

The University of Manitoba

PHYSICOCHEMICAL STUDIES OF THE MECHANISM OF COMPLEXATION AND
RELEASE OF 4-CHLOROANILINE FROM SOIL AND SOIL HUMIC ACIDS

by

Brian L. Worobey

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Submitted to the Faculty of Graduate Studies in Partial
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ABSTRACT

Research was conducted to develop a method to release and quantitate bound or tightly complexed 4-chloroaniline from soil humic acids. The nature of the humic acid adsorbent was studied to elucidate possible sites and mechanisms of its interaction with 4-CA. Mild extraction techniques were used to isolate the humic acid from soil and its structure was characterized using infrared spectrophotometry (IR) and ^{13}C -Fourier transform-nuclear magnetic resonance (^{13}C -FT-NMR or ^{13}C -NMR). Finally an attempt was made to explain the mechanism of binding of 4-CA to soil humic acids.

Pesticide-derived 4-chloroaniline residues are immobilized in soil by interaction with the soil matrix and its components, especially humic acids and clays. Tightly complexed 4-chloroaniline was released from treated soil humic acids by pyrolysis techniques; however, recoveries were low and nonreproducible precluding the use of this procedure as an analytical method. An evaluation of several alternative methods of release resulted in development of an analytically acceptable method based upon alkaline hydrolysis and quantitation of 4-chloroaniline as its N-heptafluorobutryl (HFB) derivative by electron capture-gas-liquid chromatography (EC-GLC). A lower detectable limit of 75 fg (75×10^{-15} g) was possible. Alkaline hydrolysis released 80% of the applied radioactivity; with 46% of the applied compound quantitated as 4-CA-HFB. "Nonhydrolyzable" 4-chloroaniline residue in soil humic acids is probably an artifact of the hydrolysis procedure in short term

experiments, and not the result of indigenous reactions of 4-chloroaniline with soil humic substances.

Different methods of extracting humic acids from soil were found to yield preparations of varying physicochemical characteristics. A mild extraction procedure ($\text{Na}_4\text{P}_2\text{O}_7$ -pH 7 precipitation with acetone) yielded the least aromatic and most aliphatic humic acid, whereas, increasing harshness of extraction resulted in humic acids of increasing aromaticity. Analysis of these humic acid structures by a novel technique, ^{13}C -FT-NMR, supported IR evidence that aliphatic and alicyclic substances are important components of indigenous humic acid.

Fulvic acid spectra appeared to be less aromatic cf. humic acid from the same soil. Among several models and precursors of humic acid investigated, only cellulose yielded a precipitate via the classical (NaOH) extraction procedure. Its spectral characteristics were (IR, ^{13}C -NMR) most similar to a fulvic acid isolated by the same procedure. A α 1-4, α 1-6-glucan exhibited a ^{13}C -NMR spectrum very similar to a cellulose component ^{13}C -NMR spectrum. Hemicellulosic compounds may, therefore, be essential components of fulvic acids, and, possibly, humic acids.

Soils of varying organic matter (OM) concentrations and clay (C) concentrations were reacted with 4-chloroaniline, and OM concentration evidently controlled the amount complexed. The quantity of the tightly complexed fraction released by pyrolysis was independent of clay concentration but dependent on the concentration of OM.

The micellar nature of these humic acids in solution was implied by the observation that three molecular weight ranges of humic acid,

prepared by ultrafiltration, exhibited similar IR spectra.

Aliphatic benzoic acid-type structures may form micelles in solution and so explain the physicochemical properties of HA which characterize its ability to complex 4-chloroaniline, xenobiotics, and various other soil components.

The results of these studies have provided some insight into the mechanism(s) of 4-chloroaniline interaction(s) with soil and especially soil humic substances, culminating in a de novo (from basic principles) theory of complexation. According to this theory 4-chloroaniline and possibly other xenobiotics interact with the soil matrix via three prominent mechanisms mediated by interaction of 4-CA with soil humic acid micelles:

- (1) "loosely" complexed and physically sorbed organic solvent-extractable surface complexes;
- (2) "tightly" complexed micellar inclusion products (physically and/or chemically sorbed) released by hydrolysis: and
- (3) "irreversibly" complexed residues due to hydrolytic action or long term biological incorporation.

The relative importance of each kind of complexation will depend on the physicochemical characteristics of the soil and xenobiotic involved. For 4-chloroaniline, mechanism (2) appears to be predominant.

