Membrane-Micelle Model for Humus in Soils and Sediments and Its Relation to Humification

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By ROBERT L. WERSHAW

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CONVERSION FACTORS

Multiply	By	To obtain
dalton	1.660×10^{-24}	gram
gram (g)	2.205×10^{-3}	pound
hectare (ha)	2.470	acre
liter (L)	0.264	gallon
nanometer (nm)	3.937×10^{-8}	inch
square nanometer (nm^2)	1.550×10^{-15}	square inch

Membrane-Micelle Model for Humus in Soils and Sediments and Its Relation to Humification

By Robert L. Wershaw

Abstract

Humification, the process whereby biomass consisting of dead plant and animal remains is converted into soil organic matter (humus), is one of the basic processes of the carbon cycle. The organic compounds that make up plant and animal tissue are thermodynamically unstable in the oxidizing atmosphere at the surface of the Earth. After the organisms in which they are incorporated die, the compounds are converted back to carbon dioxide and water by degradation reactions catalyzed by enzymes secreted by micro-organisms. However, not all the organic compounds in the dead biomass are immediately converted; some of the material is only partially oxidized. The residue left after partial oxidative degradation of the dead biomass is the source of the organic compounds that accumulate in soils and sediments as humus.

Previously, humification was thought to involve a conversion of degradation products by a series of polymerization reactions into new types of polymeric species that are different from the precursor molecular species in the original biomass. However, it is proposed here that the depolymerization and oxidation reactions that take place during the enzymatic degradation of biopolymers produce amphiphiles—molecules that have a polar (hydrophilic) part and a nonpolar (hydrophobic) part. These amphiphiles that result from the partial oxidative degradation of dead biomass assemble spontaneously into ordered aggregates in which the hydrophobic parts of the molecules form the interiors and the hydrophilic parts of the molecules make up the exterior surfaces of the aggregates. These ordered aggregates constitute the humus in soils and sediments. Humus ordered aggregates most likely exist as bilayer membranes coating mineral grains and as micelles in solution.

INTRODUCTION

The importance of humus as a major factor in controlling the physical and chemical properties of soils has long been recognized (Waksman, 1936). Properties such as buffering capacity, metal-binding capacity, sorption of hydrophobic organic compounds, stability of aggregates of soil particles, and water-holding capacity are determined, to a large extent, by the amount of humus present in a soil. In soils of identical mineralogical composition and grain-size distribution, water permeability will generally be higher in the soil of higher humus content. This is especially the case in fine-grained soils. Humus content is also important in maintaining soil fertility in both temperate and tropical regions. In the tropics, the absence of a significant concentration of humus generally renders the soil infertile (Coleman and others, 1989).

There is no universally accepted definition of humus. Stevenson (1982, p. 35) considered humus to be synonymous with soil organic matter but excluded "the remains of plant residues and their partial decomposition products." In contrast with this viewpoint, it will be shown in this report that all the major organic components in soils, sediments, and natural water systems interact with each other to form the humus aggregates and, therefore, the products of partial decomposition of plant tissue are included in humus. Indeed, it will be demonstrated that humus consists

mainly of the partially decomposed chemical constituents of plant tissue. However, intact plant parts are not considered here as constituents of humus.

The process of humus formation is called humification. In natural systems, dead plant and animal remains accumulate as litter layers on soil surfaces. These litter accumulations then undergo enzymatic degradation, and the degradation products are carried down into the soil zone by rainwater. Some of the degradation products are sorbed by the soil particles and some are transported through the unsaturated zone into the saturated zone, where they can react with aquifer materials or remain dissolved in, and move with, the ground water. In addition to natural humification, humification in the form of composting is being used increasingly as a means of solid waste disposal. In order to predict the fate of hazardous substances undergoing composting, a detailed knowledge of the chemical and physical processes of humification is necessary.

