

STRUCTURAL STUDIES OF HUMIC ACID
USING ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

by

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ABSTRACT

Solid soil humic acid contains stable organic free radicals, on the order of 10^{18} radicals/g. Degradation studies using CuO-NaOH reveal that these radicals are not caused by impurities or by unstable species trapped in the solid humic acid polymer; but rather are an integral part of the molecule. From this study, estimates are made as to the per cent of the semiquinones present in the ortho or para form. Analysis of the EPR spectrum, after reduction of the sample, and after sodium salt formation, yielded evidence that two stable free radicals coexist in humic acid. One of these is a semiquinone of a catechol or hydroquinone type; the other could be a quinhydrone type radical. It is also found that humic acid exhibits saturation effects at high power values.

Correlations are also made between the EPR data and the IR spectra of the samples.

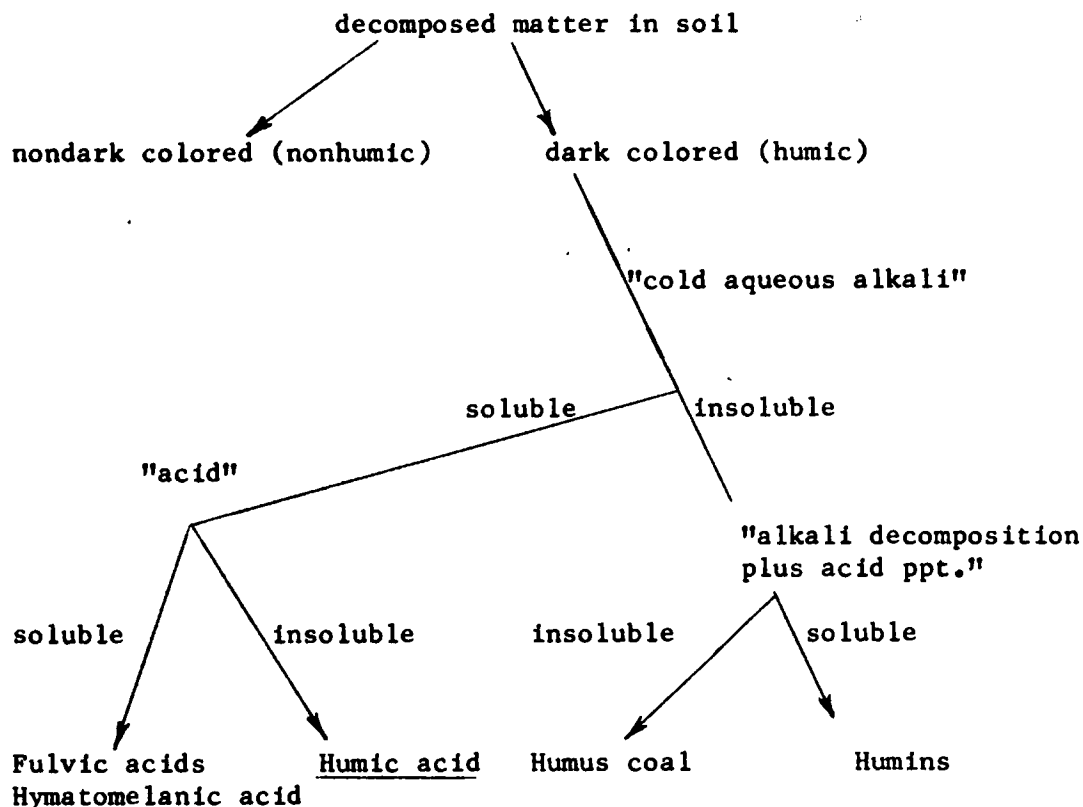
I INTRODUCTION

Definition

The problem of defining humic acid has plagued scientists for the past two hundred years. It can not be defined in the manner in which most organic compounds are defined. That is, it can not be given a definite structure; it has no unique melting point; molecular weights have been given by different workers (1, 2) which range from 500 to 50,000 but most commonly cited molecular weight is in the range $2.0-5.0 \times 10^4$ (3), and it contains little or no crystalline structure (this will be discussed in more detail later).

The commonly accepted definition of humic acid is "that portion of the soil organic matter which is soluble in base and insoluble in mineral acid and alcohol". This definition has been criticized by many investigators for three main reasons. First, other non-humic substances such as polyuronic acids, complexed proteins, and metallichions co-ordinated with the humic acid may be separated from the soil by basic extraction. Therefore, the definition may be amended to read "that portion of the soil organic matter which is soluble in base, insoluble in mineral acid, and remains after mild acid hydrolysis". The second main objection to this definition is that it is too broad in that it does not include the source. It has been shown by many investigators (4, 5, 6) that slight changes are apparent in humic acid

from different sources. Variation in the apparent degree of condensation is the probable cause of this change. Thus, the definition should be amended again to include the source, especially since Raudnitz (7, 8) has reported recently the occurrence of humic acid in living plants. The third objection to the definition of humic acid is not that it is too broad, but rather that it is too narrow. This opinion is voiced, because as seen in the following extraction scheme advanced by Scheffer and Welte (9), other fractions result which many workers feel are the same or very similar to humic acid.



It has been shown by chromatographic and optical studies (10, 11, 12, 13) that fulvic acids are probably the initial forms or the decomposition products of humic acid, and that the fractionation of extracts of soil organic matter into acid precipitable ("humic") and acid soluble ("fulvic") material is a very arbitrary procedure. For example, Laatsch (14) observed the formation of a brown flocculent precipitate characteristic of humic acids after prolonged dialysis of a solution of fulvic acids (from strongly podzolic soil) followed by evaporation to 1/10 volume. Other workers (15, 16) have been able to show with some success that humins are not a separate group of humic substances, but are apparently humic acids occurring in a stable link with the mineral part of the soil. This calls for a different method of extraction for humic acid, which is being investigated by different workers (17).

The definition of humic acid is very unsatisfactory at this point and it is hoped that the present work being carried out on humic acid using electron paramagnetic resonance spectrometry will help in the formulation of a more meaningful definition for humic acid.

History of Soil Humus Study

Humic acid was first extracted by Achard (18) in 1786, by a method which is almost the same as that used today. By the end of the 18th century, it was established that humic acid was a plant nutrient and that it was a unique natural compound.

Berzelius and his pupil Mulder (19) were two of the main proponents, in the 19th century, of many different soil acids of definite chemical structure which they thought were derivatives of carbohydrates.

Ulmin	$C_{40} H_{32} O_{11}$
Humin	$C_{40} H_{30} O_{15}$
Ulmic	$C_{40} H_{28} O_{12}$
Humic	$C_{40} H_{24} O_{12}$
Crenic	$C_{40} H_{24} O_{16}$
Apocrenic	$C_{24} H_{12} O_{12}$

The classification by extraction techniques showed that investigators in the 19th century were looking at different soil extracts and trying to classify them, but assignment of specific chemical formulas caused much confusion, for they started to find a multitude of different compounds due to the absence of detailed investigations.

At the end of the 19th century, three theories were prevalent which were to receive much attention in the 20th century: 1) humic acid is the result of carbohydrate reactions or the decomposition of cellulose (20), 2) humic acid is prepared by microbiological synthesis (21), and 3) humic acid is a ligno-protein complex (22).

In 1914 Shmuk (23) showed that the nitrogen part of humic acid is probably of protein origin and that humic acid from the soil is aromatic in nature. He postulated that humic acid is obtained by the enzymatic oxidation of aromatic compounds to quinones and then the condensation of these compounds with each other.

During this time Fischer and Schrader (24) were presenting evidence in favor of the fact that the aromatic compounds, which formed humic acid, are mainly from lignin, in contrast to the theory that cellulose was the main precursor. The important arguments in favor of this theory were:

1. The similarity between the structural units of both lignin and humic acid.
2. The fact that humic type substances were obtained by alkaline oxidation of lignin in an autoclave. Willstätter and Zechmeister (25) obtained only weak colored low-molecular weight compounds from cellulose under similar conditions.
3. The resistance of lignin to microbial attack while cellulose is rapidly decomposed to low-molecular weight substances.

Along this same line, Waksman (26) went so far as to propose that humic acid is the Schiff's base product from the reaction between lignin and proteins.

Lignin

Since humic acid is supposed to be derived (at least in part) from lignin, a brief description will be given of lignin at this time.

Lignin is the binding material for plant walls and can be thought of as encrusting the cellulose fibers and giving them their structural strength. The name, lignin, is derived from

the word "wood-forming".

A formal definition of lignin is that given by Brauns (27) in 1952: "Lignin is that encrusting material of the plant which is built up mainly, if not entirely, of phenyl propane building stones, carries the major part of the methoxy content of the wood, is unhydrolyzable by acids, readily oxidizable, soluble in hot alkali and bisulfate, and readily condenses with plant and thio compounds."

Lignin is also defined by its source and the method for its extraction.

One method of obtaining lignin is by dispersing the wood in toluene and then grinding it in a ball mill for 48 hours. The toluene is then removed and a lignin called Bjorkman lignin is extracted with dioxane and water.

Another method employs 72% sulfuric acid which dissolves the cellulose from the wood and leaves the lignin behind. The product so obtained is called Klason lignin.

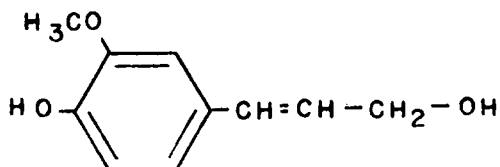
Although many other procedures are commonly used, the above illustrates the diversity of extraction conditions.

In the case of Bjorkman lignin the conditions are quite mild, while in the case of the Klason lignin the conditions are quite severe. The important point is that information as to the structure can be obtained from both of the different lignin samples.

Nomenclature based on the extraction method is not used for humic acid. One reason is that the extraction methods (the use of NaOH, NaF, or $\text{Na}_4\text{P}_2\text{O}_7$ solutions) are not really too different. Another reason is that the modern trend is to use dilute solutions (approximately 0.1 N), and to minimize the time which the sample is in contact with the solution. The humic acid which results from the different extraction methods, appears (by IR and degradation studies) to be the same in all cases.

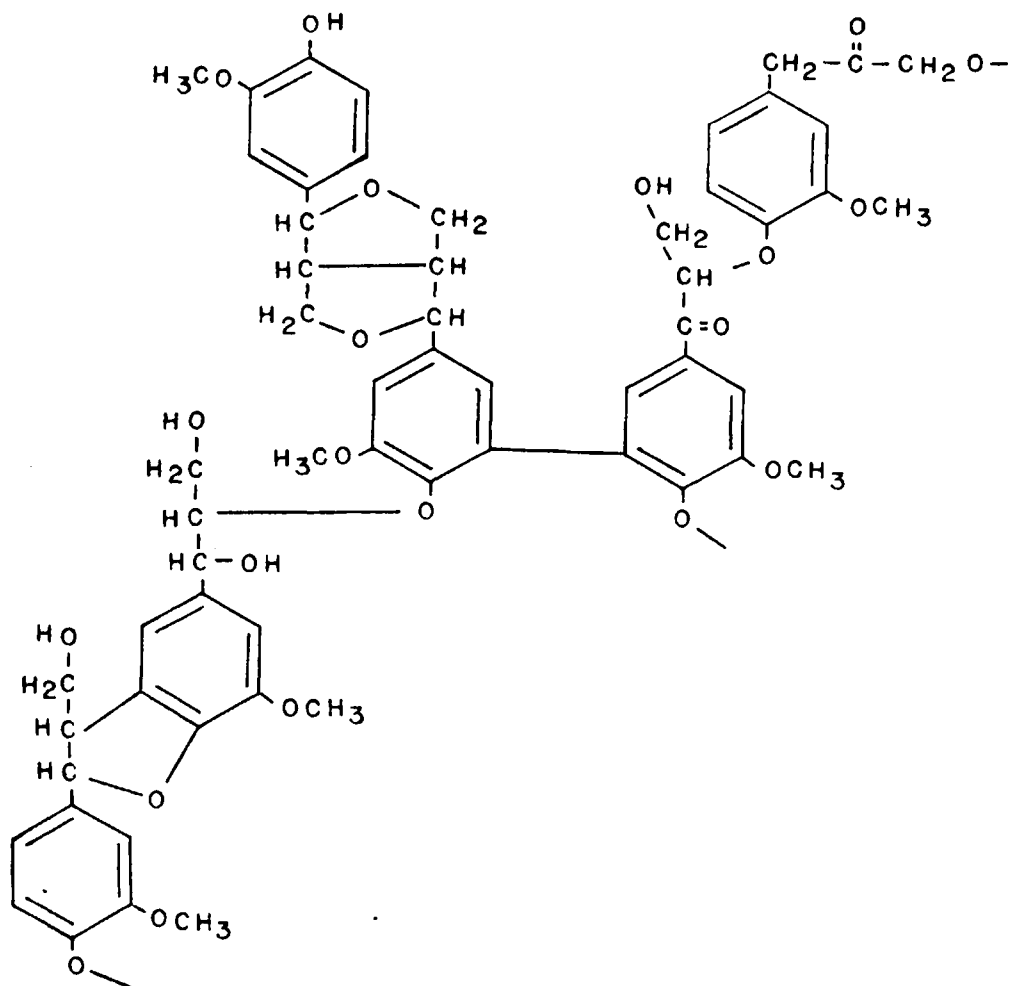
From the standpoint of source, however, both lignin and humic acid require a description such as "Western Hemlock Klason lignin; humic acid from a podzol-B pine forest soil".