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To my wife

CAROL

Her sacrifice and faith
made this endeavor a reality.

TABLE OF CONTENTS

	<u>PAGE</u>
I. INTRODUCTION	1
II. LITERATURE REVIEW	5
A. Soil Organic Matter Chemistry	5
A.1 Introduction	5
A.2 Genesis of humic substances	6
A.3 Postulated structures of humic substances	9
A.4 Extraction and fractionation of humic substances	14
A.5 Characterization of humic and fulvic acids	18
A.5.1 Infrared Spectrophotometric analysis of humic substances	18
A.5.2 ^1H -NMR versus ^{13}C -NMR analysis of humic and fulvic acids	19
A.5.3 Pyrolysis-gas-liquid chromatography/mass spectrometry (Py-GLC-MS)	26
B. Humic-like Substances in Plants and the Humification Process	28
B.1 Soil carbohydrates-especially cellulose and hemicellulose	31
B.1.1 Action of acid and base on carbohydrates	33

	<u>PAGE</u>
B.2 Fatty acids and other soil lipids	36
C. Humic and Fulvic Acid Physicochemical Properties	39
C.1 Colloidal chemical properties of humic substances	46
C.2 Free radicals in humic substances	47
D. Interaction of Xenobiotics with Soil Organic Matter	48
D.1 Relationship between OM and clay to adsorption of pesticides	58
D.2 Hysteresis effects	63
D.3 Bound pesticide residues	65
D.4 Physicochemical effects of the binding of xenobiotics to soil	69
E. Binding of Chloranilines to Soil	88
E.1 Chloroaniline binding mechanisms	99
E.2 Model reactions of ammonia and amino acids with soil	106
III. EXPERIMENTAL	109
A. Studies on the release of bound 4-CA from soil humic acids and whole soil	109
A.1 Introduction	109

	<u>PAGE</u>
A.2 Materials and Methods	109
A.2.1 Reagents	109
A.2.2 Apparatus	111
A.2.3 Methodology	112
A.3 Results and Discussion	123
A.3.1 Hydrolysis methodology	123
A.3.2 The nature of hydrolyzable residues	126
A.4 Other methods for release of tightly complexed and nonhydrolyzable 4-CA*HA	131
A.4.1 BF ₃ methanol extraction	132
A.4.2 20% Heptafluorobutyric anhydride in acetone extraction	133
A.4.3 Diazomethane methylation and extraction	133
A.4.4 Ultrasonic Extraction	135
A.5 Pyrolysis methodology	135
A.5.1 Pyrolysis of the base hydrolyzed HA-4-CA complex	135
A.5.2 Pyrolysis of the nonhydrolyzed HA*4-CA complex	138
A.5.3 Other pyrolysis results	139
A.6 Methodology Conclusions	140
 B. Studies on the Binding of 4-CA to Three Different Soils and its Release by Pyrolysis	 146

	<u>PAGE</u>
B.1 Introduction	146
B.2 Materials and Methods	146
B.2.1 Reagents	146
B.2.2 Apparatus	149
B.2.3 Methodology	150
B.3 Results and Discussion	151
B.3.1 The influence of 4-CA concentration applied on binding and release	151
B.3.2 The influence of varying concentrations of soil organic matter and clay	157
B.3.3 The influence of pyrolysis and different pyrolysis probes on release of bound 4-CA residues	158
C. Mechanism of Binding as Related to the Nature of Humic Acid Structure	160
C.1 Introduction	160
C.2 Materials and Methods	161
C.2.1 Reagents	161
C.2.2 Apparatus	162
C.2.3 Methodology	163
C.3 Results and Discussion	171
C.3.1 Extraction of humic acids	171

	<u>PAGE</u>
C.4 Characterization of humic and fulvic acids	174
C.4.1 IR Spectra of humic and fulvic acids	174
C.4.2 ^{14}C NMR spectra of humic and fulvic acids	184
C.4.3 IR and ^{13}C -NMR spectra of model or precursor humic substances	191
D. Physicochemical mechanisms of 4-CA, and other xenobiotics binding to and release from soil—a <u>de novo</u> theory	196
D.0 Introduction	196
D.1 Thermodynamics	198
D.2 Sorption isotherms	207
D.3 Diffusion controlled mechanisms	214
D.4 Kinetics	219
D.5 Inclusion phenomena	223
D.6 Humic substances as micelles and micellization as a binding mechanism	227
D.6.1 Mechanism of alkaline hydrolytic release of bound 4-CA	243
D.7 <u>De novo</u> theory of 4-CA and xenobiotic interaction with soil	244