Purpose and Scope

Humus is probably the most chemically and physically active component of soils and sediments but it is also the least understood; even the chemical structures of the constituents of humus are still a matter of considerable controversy. In this report, a new model of humus is described, along with an extensive discussion of the evidence that has led to the development of the model. The report describes the humification process from the initial degradation of plant litter that has accumulated on soil surfaces to the incorporation of humus into soil horizons. This discussion is followed by a detailed exposition of the evidence that led to the understanding of the humification process presented here and to the creation of the membrane-micelle model of humus. These results provide a new working hypothesis for the study of humus and its interactions in natural systems.

Humus and Soil Formation

In the first stage of soil formation, rocks are degraded into mineral particles that make up the inorganic components of soil. The mineral grains may be derived from the underlying rocks or they may be derived from rocks that are far removed from the location of the soil. In either case, further development of the soil occurs in place after primary rock decomposition. This soil development results in the formation of different horizons in the soil profile (Russell, 1961).

Plant degradation products, which ultimately form humus, are very important in the formation of the characteristic horizons of most soil profiles. Following the initial breakdown of the rock, plants establish themselves in the weathered rock. As these plants die or lose their leaves, plant debris accumulates in the litter layer (O horizon) at the top of the soil profile and undergoes degradation (a generalized diagram of a typical soil profile is given in fig. 1). Living organisms mix plant residues from the O horizon with weathered rock fragments to form the first soil horizon, the so-called A horizon (Buckman and Brady, 1969). Soluble organic degradation products from the litter layer are carried down into lower horizons by percolating rainwater. These soluble products are mainly organic acids that enhance the breakdown of rock components, yielding soluble nutrients and secondary minerals such as clays and hydrous metal oxides.

Not every soil horizon depicted in figure 1 is developed in all soils. The presence or absence of these horizons is dependent on the climate, drainage, topography, mineral composition of the source rocks, fauna and flora of the area, and maturity of the soil. However, in general, during the development of a soil profile, clay minerals and hydrous metal oxides are leached from the A horizon and deposited in the

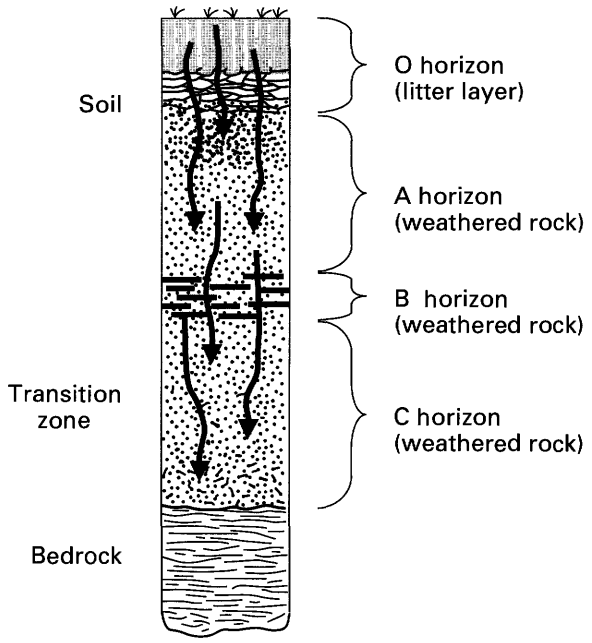

Figure 1. Generalized soil profile.

B horizon. The leaching process, which is called eluviation, is enhanced by the presence of organic acids. Eluviation results in the concentration of quartz and other resistant minerals in the A horizon. Humus also is generally depleted in the A horizon, probably because the organic molecules that make up the humus are not strongly adsorbed by the remaining minerals of the A horizon. On the other hand, the clay minerals and hydrous metal oxides that are deposited by the process called illuviation in the B horizon strongly adsorb humus molecules, thereby concentrating soil organic matter in the B horizon. The humus coatings on the mineral grains render this horizon darker than the A horizon. In calcareous soils, soluble humus components from the decaying litter layer are precipitated by divalent calcium ions in the underlying soil horizons (Zech and others, 1990b). The transition zone between the weathered and unweathered rock is called the C horizon.