It was proposed by Klason (28) in 1897 that lignin is built of coniferyl alcohol (I) derivatives. From studies of model

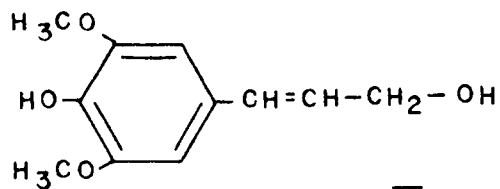


I

lignin degradation products, composite structures have been proposed. The following is one which is proposed by Erich Adler (29), which shows many of the structural units of soft wood lignin and bears out Klason's proposal.



In the case of hard woods, the oxidation pattern is only slightly different in that compounds like II are thought to make up the main body of the structure (30).



II

The Role of Lignin, Protein and Carbohydrate

While it is felt by most that humic acid is a polymer resulting from the condensation of polyphenolic units, there still remains the question as to the source of these units. Many schemes such as the one below have been proposed to show the possibilities.

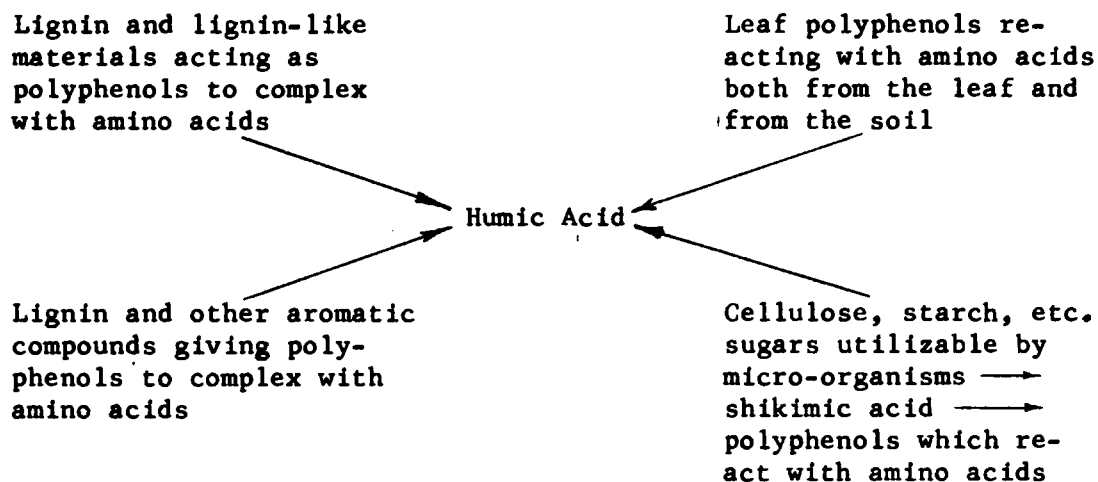


FIGURE I (31)

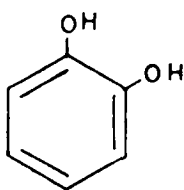
As was already indicated, many of the workers in the first part of this century felt that lignin is the main structural unit in humic acid and that it is relatively unchanged.

Breger (32) claimed that cellulose is probably removed from sediments by biochemical processes, while lignin is not destroyed and is incorporated into humic acid. Lynch (33) found that both lignin and humic acid are resistant to microbiological attack and that lignin complexed with protein resists attack on the protein. Thus, in accordance with the idea that lignin is chemically stable,

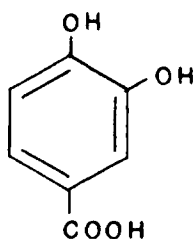
its conversion into humic acid was regarded as a physico-chemical process rather than a biochemical process. It was felt that the only structural changes are merely the loss of some functional group and the gain of new ones.

However, recent investigations (34, 35) on the utilization of aromatic compounds by micro-organisms as a source of energy makes it necessary to reconsider the question of the participation of lignin in humus formation. For example, microbiological attack could degrade lignin to simpler molecules; these could condense with other plant phenolics to form the humic acid polymer.

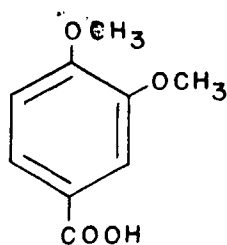
Until 1960, however, the only products obtained by oxidative degradation (36, 37, 38, 39, 40) of humic acid were typical lignin breakdown products, III through X, (with the exception of indole-derived compounds reported by Flaig (41) in 1956).



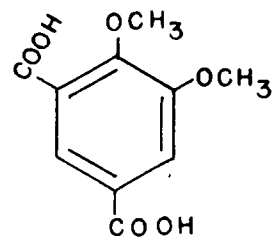
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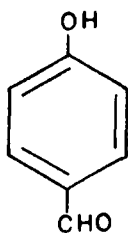
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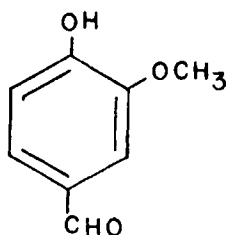
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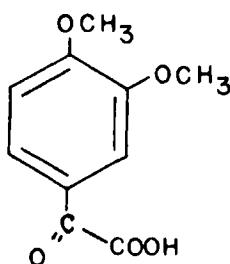
VI



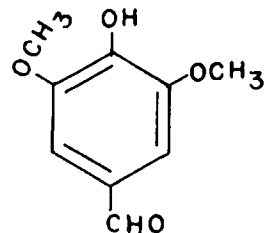
VII



VIII

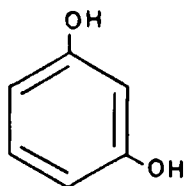


IX

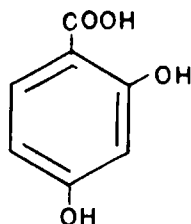


X

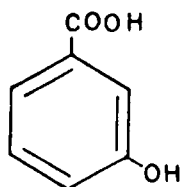
In 1960 Steelink, Nordby, Ho and Berry (42) identified resorcinol (XI) from a KOH fusion of humic acid from podzol-B forest soil, and almost simultaneously Coffin, DeLong and Wakentin (43) reported 2,4- dihydroxy-(XII), m-hydroxy-(XIII), and 3,5- dihydroxy benzoic acids (XIV) from a similar degradation.



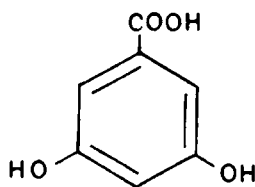
XI



XII



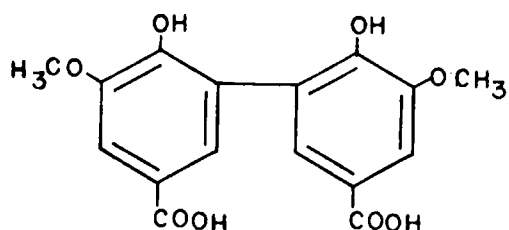
XIII



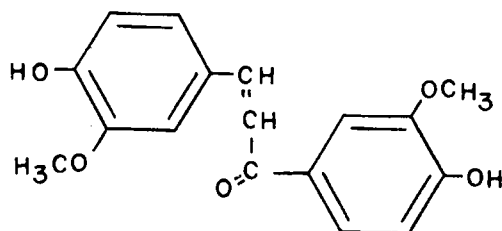
XIV

The presence of resorcinol and resorcinol derivatives would tend to indicate that part of the humic acid molecule could have been derived from catechin, tannin, or flavone structures. The fact that these moieties form a significant part of the macromolecule was shown by Steelink and Green (44) in 1962. They showed, for the first time, the co-occurrence of guaiacyl-derived products and resorcinol-derived products in a humic acid degradation mixture and that the concentration of resorcinol-derived compounds was comparable to that of the guaiacyl-derived substances.

The humic acid used by Steelink and Green was an acid hydrolyzed (2N HCl for 4 hours) humic acid from a podzol-B soil. The reason they were able to obtain XIII and XIV compounds was due to the mild oxidative technique (aqueous alkaline cupric oxide) which Pearl and Beyer (45 a, b) had used on lignin (yielding compounds XV and XVI).

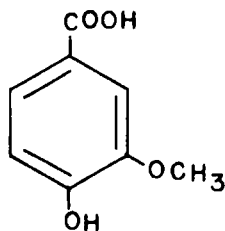


XV

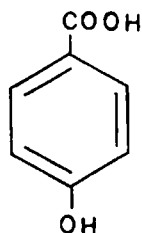


XVI

The substances which Steelink and Green reported were vanillin (VIII), p-hydroxybenzaldehyde (VII), syringaldehyde (X), m-hydroxybenzoic acid (XIII), 3, 5 dihydroxybenzoic acid (XIV), vanillic acid (XVII), p-hydroxybenzoic acid (XVIII).



XVII



XVIII

Until recently, very little was known about the role of cellulose and sugars in the formation of humic acid. Substances of an aromatic nature were detected in sugar-containing liquids by Mishustin, Dragunov and Pushkinskaya (46) using cultures of penicillium, and by Kononova and Aleksandrova (47) using

cultures of *aspergillus niger*.

In 1959, however, in a carbon 14 study by Mayaudon and Simonart (48), it was shown that cellulose and hemicellulose did not play an important part in humic acid formation. The cellulose was extracted from rice and the hemicellulose from rye grasses. These results can be seen in Table I, in which F=folic acid, HH=the hydrolyzable portion of humic acid, H=humic acid, and RH=the humin part of the soil. In the case of the cellulose and lignin which were used, both were allowed to decompose in the soil for the same amount of time. At the end of this time, 80% of the cellulose and 30% of the lignin had decomposed, and it was found that 5.9% of the cellulose and 34.2% of the lignin was incorporated into the humic acid. It is interesting to note that although cellulose and hemicellulose did not play an important part in humic acid formation, syringaldehyde and vanillin, which can be thought of as degraded lignin, did play an important part.

	F	HH	H	RH *
C ₁₄ labeled proteinaceous material	18.0	36.0	7.5	39.0
C ₁₄ labeled glucose	20.0	30.0	4.7	45.0
C ₁₄ labeled cellulose	25.3	34.6	5.0	45.2
C ₁₄ labeled lignin	10.3	13.0	35.7	41.0
C ₁₄ labeled hemicellulose	22.3	31.8	9.9	35.7
C ₁₄ labeled syringaldehyde	25.2	13.0	28.3	33.4
C ₁₄ labeled vanillin	31.3	10.4	29.6	28.0

*All numbers represent relative percentages of the C₁₄ of the decomposed substance as found in the different extracts.