	<u>PAGE</u>
IV. SUMMARY	249
V. APPENDICES	254
A. Contributions to original research	254
B. Consequences of research ,	255
C. Definitions	255
D. Common and chemical names	263
E. References	266

LIST OF TABLES

TABLE	<u>Page</u>
1. ^{13}C -NMR Spectra of Soil Humic and Fulvic Acids	22
2. Analysis of 4-CA*HA complexes	129
3. Selected pyrolysis results	136
4. Physicochemical properties of the soils used	148
5. Extractability of [^{14}C]4-CA from several soils	154
6. Pyrolytic results for bound [^{14}C]4-CA complexes in various soils	155
7. Summary of binding of 4-CA to three soils of varying OM and clay contents	156
8. Yield and ash content of fractions extracted by various techniques	177
9. Major IR absorptions for isolated humic acids	181
10. Transition metal ion contents for humic acids and fulvic acids	185
11. Major ^{13}C -NMR humic acid absorptions	187
12. Fulvic acid and a cellulose component ^{13}C -NMR chemical shifts	195

LIST OF FIGURES

FIGURE	<u>Page</u>
1. Structure of humic acid according to Fuchs	10
2. Structure of humic acid according to Dragunov	11
3. Type structure of humic acid according to Stevenson	13
4. Classical fractionation procedure for humic substances	15
5. Interaction of soil organic and inorganic fractions	50
6. Structure of FA as proposed by Schnitzer	54
7. Critical organic matter concentration	62
8. Hysteresis illustrated by sorption-desorption curves	64
9. Isolation of humic and fulvic acids from a Manitoba Carroll clay loam soil	113
10. Treatment of humic acids and hydrolysis	115
11. BF ₃ methanol, HFBA-(CH ₃) ₂ CO, and ultrasound procedures and results	117
12. Apparatus used for pyrolysis of tightly-complexed 4-CA substrates	121
13. Hydrolysis procedure and results for HA*4-CA complexes (tightly complexed)	124
14. Soil versus HA*4-CA complexes, extractability studies	134
15. Extractability of tightly complexed 4-CA from treated soil humic acids	145
16. Binding, pyrolytic release, and extractable [¹⁴ C]4-CA from three different soils	152

FIGURE	<u>Page</u>
17. Effect of OM on percentage [¹⁴ C]4-CA bound	153
18. Preparation of humic acid by the classical NaOH soil extraction and acid precipitation	166
19. Na ₄ P ₂ O ₇ soil extraction and acid precipitation	167
20. Na ₄ P ₂ O ₇ soil extraction and acetone precipitation	168
21. IR spectra of humic acids and ash contents	173
22. IR spectra of ultrafiltered HA-III	175
23. IR spectra of humic acids isolated using different extractants	176
24. ¹³ C-NMR spectra of isolated humic acids	188
25. Composite IR spectra of HA, FA and a cellulose component	193
26. ¹³ C-NMR a FA spectra of a cellulose component, and a glucan	194
27. Derivation of a proposed humic monomer model	197
28. Flow diagram of these results leading to a <u>de novo</u> theory of 4-CA binding to soil and soil humic acids	199
29. Langmuir sorption isotherms for 4-CA in soil	208
30. Monuron incorporation into micelles	229
31. Illustration of ionic spherical micelle	232
32. Solvent stabilization of micelles and hydrolytic release	241
33. Proposed humic or fulvic acid monomers	242
34. Proposed mechanisms of 4-CA interactions with soil	247

I INTRODUCTION

Mankind has become progressively more dependent upon the use of pesticides for the control and eradication of crop pests (plants, animals, insects, fungi, bacteria, nematodes, etc.). In the United States alone, the sales of just one class of pesticides, herbicides, has been estimated to be 492 million lbs. in 1975 (Rawis, 1975).

Increased pesticide usage in the past thirty years or so, has led to concern for their environmental fate and toxicity as contaminants. Knowledge of soil-pesticide interaction is crucial to an understanding of their potential or present toxicological hazards. Soil and sediment (dissolved and undissolved forms) are the environmental "sink" for all xenobiotics.