Effects of Humus on Soil Properties

Many important physical and chemical properties of soils that affect agriculture are dependent on the presence of humus in the soils. MacCarthy and others (1990) have pointed out that although in most agricultural soils humus is present in much lower concentrations than mineral constituents, it contributes about one-half the cation exchange capacity of the soils. The exchange sites on the humus bind both macronutrients and micronutrients and prevent them from being rapidly leached from the soils. Thus, humus-bound nutrient ions are retained in the soil for slow release to plants. Soil humus also functions as a reservoir for elements such as nitrogen, phosphorus, and sulfur, which generally are not bound by ion exchange sites, and provides a substantial part of the buffering capacity of many soils.

The water-holding capacity and porosity of soils are also controlled to a large extent by their humus content. Soils that are high in humus generally have high water-holding capacity. In addition, soil organic matter has been shown to increase the availability of water to plants (Russell, 1961), which is probably due mainly to increased porosity. The porosity of a given soil is a function of the ability of the soil to form stable aggregates or crumbs (Haynes and Swift, 1990). It has been well known for a long time that humus promotes the formation of water-stable crumbs in soils (Russell, 1961). More recently, research has been done to determine which components of humus are most active in forming aggregates. Haynes and Swift (1990) found in the two soils they studied that aggregate stability was more closely correlated with water-extractable carbohydrate content than with organic carbon content or total hydrolyzable carbohydrate content. In contrast, Capriel and others (1990) found that aggregate stability in the soils they studied was highly correlated to the amount of hydrophobic aliphatic compounds that was extracted with supercritical hexane. Because Haynes and Swift (1990) and Capriel and others (1990) used different extraction procedures, direct comparison of their results is not possible. It may be that both water-extractable carbohydrates and hydrophobic aliphatic compounds contribute to aggregate stability in both sets of soils. Importantly, however, both these studies clearly demonstrate that small amounts (less than 0.1 percent) of specific organic compound types can significantly increase the aggregate stability of a soil.

Stable soil aggregates also are important in preventing soil erosion because they help to maintain soil permeability during rainfall (Russell, 1961). The rainwater can therefore infiltrate the soil rather than run off with some of the finer soil constituents. In addition to reducing erosion, this increased infiltration makes more water available for plant use.

An interesting example of the importance of humus in soil fertility is provided by the Terra Preta soils of the tropics. These highly fertile soils are generally found in small (less than 2 hectares), isolated areas surrounded by much less fertile Oxisols and Ultisols (Zech and others, 1990a). Zech and others (1990a) have studied a Terra Preta soil surrounded by an Oxisol in the Amazon region of Brazil. This is a typical Terra Preta soil in that it has the same mineralogical composition as the soil surrounding it but has a much higher humus concentration. The presence of the humus renders the soil much more fertile than the surrounding Oxisol by increasing the concentration of plant nutrients in the soil. Nuclear magnetic resonance (NMR) spectra of both soils indicate that the Terra Preta humus has a higher concentration of aromatic functional groups than does the Oxisol humus. The high humus concentration of the Terra Preta soil apparently is the result of heavy applications of manure, household garbage, and bones by Indians who had built settlements close by. The higher aromatic concentration in the humus of the Terra Preta soil is consistent with an increased concentration of lignin components in cattle manure and composts (Inbar and others, 1989; Deiana and others, 1990). Zech and

others (1990a) point out that Terra Preta soils are of anthropogenic origin, and that similar soils have been found not only in other parts of South America but also near former settlements in West Africa.

Another example of increased fertility caused by increased humus concentration is found in the sandy soils of the Nile Valley. Application of sewage sludge and raw sewage to the sandy soils has markedly increased their humus concentration and productivity. This increased productivity is probably due to both increased nutrient concentrations and increased water-holding capacity.