TABLE I

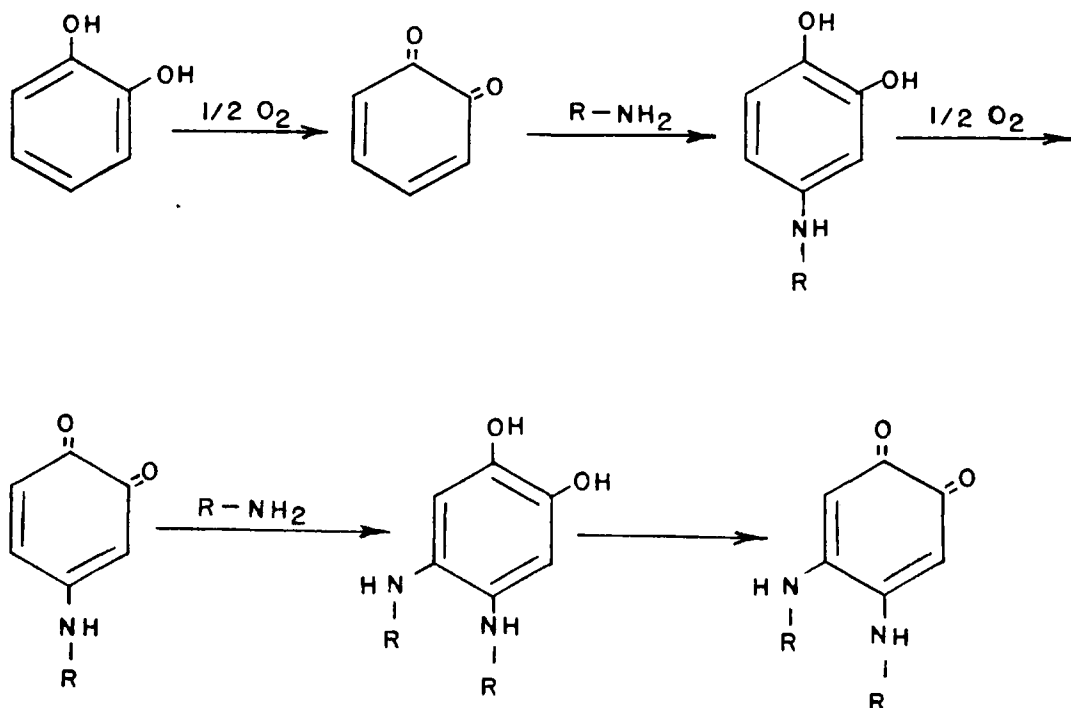
The nitrogen content of humic acid has been found to vary from 0.5% to 7.0% with most workers reporting values of around 3.0%. It has been shown by many workers (49, 50) that most of the nitrogen is hydrolyzable. This hydrolyzed portion is in the form of amino acids (over 23 different ones have been reported) which may be from bound protein material. It is interesting to note that Anderson (51) has also reported nucleic acid bases in the hydrolyzed portion of humic acid. These bases were not in the free state but were released by HClO_4 hydrolysis.

There is, however, a small amount of nitrogen left after hydrolysis. Due to the fact that Flaig and Breyham (41) were able to obtain indole derivatives from oxidative degradation studies on humic acid, the residue after hydrolysis could contain cyclic nitrogen.

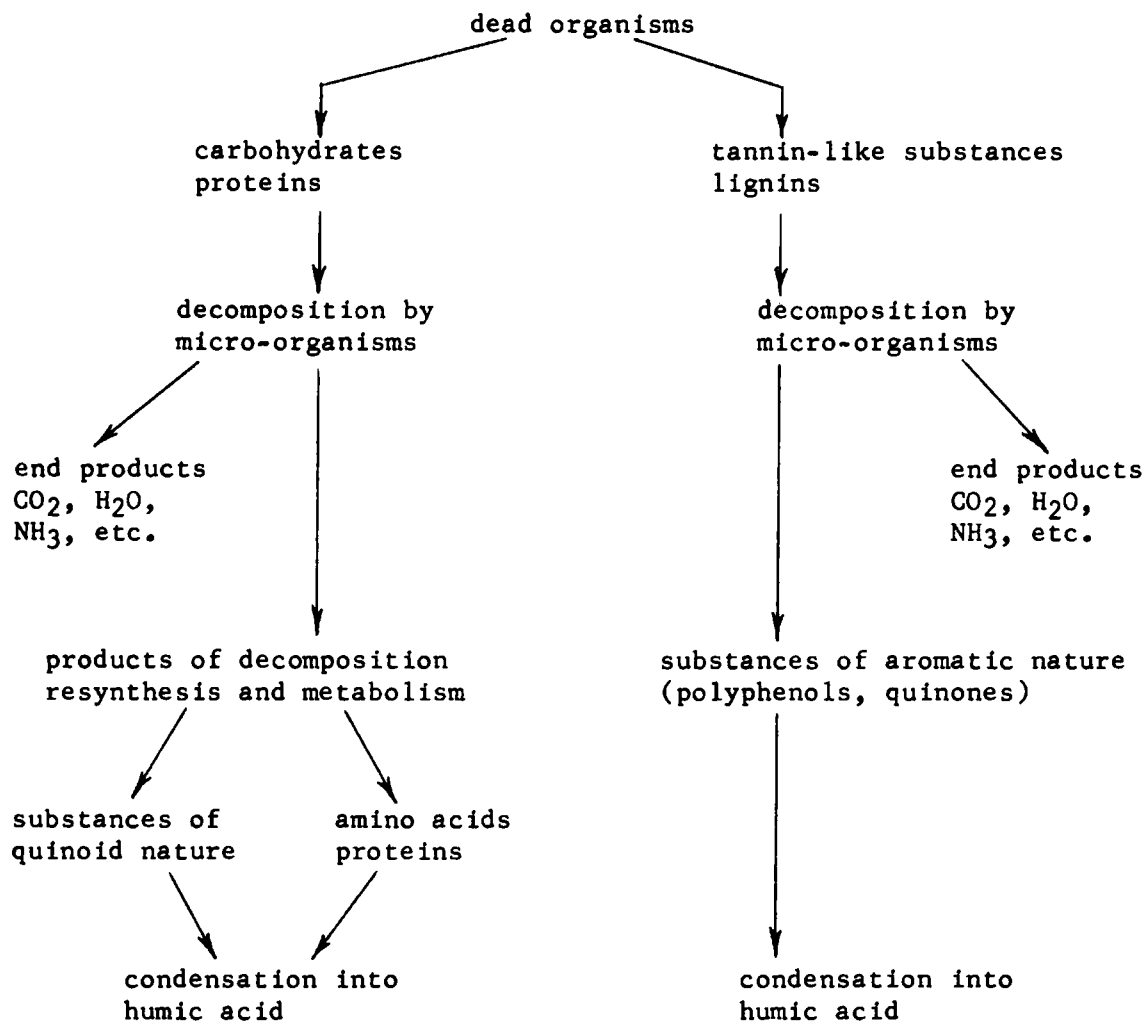
It was found by Burges (52) that humic acid, extracted with 100% lactic acid and then precipitated with ether, had a small (0.7%) nitrogen content. If nitrogen poor humic acid was added to cultures which had certain soil fungi present, it would incorporate up to 4% nitrogen. Burges concluded from this information that the "pure" humic acid is nitrogen free.

It would seem that "pure" humic acid is initially nitrogen free; then it reacts further with nitrogen compounds in the soil (amino acids, peptides, amino sugars), which are probably the products of re-synthesis since the majority of plant and animal residues are easily decomposed by microorganisms. A scheme for the condensation of amino acids with pyrocatechol has been presented

by Flaig (53) as shown below:



Although much work has been done on the relation of lignin, carbohydrates and nitrogen to humic acid, there is still much uncertainty as to the biosynthetic process. An illustration of the diverse ways in which humic substances can be formed during the humification of plant residues can be seen in the following scheme proposed by Kononova (54).



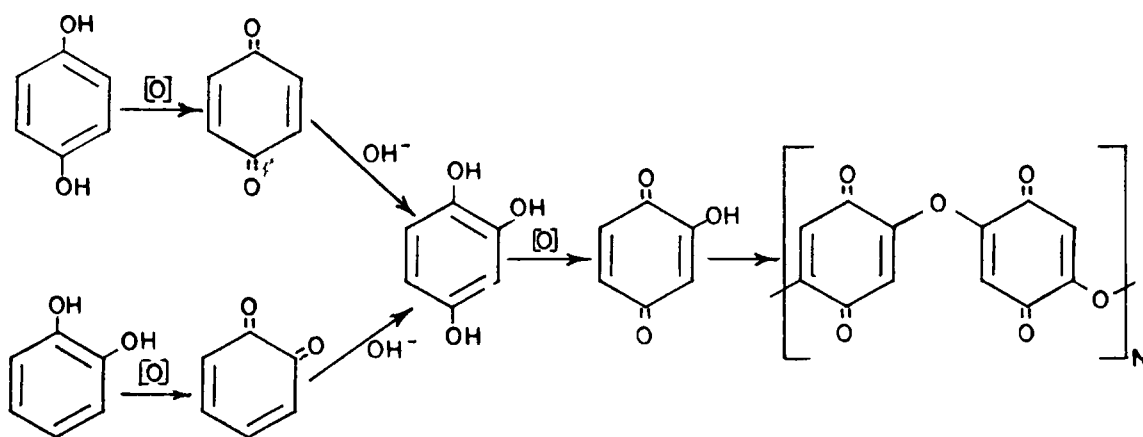
It should be mentioned, however, before going into the next section, that it is still felt by some people that humic acid is formed during the extraction process. A method is therefore needed for the proof that humic acid does exist in the soil.

The Importance of Quinones in Humic Acid

The fact that quinones participate in the formation of humic acid was first postulated by Trusov (55) in 1916. He felt the course of humification was carried out as indicated by the following

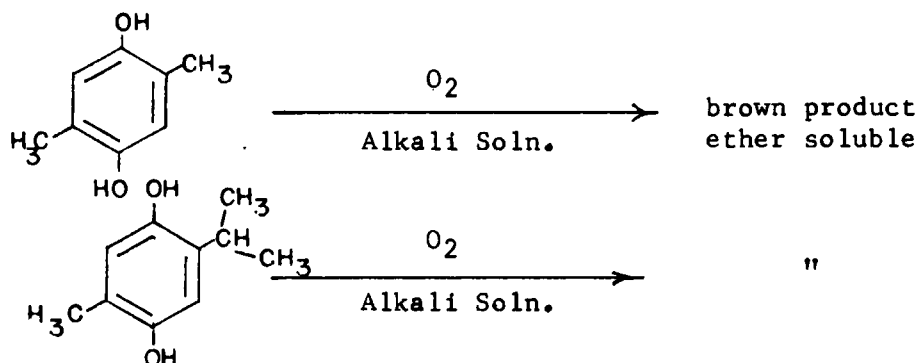
sequence: 1) hydrolytic decomposition with the formation of simpler substances of aromatic nature; 2) oxidation of the latter with the formation of quinones; 3) further condensation of the quinones and their conversion into dark-colored complex products (humic substances).

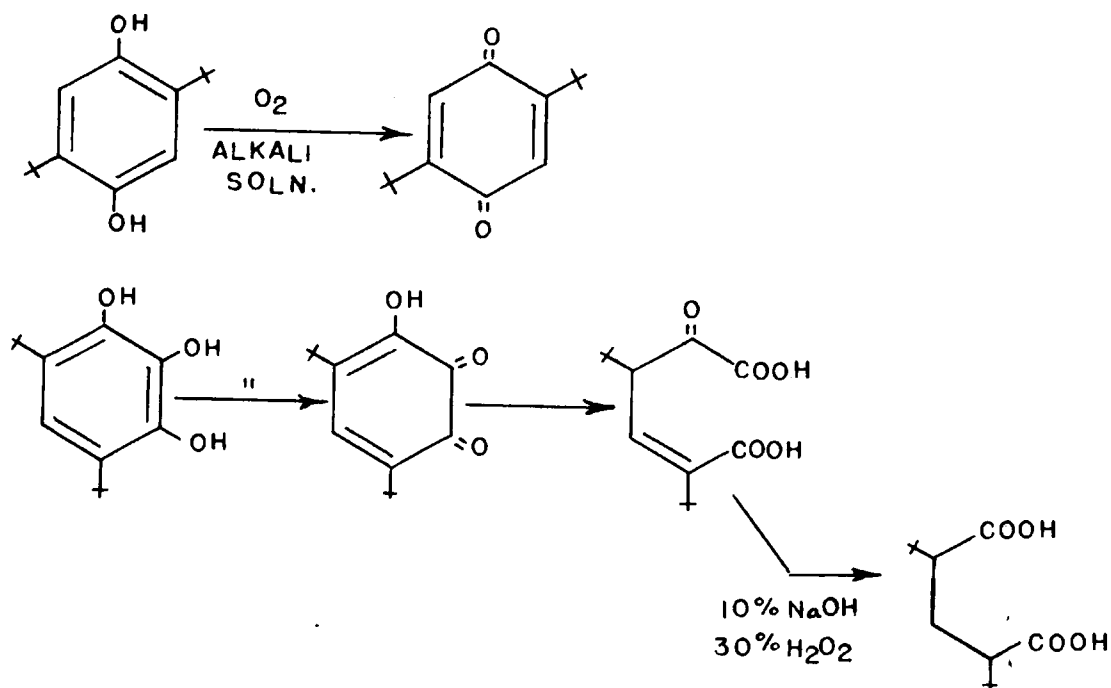
With Trusov's hypothesis, much early work was carried out on the synthesis of model humic acids from hydroquinone (56, 57). It was felt by Flaig (58) that hydroquinone and catechol polymerize to model humic acids according to the following scheme:



He also found that resorcinol did not react under the same conditions.