Of special concern are the bioactivity of the pesticides and their persistence or recalcitrance. The finding that fulvic acids and humic acids can interact with substantial amounts of hydrophobic dialkyl phthalates and solubilize them in water suggests that pesticides and other xenobiotics with low water solubilities may also be complexed and solubilized by this mechanism (inclusion); Ogner and Schnitzer, 1970a,b; Ballard, 1971, Kirkland, 1973.

Several toxicological hazards have been described for chlorinated anilines. Substituted anilines arising from degradation of pesticides are acetylated to form acylamides (Tweedy *et al.*, 1970; Kearney and Plimmer, 1972). Hoffman and Allen (1960) observed that N-acetyl derivatives of 3,4-dichloroaniline (3,4-DCA) were phyto-

toxic. Other compounds formed by the condensation of 4-CA and 3,4-DCA are chloroazobenzenes, anilinobenzenes and azoxybenzenes, all of which have been reported to be carcinogenic (Neish, 1964; Hall, 1977) and toxic to mammals (Bartha and Pramer, 1967; Weisburger and Weisburger, 1966) and microbes (Corke and Thompson, 1970). Many of the parent pesticides and/or their degradation products persist in the soil, so that environmental contamination must be a concern. Concern has been expressed for the long range effects of "poly-halogenated" soil organic matter on vital soil, sediment, or dissolved organic matter processes and conditions. Continued buildup of bound residues may affect physical and biochemical characteristics such as water-holding capacity, soil structure, soil organisms, and the processes of nitrification, ammonification, and cellulose decomposition. Once mechanisms of binding are clear, an assessment of potential release would be possible. For the present, attempts to elucidate the bound residue problem have centered around methods for analysis of bound compounds which resist extraction by the techniques normally used.

Changes in cultural practices may liberate bound residues, re-introducing them into the soil solution and thereby allowing subsequent uptake and translocation into the economic portions of plants, for example, in rice and soybeans (Nashed and Ilmicki, 1970; Still, 1968; Tweedy, et al., 1970), cotton (Smith and Sheets, 1967), rape, barley and rye (Guess, 1974). All these examples apply to substituted chloroanilines derived from several pesticides,

especially phenylurea, phenylcarbamate and anilide herbicides.

A soil bound residue has been defined as the chemically unidentified pesticide residue or its degradation product remaining in fulvic acid, humic acid, and humin soil fractions after exhaustive sequential extraction with nonpolar and polar solvents (Kaufman et al, 1976). The precise definition of a bound residue has yet to be wholly agreed upon. The complexity of the systems involved has frustrated research in this area. Analysis of soil bound residues is of paramount importance because of the potential accumulation of these residues in soil organic matter. Knowledge of the various interactions between xenobiotics and soil components may be gained by isolating those fractions most important in complexation realizing that a combination of fractions (i.e. fractions interacting with each other) may be especially important in the binding of residues.

The work described in this study involves 4-chloroaniline (4-CA), a common and abundant degradation product of many pesticides which is bound rapidly and tenaciously to soil components, especially humic substances (Hsu and Bartha, 1974a, 1974b, 1976; Worobey, 1977; Moreale and Van Bladel, 1976). The research presented herein describes experiments conducted to release and quantitate 4-CA bound to soil humic acids. The nature of this binding was investigated in relation to indigenous humic acid structure and the physicochemical properties of humic acids.

The objectives of the studies reported herein may be divided into the following three main areas:

1. To investigate methodology for release of soil (humic acid) bound 4-CA, residues, including so-called "nonhydrolyzable" residues;
2. To determine the nature of the humic acid substrate regarding the nature of its interaction with 4-CA;
3. To elucidate the mechanisms of complexation or "binding" of 4-CA by soil humic acids.

II LITERATURE REVIEW

A Soil Organic Matter Chemistry

A.1 Introduction

The organic matter in soils includes a complete spectrum of materials ranging from relatively undecomposed plant litter to the various decomposition and excretion products derived from plants, animals and microorganisms. Soil organic matter may be broadly classified into non-humic and humic (major organic component, usually) substances. The former consists of the recognizable remains of soil flora and fauna and the latter are formed by biological and nonbiological degradative and synthetic chemical reactions. Soil organic matter, which generally represents less than five percent of dry weight of mineral soils, is very resistant to further degradation and can be intimately associated with the mineral components of the soil. The general group of compounds included under the term "humic matter" or "humic substances" can be broken down into several subgroups, namely, humic and fulvic acid, humatomelanic acid and humin (Schnitzer and Khan, 1972).