In addition to enhancing mineral nutrition and water-holding capacity, humus provides other benefits to plant growth. Chen and Aviad (1990, p. 162) have reviewed the effects of humic substances on plant growth and pointed out that "humic substances * * * under conditions of adequate mineral nutrition, consistently show positive effects on plant biomass." Much of the increased plant biomass is the result of stimulation of root growth, although some stimulation of shoot development also has been observed. Chen and Aviad (1990, p. 180) have concluded that:

> The modes of action of humic substances on plant growth can be divided into direct (requiring uptake by plant tissue) and indirect effects as follows:
>
> Direct effects:
>
> 1. Effects on membranes resulting in improved transport of nutritional elements.
> 2. Enhanced protein synthesis.
> 3. Plant-hormonelike activity.
> 4. Enhanced photosynthesis.
> 5. Effects on enzyme activity.
>
> Indirect effects:
>
> 1. Solubilization of microelements (e.g., Fe, Zn, Mn) and some macroelements (e.g., K, Ca, P).
> 2. Reduction of active levels of toxic elements.
> 3. Enhancement of microbial populations.

The mechanisms of these processes are related to the physical-chemical properties of the humus, which are discussed in general terms below in relation to the membrane model of humus. Detailed discussions of the mechanisms of interaction can be found in Vaughan and Malcolm (1985). It is interesting to note that Vaughan and McDonald (1971, 1976) have proposed that the stimulation of ion uptake by plants, brought about by the presence of humic substances, is caused by increased membrane permeability. Chen and Aviad (1990, p. 174) have proposed that surface-active humic substances "may interact with the phospholipid structures of cell membranes and react as carriers of nutrients through them." In light of the membrane-forming characteristics of humus, it is not unreasonable to expect that some of the components of humus will enter into the cell membranes of plant organs—especially the roots.

In addition to the effects of humus on plant growth, Xudan (1986) found that foliar spraying of fulvic acid improved the drought resistance of wheat. This improvement was associated with a partial closure of the stomata in the leaves of the plants, which reduced transpirational losses. The fulvic acid also slowed leaf senescence, and leaf function was maintained for longer periods of time.

Structure of Humus

Historically, humus has been fractionated into three groups of so-called humic substances—humic acid, fulvic acid, and humin—on the basis of solubility in acidic and basic solutions (Kononova, 1966). In this classification, humic acid is that fraction of soil humus that is soluble in strong base but precipitates at pH values less than 2; fulvic acid is soluble in both basic and acidic solution; and humin is insoluble in both basic and acidic solutions. Most workers in the field have assumed, either implicitly or explicitly, that humic acid, fulvic acid, and humin are structurally different chemical entities that can be represented by three different generalized chemical structures.

Various interpretations have been put forth on the chemical structures of the different humic substances. The consensus of many workers in the field is that humic substances are mixtures of highly cross-linked polymers of different molecular weights and charge densities (Stevenson, 1986). Many of these workers appear to believe that it is possible to represent the structure of each of the three humic substances by using a generalized structural diagram of covalently bonded functional groups similar to that which has been used to represent the chemical structure of lignin. The relative solubilities of the three different humic substances then result from differences in molecular weight and charge density.

In addition to the distinctions between humic acid, fulvic acid, and humin, many investigators have

attempted to distinguish between humic and nonhumic substances in soils and sediments by considering such well-defined molecular species as proteins, carbohydrates, and lipids as nonhumic substances (Stevenson, 1982). Other workers, however, believe humic substances arise from a Maillard-like reaction between reducing sugars and amino acids (Rubinsztain and others, 1986). Furthermore, peptides and carbohydrates are frequently present in fulvic- and humic-acid isolates that have been described in the literature (Anderson and others, 1989; Wershaw and others, 1990).

It will be demonstrated here that the distinctions between humic and nonhumic substances and between humic acid, fulvic acid, and humin are artificial, and that it is necessary to consider the various weak bonding interactions between the organic compounds in any model of soil organic matter. In order to take into account these interactions, Wershaw (1986) developed a unified model in which humus is pictured as being made up of ordered aggregates of plant degradation products. The depolymerization and oxidation reactions that take place during the enzymatic degradation of biopolymers produce amphiphiles, that is, molecules that have a polar (hydrophilic) part and a nonpolar (hydrophobic) part. In aqueous systems, these amphiphilic molecules, by virtue of the fact that they consist of these parts, will assemble spontaneously into ordered aggregates in which the hydrophobic parts will be in the interiors of the aggregates away from the water phase, and the hydrophilic parts of the molecules will be on the exterior surfaces of the aggregates in contact with the water phase or with polar groups on the surfaces of mineral grains. It is these ordered aggregates of organic molecules that constitute the humus in soils and sediments. These humus aggregates, like other amphiphile aggregates, can assume a variety of different configurations such as micelles, bilayer membranes, and liquid crystals (Leibler, 1990). In soils and sediments, humus ordered aggregates most likely exist as bilayer membranes coating mineral grains and as micelles in solution.