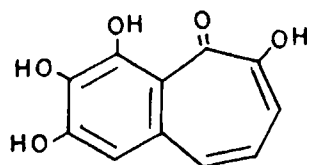
Flaig (59) later showed the effect of alkyl substituents on the course of their condensation reactions.





Under somewhat similar conditions, it was reported in 1955 (60), that synthetic humic acids from quinone had molecular weights of from 600 to 1000 and gave different fractions upon electrophoresis. They also yielded CO_2 , oxalic acid and acetic acid upon degradation which would agree with the reactions shown above, where aliphatic compounds are found.

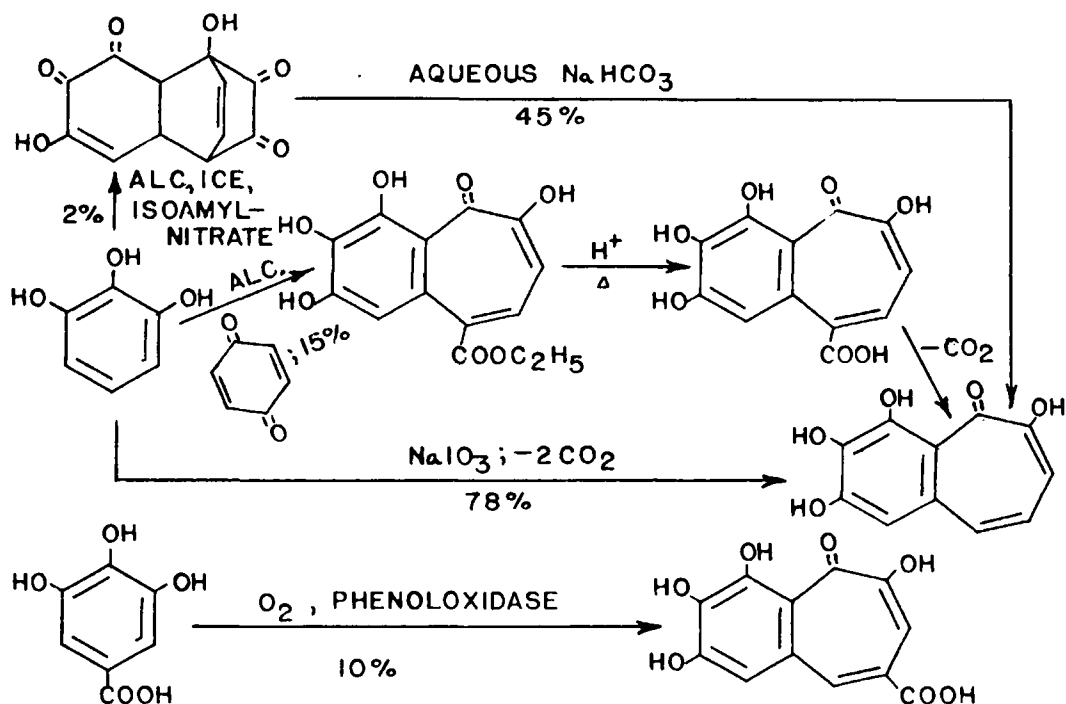
In 1957 the formation of purpurogallin (XIX) was first



XIX

reported (61), and in 1958 Flaig (62) showed different ways for the preparation of purpurogallin from lignin type breakdown products.

Of special interest is the use of phenoloxidase which occurs in soils.



Purpurogallin was also found in the alkaline degradation products of model humic acids, by chromatographic methods (63), and by the use of IR. Scharpenseel (64) showed that purpurogallin is present in soil humic acid along with pyrogallol and pyrocatechol.

Harries(65) showed in 1902 that pyrogallol forms hexahydroxydiphenyl by aerial oxidation in the presence of barium hydroxide, and Flaig showed (66) by the ultraviolet absorption spectra, that for both substituted and unsubstituted pyrogallol the first oxidation step is an α -hydroxy-o-benzoquinone. Flaig then presented several schemes (67, 68) which accounted for all of the above information and later he used this information in the formulation of a model humic acid (Figure 2) (69).

It was then postulated by Gutowsky that dimeric species (see Figure 4) were being formed, and that the odd electron was going from one ring to another by means of fast electron transfer. Coupled products of type XX and XXI were suggested for hydroquinone oxidation, while compounds of type XXII were proposed to form during quinone reduction. The possibility of alkoxyl attack was rejected because of the narrower spectra which Gutowsky thought was also explained by the electron transfer hypothesis.

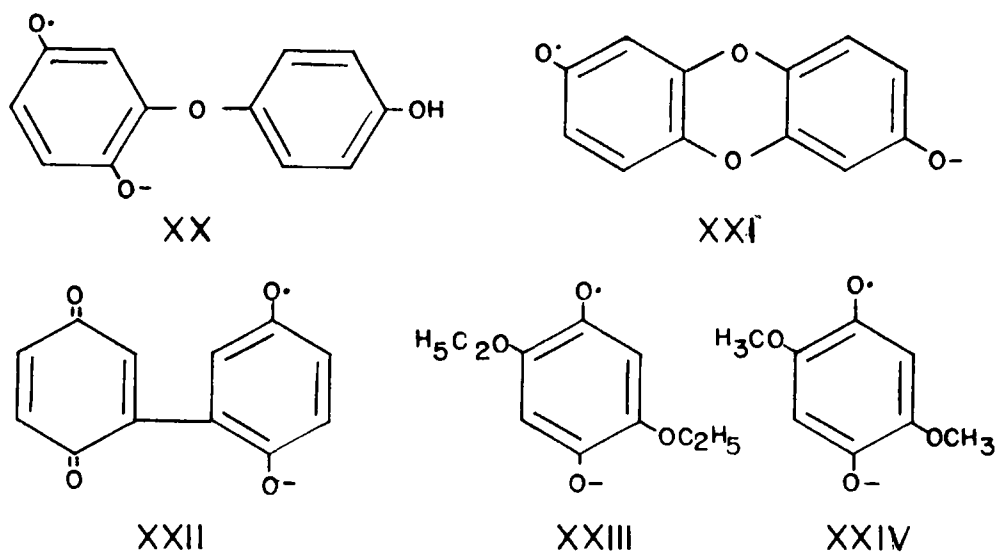
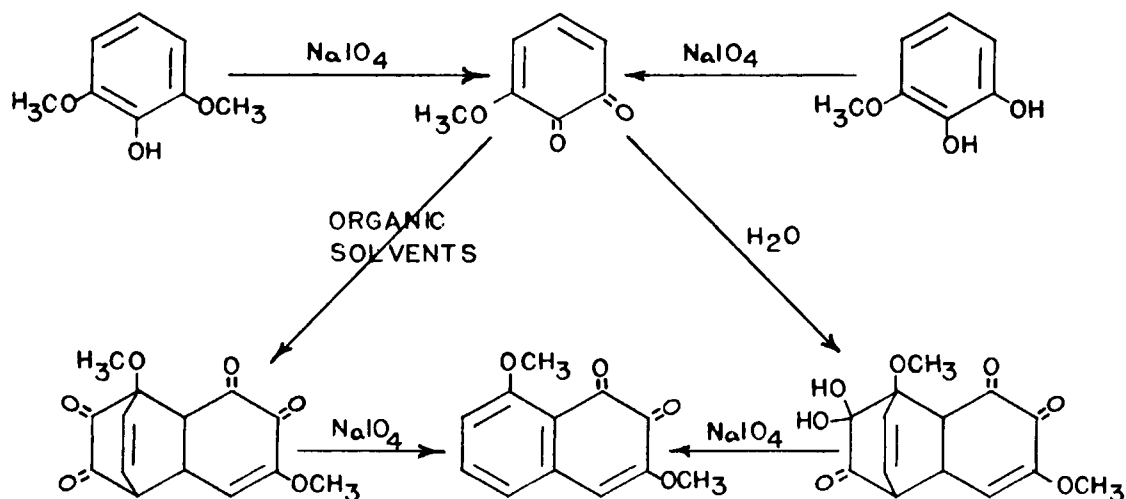


Figure 4

A further study of the dimerization of quinones was done recently by J. E. Wertz and co-workers (71). In this article it was shown that the spectra obtained from a solution of p-benzoquinone in ethanol or methanol (after disappearance of the original five-line spectrum) have exactly the same splitting and g-values as XXIII and XXIV respectively. The ring proton splittings of XXIII and XXIV are about eight times smaller than in unsubstituted benzosemiquinones, therefore, it was felt that

alkoxyl attack could not be rejected as Gutowsky and co-workers did because of the small line width of the EPR spectra of the products. They also prepared an authentic sample of XXII and found that when it was placed in 10% NaOH solution its spectrum was identical with the spectra obtained from either hydroquinone or quinone in 10% NaOH.

Recently Adler and Berggren (72) carried out a series of oxidation studies on quinones and found that it was possible to convert dimeric o-benzoquinones into β -naphthoquinones, as seen in the following scheme.



The above reactions are interesting in the light of the recent report on anthraquinone from humic acid degradation (73). Anthraquinone was found in the decarboxylation products of the water-soluble acid fraction obtained by the alkaline permanganate oxidation of humic acids extracted with 0.5% NaOH at 100°C for 30 minutes from volcanic ash soils. It would therefore seem

that the anthraquinone was probably formed in the above manner during the degradation of the humic acid.

From the above discussion, it can be seen that quinones probably play a major part in the formation of humic acid, although conclusive proof is still not available as to their existence in the humic acid molecule. This fact will be discussed in more detail later.

The Study of Undegraded Humic Acid

X-Ray Diffraction

Sedletzky and Brunowsky (74) found, by means of X-ray analysis that humic acids from soils and peats give diffuse X-ray diffraction lines. They regarded these results as evidence of the crystalline structure of humic acid. Gorbunov (75), however, concludes from his work that the diffuse lines are due to intramolecular diffraction.

Kononova (76) found these diffraction lines are more distinct from humic acids of more mature soil. Similar results were obtained by Inoue (77), using humic acid from coal. He also found that by adding substituents to the humic acids (by reacting the humic acid with azo-compounds), he could change the diffraction pattern.

The above results would tend to show that humic acid becomes more crystalline in nature as it matures; however, a more recent article by Fontana and Burana (78) states that after studying seven different humic acids, they failed to find any lines at all. They attribute the lines found by other workers to impurities.

Electron Microscope

Studies on the crystalline nature of humic acid have also been carried out by Flaig and Beutelspacher (79) using an electron microscope. From this work they concluded that humic acids consists of tiny spherical particles capable of uniting into chains and forming aggregate structures. These same results were also found by Kasatochkin, Zolotarevskaya and Lukin (80).

Ultra Sonics

Ultra sonics was first used (81) in order to degrade the humic acid molecule. However, at low frequencies sodium humate and humic acid suffered no molecular degradation, and at high frequencies only acetic acid and formic acid were found. It was concluded by Srivastava and Bukowitz (82) after comparing the results of a humic acid study with those of krilium (a linear polyelectrolyte) and bovine plasma albumen (a tightly coiled protein), which degrade readily, that a linear molecular structure (both coiled and uncoiled) must be ruled out for humic acid.

Differential Thermal Analysis

Bouilloux (83) found in 1947 that glucosidic humic acids differ from the phenolic and natural humic acids by the existence of a violent exothermic reaction in the region of 350° C. It was concluded from this work that sugars do not have an important role in humification, but nothing opposes a phenolic origin of natural humic acids.

Although absolute results can not be compared, an investigation by Mitchell (84) seemed to confirm the above results.

Ultraviolet and Infrared Spectrometry

The ultraviolet region of the spectrum has been used for determining the degree of humification (85) and for such things as showing the similarity between fulvic acid and humic acid (86). However, as pointed out by Ziechman and Scholz (87) the ultraviolet region is monotonic due to the complex nature of humic acid, and therefore of little value in characterizing functional groups.

The infrared region, on the other hand, is used quite frequently for studying the functional groups of humic acids as can be seen in Table II. It is especially good for showing the formation of the sodium salt of the acid. This is seen in the replacement of the band at 1720 cm^{-1} with strong bands at 1600 cm^{-1} and 1580 cm^{-1} which is regarded by Bellamy (88) as evidence for the ionized carboxyl group. The infrared region has also been used for classification of humic acids according to soil type (89), studies of synthetic humic acids (90), and for comparing different substances like fulvic and humic acid (91).

Very little work has been carried out in the near and far infrared, although Ziechman and Scholz (87) have shown that spectroscopy in the near infrared shows differentiable bands which could be used for the fast determination of the quinoid and benzoid systems in humic acid.