They serve as a storehouse of chemical elements essential for plant growth, especially carbon and nitrogen, and, to a lesser extent, of phosphorus, sulfur, calcium, iron, manganese and other trace minerals. Humic substances have profound effects on soil structure, drainage, aeration, water and buffering capacity and physiological functions of plants (Linehan, 1977; De Haan, 1977;

Rotini, 1977; Hernando et al., 1977; Nopamornbodi, 1977). Although humic compounds are relatively resistant to both chemical and biological attack, they too are degraded into the simplest molecules (CO_2 , H_2O , NH_3) as a continuation of steps in the carbon and nitrogen cycles.

A.2 Genesis of humic substances

An understanding of the chemistry of humic substances and of the role they play in soil, requires some insight into the processes by which they are formed. Several hypotheses have been proposed to explain the formation of humic substances in soil (Felbeck, 1971).

a) The plant alteration hypothesis.

According to this view, the nature of the original plant compound strongly influences the nature of the humic substances (i.e., the carbohydrate, lignin, cellulose, lipid, protein, etc. of plants). The higher molecular weight humin then represents the first stages of humification followed by subsequent microbial attack to degrade the humin to humic acid, then fulvic acid, and finally mineralization (very slowly, 2-3% per year) to CO_2 and H_2O .

b) The chemical polymerization hypothesis.

Plant material is degraded microbially to small molecules, which are absorbed and utilized by the soil microbes as sources of carbon and energy. Microbes synthesize various products, usually phenols

and amino acids, which are excreted into the surrounding medium, where chemical oxidation and polymerization to humic substances take place. According to this hypothesis, the nature of the original plant tissue has no effect on the kind of humic substances finally produced.

c) The cell autolysis hypothesis.

Humification is thought to be the product of the autolysis of dead plant and microbial cells. The product is a heterogeneous substance formed by random condensation and polymerization of cellular debris such as sugars, amino acids, phenols, and other aromatic compounds, which are converted to free radicals by autolytic enzymes released on the death of the cell.

d) The microbial synthesis hypothesis.

As in the chemical polymerization hypothesis, microbes initially utilize the dead plant tissue only as a source of carbon and energy. However, microbes then synthesize various high molecular weight humic-like compounds intracellularly and these compounds are only released to the soil when the microbes die and their cells are lysed. According to this view, the higher molecular weight compounds represent the first stages of humification followed by extracellular microbial degradation to humic acid, fulvic acid, simple organic compounds, and, finally, to product of mineralization.

e) Chemical models of humic substances.

Several simple chemical monomers have been shown to yield humic-like compounds upon enzymatic or chemical oxidative polymerization (Schnitzer and Khan, 1972; Gieseking, 1975).

Hydroquinone, catechol, substituted benzene and hydroxybenzene carboxylic acids, etc., yield humic-like polymers upon alkaline or enzymatic (phenolase, tyrosinase) oxidation. It was demonstrated that the enzymatic oxidation of phenols (such as catechol, chlorogenic acid, and caffeic acid) in the presence of amino acids, peptides, or proteins yielded "humic acid" very similar to those from soil, and that the most rapid polymerization occurred at pH 7 (Swaby and Ladd, 1962; Ladd and Butter, 1966). Haider et al. (1965) oxidized phenolic compounds with phenolases in the presence of amino acids and peptides, and showed that the binding of nitrogen occurred at pH values above 6.5 and that only those phenols reacted which had no methylated hydroxyl groups. Synthetic polymers resembling humic acids have also been synthesized by heating solutions of glucose and glycine or methylglyoxal and glycine. Hayes (1960) showed that, when appropriate ratios of reactants were chosen and the polymerization process controlled, the resulting synthetic humic acids had physicochemical properties similar to humic acid isolated from a muck soil. Finally, the nonenzymatic browning reaction (Maillard reaction) of carbohydrates with amines also yields humic-like compounds (Gieseking, 1975).

These hypotheses all represent possible pathways in humic matter formation. Selection of one theory over the others is impossible, since the actual processes probably involve a combination of two or more mechanisms.

