde* 57, 193. (1952).
24. Puri, A. N., und Sarup, A.: Extraction of humus from soil with alkalies and its colorimetric estimation. *Soil Research* 6, 122 (1938-1939).
25. Remezow, N. P.: Comparative Study of methods of isolating humic acids from soil. *Pedology* 1945, 303, *Ref.:* *Chem. Abstr.* 40, 973 (1946).
26. Simec, B. G.: Neue Forschungsergebnisse in der Chemie der natürlichen Huminsäuren. *Brennstoffchemie* 9, 381. (1928)
27. Simon, K.: Die charakteristischen Humusstoffe, ihre Beurteilung und ihre Bedeutung im Stalldünger. *Z. Pflanzenbau, Pflanzenschutz* 3, 145. (1952).
28. Stach, H.: Beiträge zur Kenntnis der Wirkung von Hydrozylionen auf das chemische Verhalten von Huminsäuren. *Brennstoffchemie* 22, 25. (1941).
29. Springer, U. und Wagner, A.: Zur Kenntnis der von Pilzen gebildeten huminsäureähnlichen Substanzen unter besonderer Berücksichtigung von *Stachybotris lobulata*. *Z. Pflanzenbau, Pflanzenschutz* 3, 145. (1952).
30. Stach, H.: Beiträge zur Kenntnis der Konstitution der natürlichen Huminsäuren aus Braunkohlen. *Das Braunkohlenarchiv*, Heft 40, 1-51. (1933).
31. Tjulín, A. F.: Peptisationsanalyse der Bodenkolloide. *Kolloid-Z.* 70, 207. (1935)
32. Tjurin, I. U. und Gutkina, E. L.: Nature of "Humins" in Chernosem. *Trans. Dokutchaevev Soil Inst. USSR* 23, 41. (1940). *Ref.:* *Chem. Abstr.* 35, 8178 (1941).
33. Vernander, N. B. und Sokolowski, A. N.: Study of humus. Chernosem humus as a polydisperse system. *Pap. and Proc. 1st International Congress of Soil Science (Washington)* 3, 367 (1928).
34. Welte, E.: Über die Entstehung von Huminsäuren und Wege ihrer Reindarstellung. *A. Pflanzenern., Dung., Bodenkunde* 56, 105 (1952).
35. Zelle, K.: Zur Kenntnis der Huminsäuren. *Kolloid-Z.* 72, 211. (1935).
36. Zetsche, F., und Reinhard, H.: Über die Huminsäuren des Torfes. *Brennstoffchemie* 18, 393. (1937).
37. Scheffer, F. und Welte, E.: Die anwendung der Absorptionsspektrographie in der Humusforschung. *Z. Pflanzenern., Dung., Bodenkunde* 48, 250. (1950).
38. Scheffer, F. und Welte, E.: Über absorptionsspektrographische Untersuchungen an natürlichen Huminsäuren. *Landw. Forschung* 1, 190. (1950).

39. Fuchs, W.: Untersuchungen über Lignin, Huminsäuren und Humine. Z. Angew. Chem. 44, 111. (1931).
40. Souci, S. W.: Die Chemie des Moores, Ferd. Enke, Stuttgart. (1938).
41. Simon, K.: Humus und Huminsäuren. Practische Blätter für Pflanzenbau und Pflanzenschutz. München, 12, 363. (1935).
42. Forsyth, W. G. G.: Freezing as an aid in the drying and purification of humus and allied materials. Nature 160, 607. (1947).
43. Laatsch, W., Hoops, L., und Bieneck, O.: Über Huminsäuren des Pilzes *Spicaria elegans*. Z. Pflanzenern., Dung., Bodenkunde, 58, 258. (1952).
44. Scheffer, F.: Agrikulturchemie. Teil C: Humus und Humusdungung. Ferd. Enke, Stuttgart. (1941).
45. Beutelspacher, H.: Physikalisch-chemische Beiträge zur Humusforschung. Z. Pflanzenern., Dung., Bodenkunde 57, 57. (1952).
46. Flaig, W. und Beutelspacher, H.: Physikalische Chemie der Huminsäuren. Landbouwkundig Tijdschrift 66, 306. (1954).
47. Hock, A.: Grundsätzliches bei Farbmessungen in Humuslösungen. Z. Pflanzenern., Dung., Bodenkunde, 7, 279. (1938).
48. Farbtiefe und Farbcharakter von Humusextrakten in ihrer Abhängigkeit von der Alkalikonzentration, zugleich ein Beitrag zur Kenntnis der Humustypen. Z. Pflanzenern., Dung., Bodenkunde, 34, 1. (1934).
49. Ma cheboeuf, M: Appareil permettant la separation de fractions proteidiques par electrophorese. C. R. Soc. Biol. 135, 1241. (1941).
50. Staudinger, H., und Husemann: Über Hochpolymere Verbindungen. Ber. dtsh. chem. Ges. 68, 1691. (1935).
51. Scheffer, F., Welte, E., und Hempler, G.: über oxydimetrische Eigenschaften von Huminsäuren. Z. Pflanzenern., Dung., Bodenkunde, 58, 68. (1952).
52. Ubaldini, I.: Beitrag zur Kenntnis der Huminsäuren, Brennstoffchemie 81, 188. (1937).
53. Fuchs, W., und Stengel, W.: Zur Kenntnis der Hydroxyl- und Carboxylgruppen der Huminsäuren. Brennstoffchemie 10, 303. (1929).
54. Stadnikoff, G.: Über Huminsäuren. Kolloid-Z. 80, 60. (1937).
55. Kurschner, K.: Über die Humussaure eines Kasseler Brauns. Der Papierfabrikant I. 38, 205, 216 (1940); II. 38, 253 (1940); 39, 1 (1941); III. 39, 113, 127, 132 (1941); IV. 39, 188 (1941). Cellulose-Chem. 19, 59. (1941).
56. Fuchs, W.: Über die sogenannte "Nitro" - Huminsäure. Brennstoff-Chemie 9, 178. (1928).
57. Freudenberg, K., und Hubner, H. H.: Oxyzimtalkohole und ihre Dehydrierungspolymerisate. Chem. Ber. 85, 1181. (1952).
58. Schramm, G., und Braunitzer, G.: Tyrosin als Endgruppe des Tabakmosaikvirus. Z. Naturforsch. 86, 61. (1953).

59. Zahn, H., und Wurtz, A.: Zur quantitativen Bestimmung von Phenolen mit 2,4-Dinitrofluorbenzol. Fresenius Z. f. Analyt. Chem. 134, 183. (1951).
60. Eller, W.: Studien über Huminsäuren. Einige Eigenschaften der Huminsäuren und Humine. Liebigs Ann. Chem. 442, 160. (1925).
61. Feustel, I. C., und Byers, H. G.: The Behavior of lignin and humic acid preparations toward a bromination treatment. Soil Sci. 42, 11. (1936).
62. Leopold, H.: Ueber die Carbonylzahl einiger Huminsäuren. Brennstoffchemie 9, 215. (1928).
63. Strache, H., und Brandl, A.: Die Carbonylzahl der Kohlen und ihre Beziehungen zum Alter und Verwitterungsgrad. Brennstoffchemie 7, 341. (1926)†
64. Houben, J., und Weyl, Th.: Methoden der organischen Chemie Bd. II, S. 476 u. 477. Georg Thieme, Stuttgart 1953.