REF.	3400 CM ⁻¹	3050 CM ⁻¹	2900 CM ⁻¹	2550 CM ⁻¹	1710 CM ⁻¹	1500 - 1650 CM ⁻¹	1480 CM ⁻¹	1430 CM ⁻¹	1400 CM ⁻¹	1350 CM ⁻¹	1195 CM ⁻¹
(94)	ALC.-OH PHENOLIC -OH		ALIPHATIC C-H	O-H (ACID)	C=O (ACID)	(1650) ALIPHATIC C=C C=O (1600) AROMATIC C=C	AROMATIC -OC-H ₃			AROMATIC C-OMe	(1240) PHENOLIC C-OH
(89)	H-BONDED -OH	AROMATIC C-H	ALIPHATIC C-H	O-H (ACID)	C=O (ACID)	C=C	ALIPHATIC C-H	ALIPHATIC C-H O-H	ALIPHATIC C-H PHENOLS	C-O ESTERS ETHERS QUINONES PHENOLS	ALIPHATIC C-O-C
(90)				O-H (ACID)	C=O (ACID)	(1600) C=C (1520) AROMATIC C-H	$\begin{array}{c} \text{H} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{H} \end{array}$ $\begin{array}{c} \text{H} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{H} \end{array}$		-CH ₃		AROMATIC SUBST.
(96)					C=O (ACID)						(1240) PHENOLIC -C-OH
(97)					C=O (ACID)	(1600) C=C (1520) NH ₂		COOH			
(98)						(1640) QUINONE C=O					
(62)					C=O (ACID)	(1600) C=C	$\begin{array}{c} \text{H} \\ \diagup \\ \text{C} \\ \diagdown \\ \text{H} \end{array}$	-C-O-H	-CH ₂ -	PHENOLIC -C-OH	AROMATIC SUBST.
(95)		PHENOLIC - OH			C=O (ACID)	(1600) C=O O ⁻					

TABLE 2. INFRARED BAND ASSIGNMENTS FOR HUMIC ACID

Purpose of This Investigation

In 1960 Rex (92) reported the existence of free radicals in lignin and soil organic matter. The fact that humic acid possesses a stable free radical was established by Steelink and Tollin (93) in 1962, using electron paramagnetic resonance spectroscopy.

Based upon their study of soil humic acid, Steelink and Tollin showed for the first time that humic acid actually exists in the soil (that it was not created upon extraction) and also showed that EPR could be used to determine the chemical nature of humic acid. They proposed that humic acid may consist of a mixture of ordinary semi-quinone type radicals and electron-transfer complexes of the quinhydrone-type and that such a system could be investigated by observing the effects of chemical treatment, such as oxidation, reduction, and hydrolysis on the EPR spectra.

The purpose of this investigation is, therefore, to study the effects of oxidation, reduction, and hydrolysis on humic acid by means of electron paramagnetic resonance spectroscopy and to some extent infrared spectroscopy. This should help to shed light upon the structure of humic acid due to the ability of electron paramagnetic resonance spectroscopy to look at the free radical portion of the molecule without the complex nature of the molecule interfering. Other methods of studying humic acid (I.R., U.V., X-ray diffraction, etc.) have yielded little information (as previously stated), due to the molecules' complex nature.

II DISCUSSION OF RESULTS

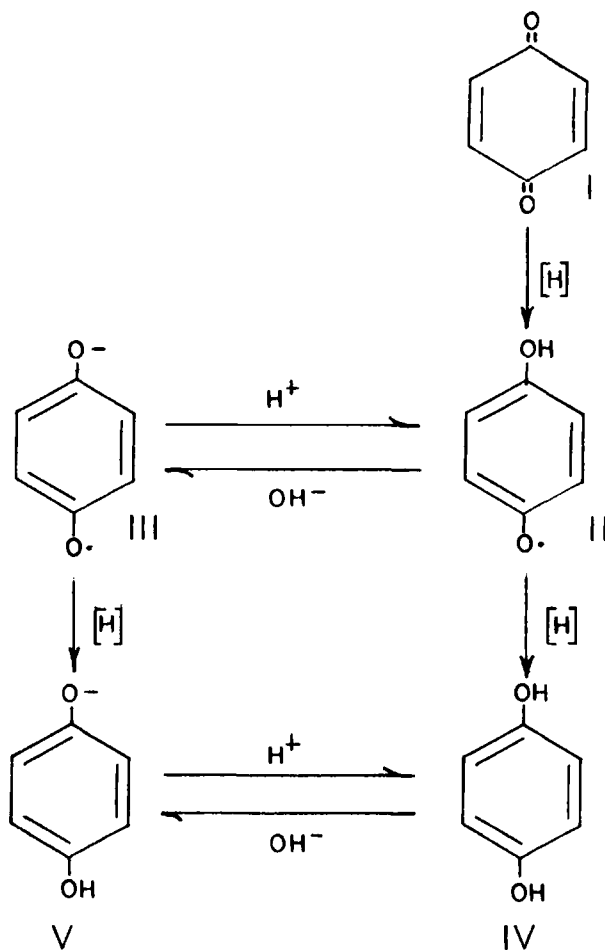
Sodium Salt Formation and Chemical Reduction

Steelink and Tollin (93) proposed that the electron paramagnetic resonance (EPR) signal of humic acid comes from two radical species, one of which is a semi-quinone type radical, and the other a quinhydrone type radical.

To test this proposal, humic acids from various soils were reduced with sodium in alcohol, in which the sample was isolated as the sodium salt. It was found (see Table III) that the products showed a substantially higher free radical content than the original sample. Also, when the sodium salt was dissolved in water and precipitated out as the acid, the resulting product had a radical content that was lower than the initial sample. The differences, however, in radical content between the initial acid sample and the reprecipitated sample were not very large.

Infrared spectra were also taken of the samples (IR spectra, 1-3). The reduced salts showed replacement of the band at 1710 cm^{-1} with strong bands at 1600 to 1590 cm^{-1} , together with increased absorption in the region of 1390 cm^{-1} , which Bellamy (88) regards as evidence for the ionized carboxyl group. In the case of the reprecipitated reduced acid, the spectrum was exactly the same as the initial acid sample.

The above results can be explained on the basis of a quinone/semi-quinone/hydroquinone model for humic acid as shown in the following scheme (104, 105):



According to the above scheme, upon reduction in basic media, two main reactions would take place:

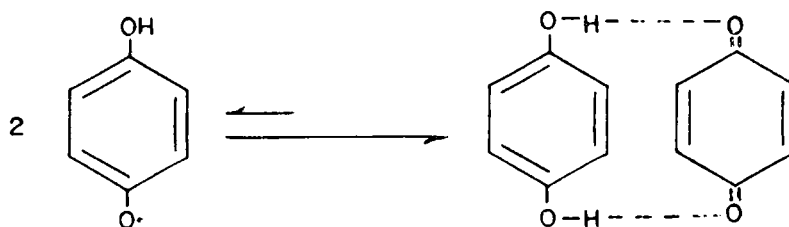
- A. The quinones (I) would be reduced to semi-quinone radical ions (III), which have been shown (70, 71, 106, 107) to be stable for extended periods of time.
- B. The semi-quinone radical ions could be reduced to hydroquinone ions (V).

The increase in radical content which was found upon reduction of humic acid, can be explained by the fact that more semi-quinone ions are formed by reaction (A) than are destroyed by reaction (B).

The decrease in radical content, below the initial amount, on reacidification of the reduced sodium humate can be explained if the semi-quinone ions formed by reaction (A) are more unstable than those that were destroyed by reaction (B). This is consistent with the known (105) instability of semi-quinone radicals in acid solution.

The fact that the infrared spectrum, of the reduced humic acid, showed no change from the initial spectrum upon reacidification of the sample could be due to the fact that the quinones which were reduced represent only a small part of the humic acid molecule. Thus, any change would not be apparent in the infrared spectra.

A second explanation for the results is based on the assumption of a quinone/semi-quinone/hydroquinone/quinhydrone model for humic acid. In this case the previous scheme would be altered to include an equilibrium of the following type:



Equilibria of this type, between a diamagnetic quinhydrone and a paramagnetic semi-quinone, are well known (108), and are shifted far to the left in basic solution. The only exceptions to this are in cases dealing with molecules like anthrarufin and quinizarin, which form intramolecular quinhydrones and are diamagnetic in basic solution (108) (see Appendix).

In order to test the effect of salt formation, sodium salts were formed of numerous humic acids (see Table IV), and a large increase in radical concentration was observed (salt formation can also be seen in infrared spectra 4-8). This then would help to substantiate the presence of a quinhydrone type moiety in humic acid.

Further evidence that a quinhydrone could be present is the fact that the signal shape (see Figure 6) shows the presence of two different free radical species whose spectra are superimposed (the shoulder on the EPR signal was not always apparent, however). Steelink and Tollin (93) also showed that a quinhydrone species could be used to explain the temperature dependence of the EPR signal.

The actual radical contribution from a quinhydrone radical (the exact nature of this radical is still unknown) would probably be very small due to the fact that quinhydrone radicals must be prepared in the solid salt form (109) or by adsorption on barium hydroxide (108) since they dissociate to free radical ions in solution. This fact could explain why the shoulder on the EPR signal is not always apparent.

Although both ortho- and para-quinones are available for quinhydrone formation, as can be seen in Figure 2, it is probable that most of the stable quinhydrones persist in the humic acid molecule as o-quinhydrones. This is because, for geometrical reasons, the two rings, in the case of p-quinones, must lie parallel to each other, and quinones with many groups attached have been shown (110, 111) to form unstable quinhydrones. In the case of o-quinones, steric factors have very little effect and o-quinhydrones formed from such molecules as phenanthrene-quinone-3-sulfonic acid are very stable (112).

The quinhydrone species would thus explain the initial increase in radical content upon sodium salt formation, while a non-interacting semi-quinone radical, in addition to the quinhydrone, would explain the radical content of the initial acid.

In order to obtain more information about the nature of the quinones in humic acid, a reduction (sodium/ethanol) study was carried out over a period of time. The results from this study can be seen in Table V.

In the case of both the Swiss and English samples, the radical concentration rose to a high value and then decreased. The initial increase is probably due to salt formation. As for the radical concentration and the line width after the initial salt formation, the fluctuations show that many complex reactions are taking place. This could be due somewhat to both alkoxyl attack (71) and the time lag between obtaining the sample and the measurement of the radical content in the instrument. These

extraneous effects could be eliminated if the reduction was carried out directly in the microwave cavity of the EPR spectrometer like the reductions carried out by Maki and Geske (113). Changes in the EPR signal could then be determined as the reduction takes place.

An electrolytic reduction of English humic acid was carried out using a dropping mercury electrode. This reduction gave a radical concentration (see Table V) which was similar to the maximum value obtained from the sodium/alcohol reduction. This reduction, however, was not carried out in the cavity so the effects of the reduction with time could not be determined.

Although further studies such as those using the in situ technique of Maki and Geske are needed for a more definite confirmation of the structure, the present study would clearly support a humic acid model containing significant contributions from quinone, semi-quinone, hydroquinone and quinhydrone systems.

Spectra of Basic Solutions

A high resolution EPR study of aqueous alkaline solutions of humic acid, carried out in this laboratory, revealed fine structure (see Figure 5) for a Swiss humic acid sample reduced by a sodium-mercury dispersion in water. Although this spectrum could not be resolved, it supports the interpretation that the spectra could be due to two separate absorbing radicals. Further work such as the in situ study mentioned above should reveal more specific information.

Chemical Oxidation

Since o-quinones have been shown (59) to break down under alkaline oxidative conditions into dicarboxylic acids, an

alkaline degradation study was carried out on humic acid.

Humic acid samples were treated with CuO-NaOH according to the procedure of Pearl (103). The pH was then adjusted to values of 6.8, 3.0, and 1.0. The solution was extracted at each pH with diethyl ether to remove low molecular weight phenolic compounds (these compounds were diamagnetic). A solid precipitate was obtained at each pH value and the radical concentration was measured (see Table VI).

To a first approximation, the total number of radicals in the degradation products of English and Swiss humic acids are equal to those in the original samples. It would appear that the oxidation has not significantly created or destroyed the radical species. This was not the case for the California humic acid, for close to 40% of the radical concentration was destroyed.

The infrared spectra of the oxidative degradation products are 9-17%.

The following facts can be inferred from the above information:

- (a) The radical species (in the case of English and Swiss humic acids) is fairly stable towards alkaline oxidation, and is probably in the p-semiquinone form.
- (b) The radical species (in the case of California humic acid) is somewhat unstable towards alkaline oxidation, and is probably in both the o- and p-semiquinone form.