A.3 Postulated structures of humic substances

Numerous attempts have been made to devise a structural formula representative of humic acids, but none were entirely satisfactory. The widely quoted model of Fuchs, Fig. 1, is untenable for several reasons (Kononova, 1966, Swain, 1963). The data obtained by x-ray diffraction excluded polycondensed systems containing more than a few rings. A polycondensed system failed to account for conformational changes implied by viscosity studies (Mukerjee and Lahiri, 1958).

Contemporary investigators favor a structural model consisting of micelles of polymeric nature, the basic structure of which is an aromatic ring of the di- or trihydroxyphenol-type bridged by -O-, -CH₂-, -NH-, -N=, -S- and other groups, and containing both free OH groups and quinone linkages. Dragunov's structure (Fig. 2) most nearly meets the above requirements. However, a fully acceptable model would also need to account for the occurrence of significant amounts of aromatic -COOH groups, some arranged in positions such that cyclic anhydrides can be formed by various chemical treatments. The presence of 5-membered ring structures and condensed elements containing two or three rings is also a distinct possibility.

Felbeck (1965a, 1965b) on the basis of his organic matter

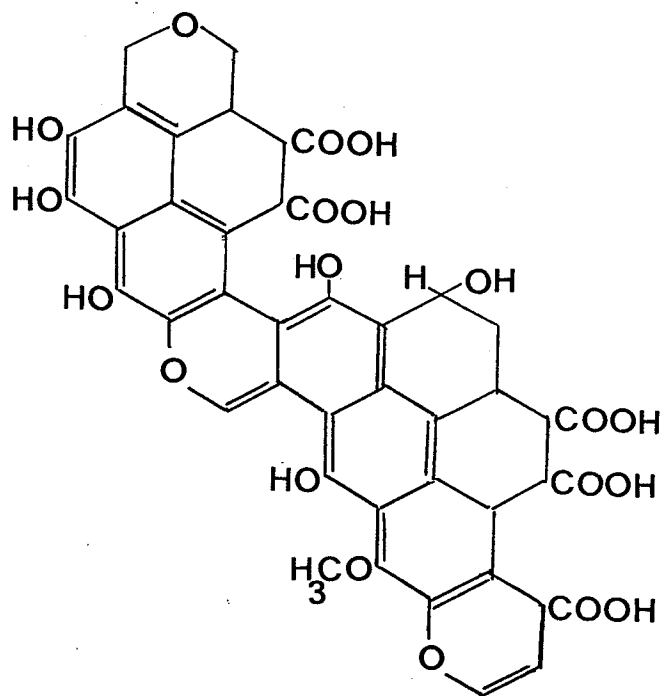


FIGURE 1. Structure of humic acid according to Fuchs (Kononova, 1966; Swain, 1963).

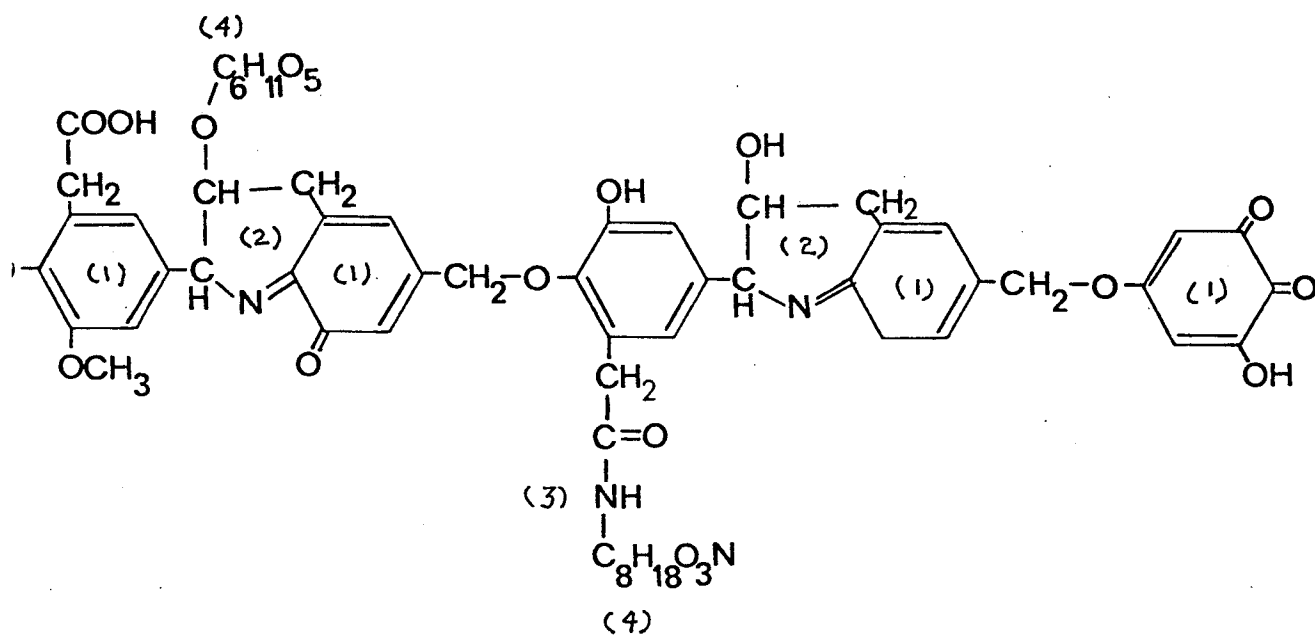
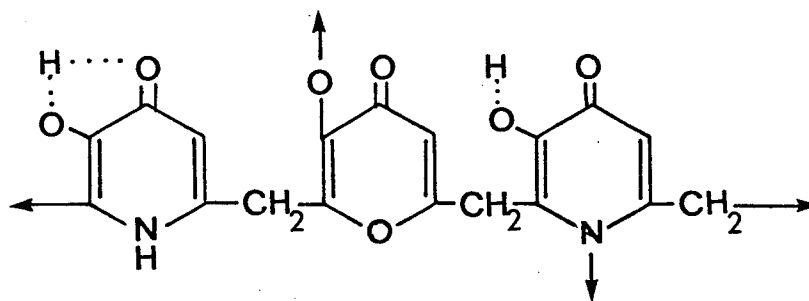