- (c) The radical concentration is more concentrated in the cases of the higher pHs which would be expected if some molecules contained more o-quinones than others, for the o-quinones would be oxidized to diacids and would cause precipitation at lower pHs than for those molecules which contained mainly p-quinones. The IR spectra show acid absorption only at the lower pHs.
- (d) Reduction in molecular weight by alkaline oxidation may contribute to the increased spin concentrations of some of the fractions. This is in agreement with the increased sharpness of the IR peaks after degradation.

Acid Hydrolysis

When humic acid is refluxed with dilute mineral acid, the hydrolysate contains a higher carbon content and a lower hydrogen, nitrogen and mineral content (42). This treatment also removes carbohydrates and protein material (114). The effect of acid hydrolysis upon humic acid was studied by EPR, and the results can be seen in Table VII.

Upon hydrolysis the radical concentration increases quite markedly with time. At the same time, the peaks in the infrared spectra (see IR spectra 17-25) become sharper and new peaks appear (both the Swiss and the English humic acid spectra behave in exactly the same manner).

The explanation that the molecular weight is becoming smaller by removal of carbohydrate and protein material, fits the above data very well.

The "residue" was a flocculant solid which appeared in the solution, and was obtained by centrifuging the solution. It has a high radical concentration, and the infrared spectra showed more fine structure than was the case for the other hydrolysates. The fact that the "residue" might be formed from the carbohydrates present was ruled out by the fact that a synthetic humic acid formed by heating dextrose gave a very different infrared spectrum (see IR spectrum 26). It is interesting to note, that somewhat similar treatment of hydroquinone (90) yielded a synthetic humic acid with a spectrum very similar to that of humic acid (see IR spectrum 27).

It was also found that the hydrolysates were somewhat insoluble in aqueous NaOH (the residue was very insoluble). This could be explained by decarboxylation, although the infrared spectra showed only slight decreases in the carboxylate region.

It would seem, therefore, that hydrolysis doesn't affect the radical itself, but tends to remove carbohydrate and protein material, leaving behind the so-called "inner core" (42, 44).

Saturation Effects of Humic Acid

If a sample is irradiated with radiation of frequency ν , some of the electrons in the lower state will absorb energy and jump to the higher state, provided that $h\nu = g\beta H$. At the same time the electrons in the upper state will be stimulated to emit radiation of frequency ν and fall to the lower state. For any system in thermal equilibrium there will be more electrons in the ground state and so this will result in a net absorption of energy. The Maxwell-Boltzmann expression,

$$\frac{N_1}{N_2} = e^{-\frac{E}{kT}}$$

in which N_1 is the number of electrons in the upper state, N_2 is the number in the lower state, ΔE is the energy separation between levels, k is the Boltzmann constant, and T is the absolute temperature, can be used to show that at room temperature there will be a deviation in the ratio of N_1 to N_2 from one, and that this deviation will increase as the temperature is lowered.

If resonance absorption is to continue, there must be some means, other than stimulated radiation, for the electrons to fall back down to the ground state and lose their energy. Without some mechanism for the electrons to relax down to ground state, the number of electrons in the two states will become equal in number due to the excess in the lower level going to the upper level. This will result in no further absorption of energy. One mechanism by which the electrons can lose their energy is called "spin-lattice interaction" and is a sharing of the energy with the thermal vibrations of the solid as a whole. The length of time necessary for the electron to lose its energy is called the spin-lattice relaxation time. In most cases, relaxation times are short and no trouble is encountered. In the case of humic acid, however, the relaxation times are long and a low power input (attenuation of 500) must be used, for it was found that humic acid samples tend to saturate readily at high power inputs.

The saturation effects of petroleum asphaltenes have been studied by Yen, Erdman, and Saraceno (115). They found an inverse relation between the population of aromatic carbon atoms per spin, and the spin-lattice relaxation time.

Similar studies on humic acid could yield information as to the amount of aromatic carbons in humic acid.

III EXPERIMENTAL

Humic Acid Samples

1. The English humic acid was graciously supplied by Professor Alan Burges, Department of Botany, University of Liverpool. The humic acid was obtained from a podzol-B soil in the Delamere Forest, Cheshire, England.
2. The Swiss humic acid was a commercial product supplied by Fluka A G, Buchs, Switzerland. The humic acid was obtained from a muck soil of a compost type prepared by the company.
3. The Wisconsin humic acid was obtained from a scrub oak forest podzol from Wisconsin, which was graciously supplied by Professor Frances Hole, Department of Soil Science, University of Wisconsin.
4. The Arizona humic acid was obtained from a brown pine forest soil lying some two feet below the surface. The soil was from the top of Kitt Peak, elevation 6,800 feet, Pima County, Arizona.
5. The California humic acid was obtained from a podzol-B soil found in the "pigmy" forest, Mendocino County, California.
6. The Leonardite humic acid was a commercial product obtained from leonardite coal.

Extraction

1. One kilogram of California podzol-B was placed in a 4 liter Erlenmeyer flask and 2.5 liters of cold 0.1M NaOH was added. The mixture was then shaken periodically for 24 hours, at the end of which time, the solution was decanted off and then centrifuged. The solid was removed and the pH of the solution was adjusted to 1.0 by the use of con. hydrochloric acid. The solution was then centrifuged and the liquid decanted off. The remaining solid was then redissolved in 0.1M NaOH and centrifuged again. The solution was decanted off and reacidified as before. The solution was then centrifuged again. The solid was removed and then mixed with one liter of a 1:1 mixture of hot benzene and ethanol. The solution was then centrifuged and the solid placed in a 90° C oven to dry for 2 hours.

The fulvic acid solution (that acid solution remaining after the final precipitation of the humic acid) was a deep red color.

2. The Arizona humic acid was extracted in the same way as the California humic acid. It was noticed, however, that the fulvic acid solution was a pale yellow in color.
3. The Wisconsin humic acid was divided into two parts. One part was extracted in the same way as the California humic acid. The other part was extracted by using cold sodium pyrophosphate as proposed by Stevenson (99).

3. (Continued)

The fulvic acid solutions resulting from the sodium pyrophosphate and the sodium hydroxide extractions was a deep wine red in color. The fulvic acid from the sodium pyrophosphate extraction was then extracted by the method of Kononova (100).

The dark red fulvic acid solution was passed through a column of activated charcoal. About 5 hours was required to get the solution to pass through the charcoal. The fulvic acid was then leached from the charcoal by means of 0.2N sodium hydroxide. The resulting solution was again a dark red in color. It was found that similar results could be obtained by merely heating the solution with activated charcoal and then filtering the charcoal off. This final method was used to purify the sample.

The volume of the basic solution was then reduced on a vacuum flask evaporator, and the thickened solution then allowed to dry in the air. A shiny black solid resulted.

4. The English humic acid was extracted from a podzol-B soil by Professor Alan Burges (101) with lactic acid and reprecipitated with ethyl ether.
5. The Swiss humic acid was extracted by the Fluka A G Company from an alkali-treated soil with 0.5N sodium hydroxide.
6. No communication was received as to the method by which the Leonardite humic acid was extracted, so it was assumed that it was obtained from the leonardite coal by the conventional

6. (Continued)

method (102) of extracting with sodium hydroxide and removing the insoluble salts by centrifuging the humate solution.

Type of Soil

The type of soil used in most of this work (in the cases of English, Wisconsin, Arizona and California) was a podzolic type. Podzolic soils have a profile: $A_0 - A_2 - B - C$. A_0 is the forest litter horizon and forms a layer about three to five centimeters thick. The humus horizon (A_1) is not usually developed. A_2 is the podzolic horizon which is of whitish color and is mainly composed of quartz grains which are resistant to decomposition. The podzolic horizon is almost devoid of humus and is poor in mineral colloids. The illuvial horizon (B) has a very high humus content which can be attributed to the high content of clay particles and mineral colloids. It is from this horizon that the samples of humic acid were obtained. Below this horizon occurs slightly changed soil-forming rock, usually designated horizon C.

Acid Hydrolysis

One gram of California humic acid was ground to a thick paste in a mortar and pestle with water of pH 8.0. This was placed in a 100 ml flask with 50 ml of 4.0N H_2SO_4 . This mixture was then made to reflux. Samples were removed from the solution periodically over a 24 hour period. Upon standing, a dark brown solid precipitated from the acid solution.

(Continued)

The precipitate was designated the "hydrolysis residue".

Swiss and English humic acids were treated by the same procedure..

Copper Oxide Oxidation

To 185 ml of water was added 41.3 g of sodium hydroxide and 57.3 g of copper sulfate (pentahydrate).. This slurry was transferred to a high speed stirring autoclave (200 ml capacity). Six grams of humic acid were then added to the mixture. The autoclave was flushed with nitrogen for several minutes and then heated to 170° C for 3 hours. The reaction mixture was allowed to cool for 12 hours. The solid was then removed by means of centrifuge, and the pH of the centrifugate was adjusted to 6.8. All pH measurements were made by means of a Beckman Model G, glass-electrode pH meter.

This oxidation was carried out according to the general procedure described by Pearl and Beyer (103).

Ether Extraction

The filtrate from the oxidation mixture (at pH = 6.8) was extracted with ethyl ether in a continuous liquid-liquid extractor (250 ml capacity). This extraction was carried out until the ether coming over was colorless. This procedure required about 3 days. An extraction was then carried out at pH = 3.0 and pH = 1.0 and the solid residue removed in each case.

Sodium/Ethanol Reduction

One gram of English humic acid was placed in 50 ml of absolute ethanol in a 200 ml flask equipped with a magnetic stirrer and a reflux condenser. To the solution was added 0.5 gram of metallic sodium and the stirrer was started. At the end of ten minutes a one ml sample was removed and 0.5 gram of sodium was added. This process was then repeated eleven times at ten minute intervals.

At the end of each ten minute period 5 ml of absolute ethanol was added to the one ml sample and it was centrifuged. The solution was then decanted off and the sample shaken with an additional 5 ml of absolute ethanol. The sample was then centrifuged, the solution poured off, and the solid dried in an 80° C oven for eight hours.

This same procedure was used for a one gram sample of Swiss humic acid.

Electrolytic Reduction

By means of a dropping mercury electrode the half-wave potential for humic acid was determined. The humic acid was then electrolytically reduced at a potential higher than its half-wave potential by means of a dropping mercury electrode. The English humic acid which was reduced was dissolved in 0.1N NaOH. The sample was washed with absolute ethanol after the reduction.

Sodium Salts

The sodium salts of the humic acid samples were prepared by dissolving approximately 0.3 gram of humic acid in 1 ml of 2N NaOH. To this solution was immediately added 20 ml of absolute ethanol. The resulting precipitate was filtered rapidly, washed with ethanol and allowed to dry.

Electron Paramagnetic Resonance Spectra

All paramagnetic resonance spectra were obtained with a Varian 100 kilocycle modulation spectrometer, Model V-4502. Spin concentrations were estimated by comparison with diphenylpicrylhydrazyl. Number of radicals were assumed to be proportional to signal height times signal width squared. All samples were dried over P_4O_{10} in an "Abderhalden Pistol" using toluene (in the case of the Wisconsin samples benzene was used). It was found that size of the particles (humic acid) had little or no effect upon the signal.

Infrared Spectra

All infrared spectra were taken with Perkin Elmer "Infracord", Model 137. Samples (approximately 4 mg) were dispersed in KBr (400 mg) by mechanical grinding for 3 minutes in a "Wig-l-bug". The samples were pressed into pellets (1.1 mm thick) with a Beckman "Pellet Die", Model #5020. The pelleting pressure used was 100,000 pounds per square inch (the gauge pressure was 20,000 pounds per square inch). It was found that the best pellets resulted when the pressure was increased very slowly over a ten minute period while the sample was under vacuum.

(Continued)

The potassium bromide used was Baker "AR" grade. It was ground to a fine powder with a mortar and pestle and then placed in a drying oven at 110° C for 2 days. After drying it was placed in a desiccator over calcium chloride.

IV SUMMARY

1. The present study would clearly support a humic acid model containing significant contributions from quinone, semi-quinone, hydroquinone, and quinhydrone systems.

2. More specific information as to the nature of the humic acid free radical could be obtained by an in situ reduction study of humic acid samples.

3. The radical species is fairly stable towards alkaline oxidation, in the case of Swiss and English humic acids, which would suggest that it exists as a para-semiquinone free radical.

4. The radical species appears somewhat unstable towards alkaline oxidation, in the case of California humic acid, which would suggest that both the ortho- and para-semiquinone forms contribute to the radical species.

5. The hydrolysis study substantiates the idea that hydrolysis does not affect the radical itself, but tends to remove carbohydrate and protein material leaving behind the so-called "inner core" of humic acid.

6. Further saturation studies on humic acid could yield information as to the aromatic nature of humic acid.

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TABLE III
FREE RADICAL CONTENT OF VARIOUS
REDUCED HUMIC ACID SAMPLES

<u>Sample</u>	<u>Chemical Treatment</u>	<u>Spins/gram</u>
English	none	1.4×10^{18}
	Sodium/alcohol salt	35×10^{18}
	Sodium/alcohol	
	(reacidified)	0.6×10^{18}
California	none	0.9×10^{18}
	Sodium/alcohol salt	9.0×10^{18}
Swiss	none	0.7×10^{18}
	Sodium/alcohol salt	14×10^{18}
	Sodium/alcohol	
	(reacidified)	0.3×10^{18}

TABLE IV
FREE RADICAL CONTENT OF HUMIC ACID
SAMPLES AND THEIR SODIUM SALTS

<u>Sample</u>	<u>Chemical Treatment</u>	<u>Spins/gram</u>
English	none	1.4×10^{18}
English	Sodium salt	23×10^{18}
Swiss	none	0.7×10^{18}
	Sodium salt	16×10^{18}
Leonardite	none	0.4×10^{18}
	Sodium salt	6.6×10^{18}
Arizona	none	0.6×10^{18}
	Sodium salt	80×10^{18}
Wisconsin ($\text{Na}_4\text{P}_2\text{O}_7$ ext.)	none	0.4×10^{18}
	Sodium salt	17×10^{18}
Fulvic Acid	none	0.4×10^{18}
	Sodium salt	0.7×10^{18}

TABLE V

FREE RADICAL CONTENT OF HUMIC ACID
 SAMPLES AFTER SODIUM/ALCOHOL REDUCTION

Sample	Elapsed Time of Reduction (min)	Spins/gram	Line Width (gauss)
English	0	1.4×10^{18}	3.6
	10	8.5×10^{18}	6.1
	20	9.2×10^{18}	6.2
	30	34.4×10^{18}	5.5
	45	6.3×10^{18}	6.3
	60	12.5×10^{18}	6.3
	90	12.2×10^{18}	7.0
	120	11.2×10^{18}	6.5
	150	11.2×10^{18}	7.0
	180	7.9×10^{18}	6.5
	210	9.2×10^{18}	7.7
	240	5.9×10^{18}	6.5
Swiss	0	0.7×10^{18}	3.9
	20	14×10^{18}	7.0
	40	9.1×10^{18}	7.0
	60	3.3×10^{18}	6.8
	80	1.4×10^{18}	7.0
	100	7.1×10^{18}	6.8
	120	13×10^{18}	6.5
	140	15×10^{18}	6.3

FREE RADICAL CONTENT OF HUMIC ACID

SAMPLE AFTER ELECTROLYTIC REDUCTION

English (Na salt)	33.5×10^{18}	8.2
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TABLE VI
FREE RADICAL CONTENT OF
OXIDATION PRODUCTS OF HUMIC ACID

Sample	pH Fraction	Spins/gram	% of Initial Radical Conc.	% of Initial Weight
English	6.8	2.8×10^{18}	70	35.0
	3.0	2.2×10^{18}	20	12.7
	Original	1.4×10^{18}	100	100
Swiss	3.0	1.5×10^{18}	71	28.2
	1.0	0.3×10^{18}	7.5	15.0
	Original	0.6×10^{18}	100	100
California	6.8	1.9×10^{18}	49.3	23.3
	3.0	0.3×10^{18}	8.2	24.5
	1.0	0.3×10^{18}	1	3.0
	Original	0.9×10^{18}	100	100

TABLE VII
FREE RADICAL CONTENT OF
HYDROLYZED HUMIC ACID SAMPLES

Sample	Length of Time (Hrs.) of Hydrolysis	Spins/gram
English	0	1.4×10^{18}
	2	7.5×10^{18}
	4	12.2×10^{18}
	8	9.3×10^{18}
	12	15.0×10^{18}
	23	19.4×10^{18}
	23 (Residue)	41.0×10^{18}
Swiss	0	0.6×10^{18}
	2	6.4×10^{18}
	4	8.2×10^{18}
	8	8.3×10^{18}
	12	12.0×10^{18}
	24	24.8×10^{18}
	24 (Residue)	93.3×10^{18}

VI SPECTRA

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EPR Spectrum of Sodium/Mercury Reduced Humic Acid in a Sodium Hydroxide Solution.....	53
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EPR Spectrum of English Humic Acid (solid).....	53
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Infrared Spectra

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2. English Humic Acid, Reduced Sodium Salt.....	54
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5. Leonardite Humic Acid, Sodium Salt.....	54
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14. English Humic Acid, Degraded pH 3.0 Fraction.....	55
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16. California Humic Acid, Degraded pH 3.0 Fraction.....	55
17. California Humic Acid, Degraded pH 1.0 Fraction.....	56
18. English Humic Acid.....	56
19. English Humic Acid, Hydrolyzed 2 hours.....	56
20. English Humic Acid, Hydrolyzed 23 hours.....	56
21. English Humic Acid, Hydrolyzed "Residue".....	56
22. Swiss Humic Acid.....	56
23. Swiss Humic Acid, Hydrolyzed 2 hours.....	56
24. Swiss Humic Acid, Hydrolyzed 23 hours.....	56
25. Swiss Humic Acid, Hydrolyzed "Residue".....	57
26. Humic Acid from Dextrose.....	57
27. Humic Acid from Hydroquinone.....	57
28. Polystyrene.....	57

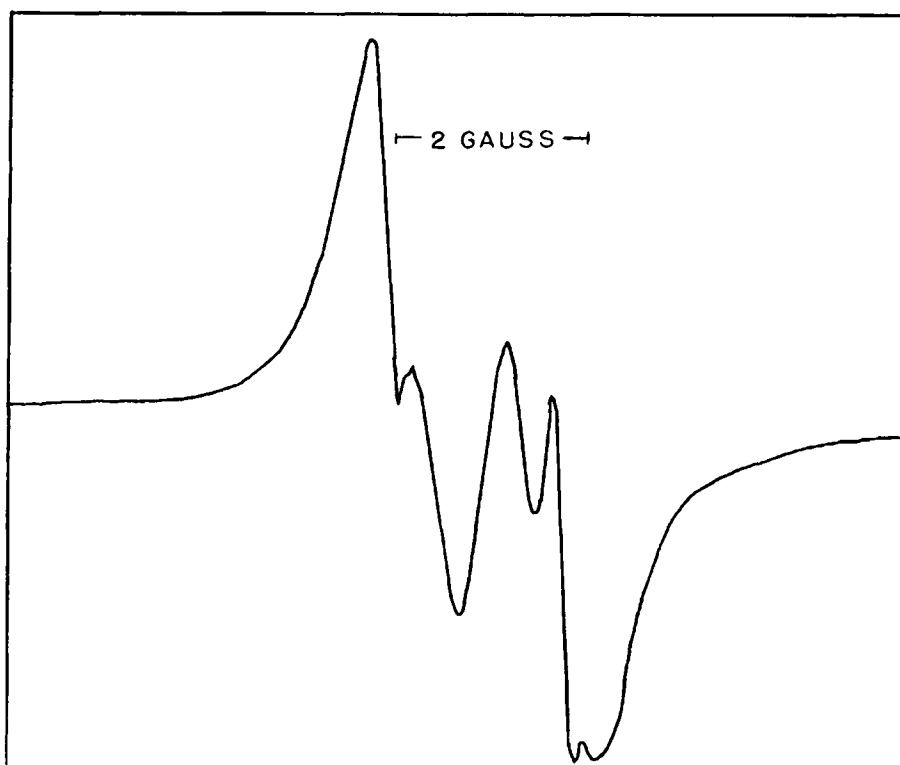


FIGURE 5. EPR Spectrum of Sodium/Mercury Reduced Humic Acid in a Sodium Hydroxide Solution.