FIGURE 2. Structure of humic acid according to Dragunov (Kononova, 1966). (1) Aromatic ring of the di- and tri-hydroxybenzene type, part of which has the double linkage of a quinone group. (2) Nitrogen in cyclic forms. (3) Nitrogen in peripheral chains. (4) Carbohydrate residues.

hydrogenation studies, proposed a humic structure in which 4-pyrones (as illustrated below) act as a central structural unit for phenolic



and amino acid side chains. However, this hypothesis lacks credibility since the molecule does not contain COOH and OH groups which can be readily methylated. Haworth and his coworkers (Cheshire *et al.*, 1967) suggested that humic acid possesses a chemically resistant polycyclic aromatic core to which polysaccharides, proteins, and phenolic acids were attached. These side chains are linked to each other. Such a molecule would have the ill-defined structure typical of humic substances, and the central core, which would contain the stable semiquinone radicals, should be resistant to microbial attack.

Stevenson (1972a,b) summarizes the structure for humic acid (Fig. 3). As can be seen, this humic acid model contains a high density of reactive functional groups (COOH, phenolic OH, and quinone C=O). While the humic acids in a given soil will vary in composition, most molecules would be expected to contain the same basic units and the same types of functional groups indicated by the model structure. Most recently Schnitzer and coworkers (1972) have

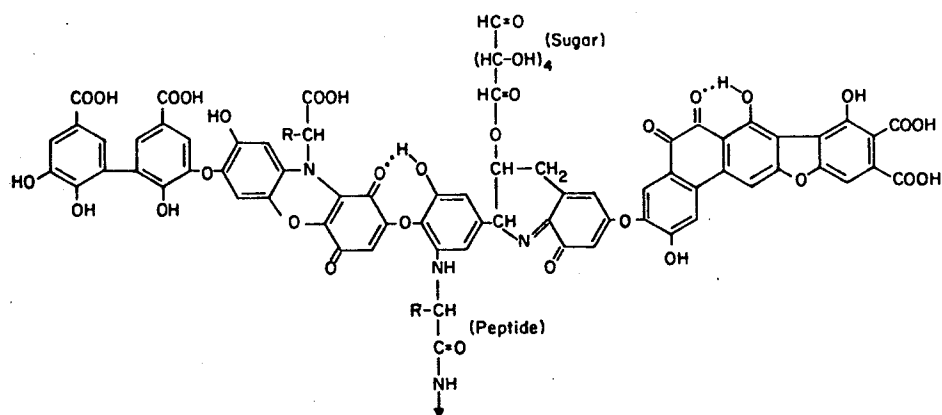


FIGURE 3. Type structure of humic acid according to Stevenson (1972).