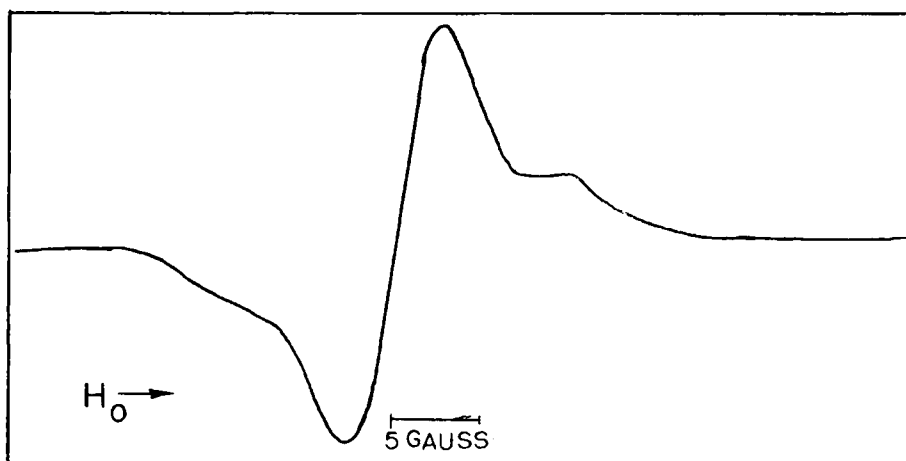
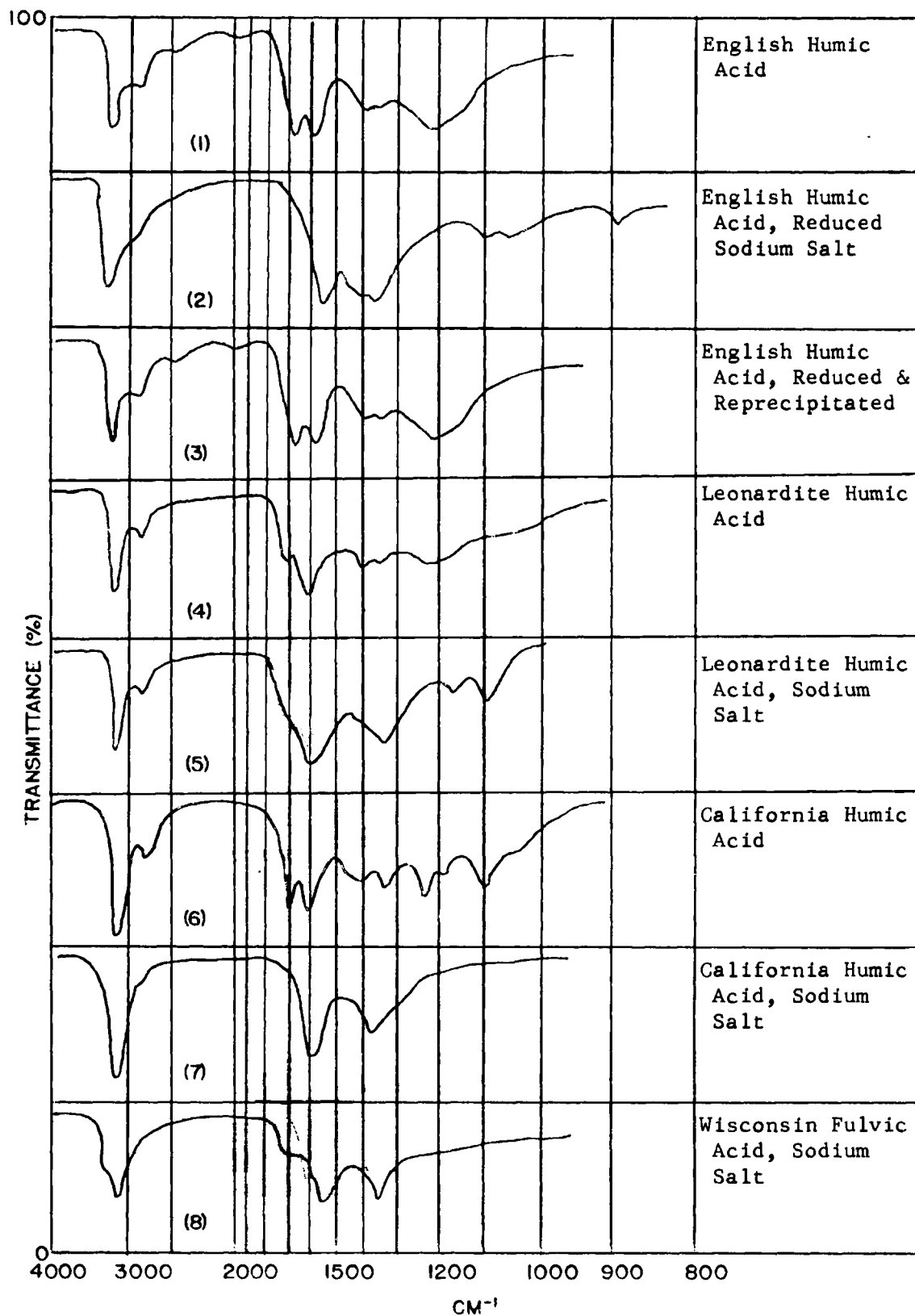
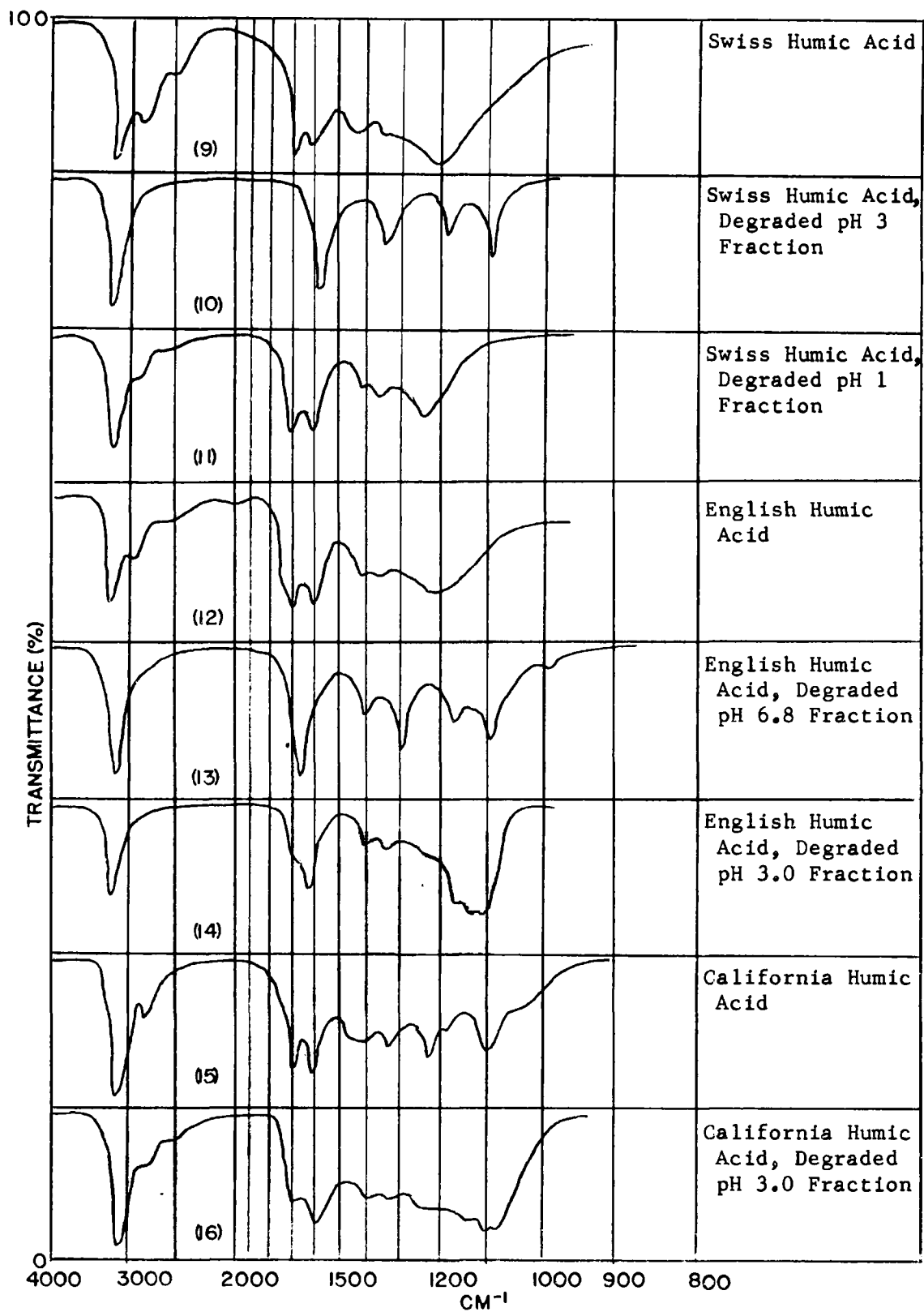
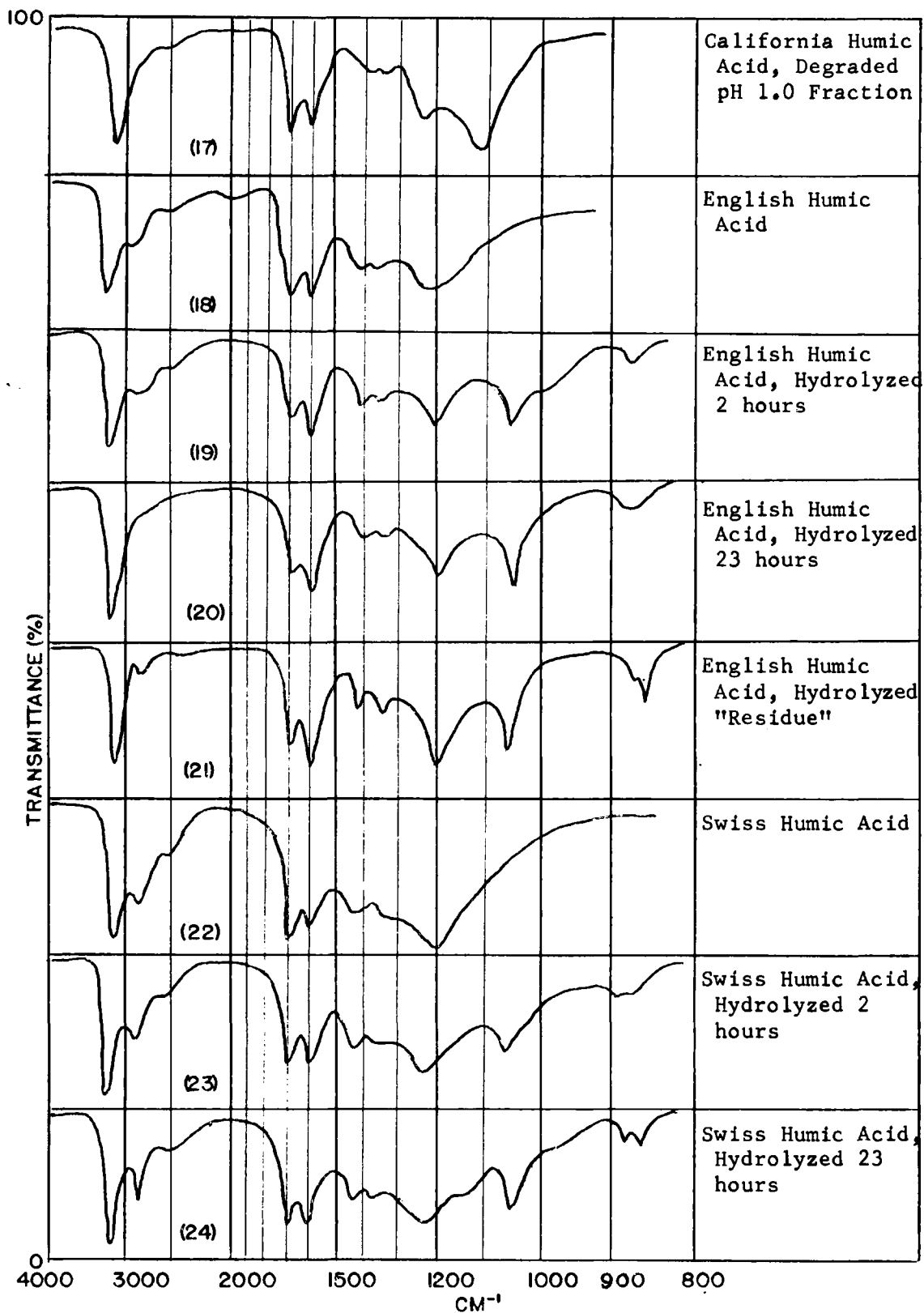
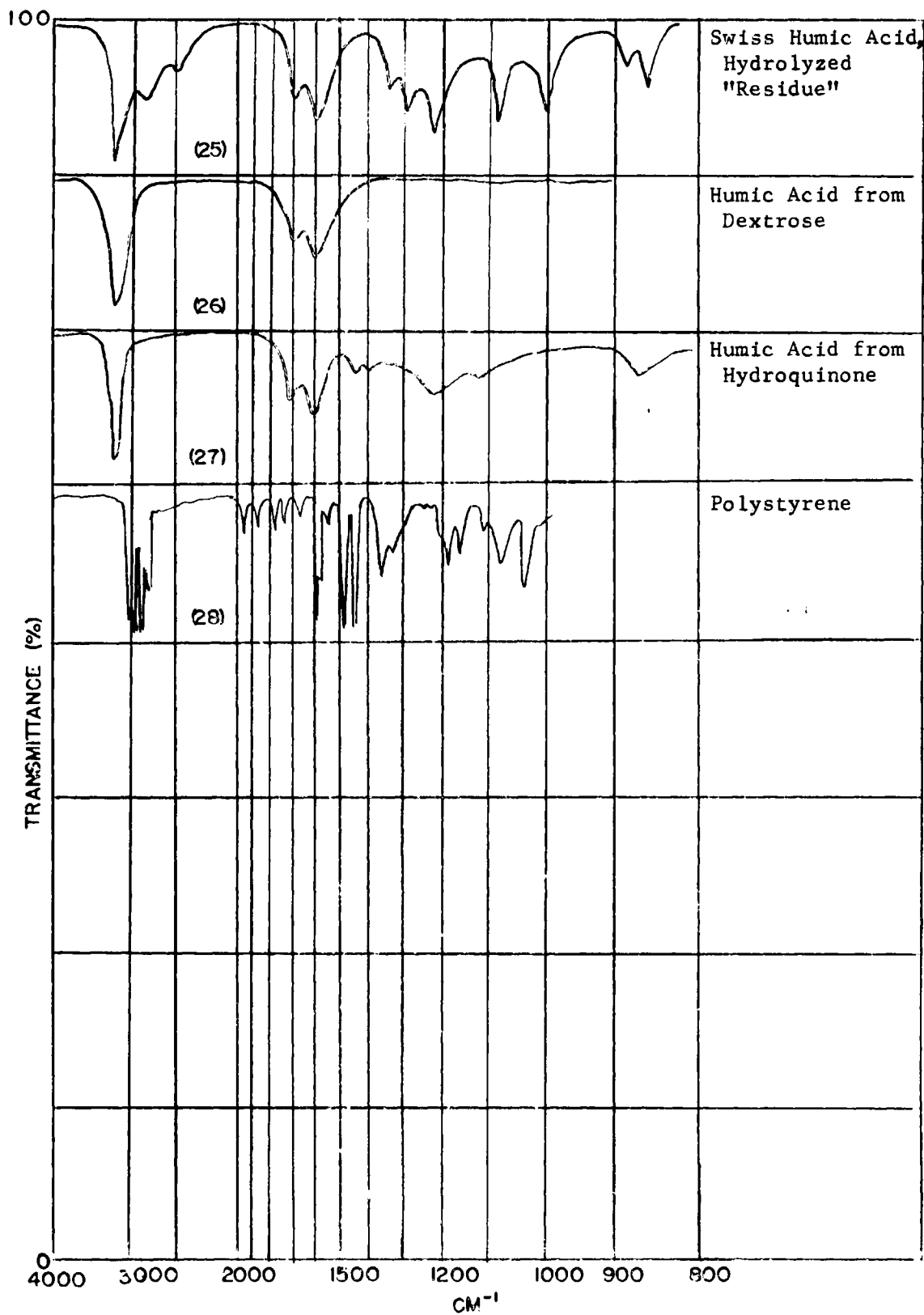


FIGURE 6. EPR Spectra of English Humic Acid (solid).









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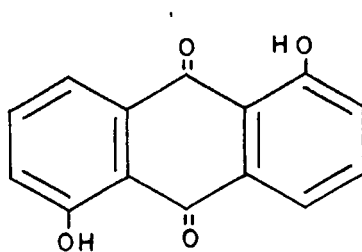
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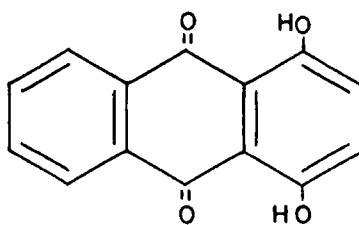
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APPENDIX



Anthrarufin



Quinizarin