According to Wershaw (1986), the commonly used extraction procedures with basic solutions partially disrupt the humus aggregates and allow extraction of some of the components of these aggregates. On acidification, some of the components reaggregate to form an insoluble phase called humic acid; the components that remain in solution are called fulvic acid. Those components of the aggregates that are resistant to dissaggregation by the extracting solvent constitute the humin fraction. Therefore, in terms of this proposed model, the division of humic substances into humic acid, fulvic acid, and humin is artificial and tends to obscure the close interactions between the natural organic constituents of soils, sediments, and natural waters. It is more proper to consider these organic constituents as parts of the humus ordered, aggregated structures. Wershaw (1986) has further proposed that the component molecules of the humic aggregates are partially degraded plant biopolymers, simple molecules from plants, and various compounds derived from soil micro-organisms. The component molecules are held together by weak interactions such as hydrogen bonding and hydrophobic forces. The physical and chemical properties of these humic aggregates are more a function of the structure of the aggregates than of the properties of the individual components.

MECHANISMS OF DEGRADATION OF PLANT MATERIALS

Dead plant tissue and, to a lesser extent, dead animal tissue accumulate on soils as a litter layer, which is the source of the organic compounds that form humus. Two processes are involved in the formation of humus from plant tissue. The first process is the degradation of the plant tissue and the second is the incorporation of the degradation products into the humus. In this section, the degradation reactions of plant material will be discussed and, in particular, those that affect the major structural components of vascular plants.

Vascular plants constitute, by far, the dominant group of plants in most terrestrial environments. The tissue of these plants consists mainly of three groups of polymers: cellulose, hemicelluloses, and lignins. Lesser quantities of aliphatic polyesters, starches, proteins, phenolic macromolecular species, and lipids are also present in the tissue of vascular plants.

In a general way, the degradation of plant polymers involves depolymerization and oxidation. The degradation of plants is most commonly brought about by the action of enzymes that catalyze the degradation reactions; however, nonenzymatic degradation also is possible. The degradation enzymes, for the most part, are secreted by micro-organisms that use the plant material as a source of energy. In addition to the direct enzymatic degradation of plant materials,

some oxidative cleavage reactions have been identified as important in the degradations of some plant components. The oxidative reactions are brought about by activated forms of oxygens or free radicals that are produced enzymatically by fungi or other micro-organisms. In order to better understand the degradation reactions, each major group of plant polymers will be considered separately.

Biologically Mediated Degradation

Cellulose

The cell walls of most vascular plants are composed primarily of cellulose; lesser amounts of hemicellulose, lignin, cutin, and other plant polymers also may be present in the cell walls of different types of plant tissues. The exact composition of the cell wall of a particular type of cell will be dependent on the function of the organ containing the cell and the function of the cell within the organ.

Cellulose is a homopolymer of β-D-glucose in which the glucose units are linked by 1,4-glucoside bonds into long, relatively straight chains. In cellulose, the chains of monomeric units are bound parallel to one another by hydrogen bonding in groups of chains called fibrils. Hydrogen bonding between the chains in a fibril is promoted by the fact that all the OH groups of the glucose units are equatorial and, therefore, the OH groups in one chain easily overlap the OH groups in another chain. The fibrils vary in diameter in different types of plant tissue; Harada and Côté (1985) have reported a range from 2.5 to 20 nm.

The enzymatic hydrolytic degradation of cellulose in natural systems has been extensively studied (see reviews by Eriksson and Wood, 1985; Wood, 1988 and 1989). In general, two different types of enzymes are involved in the hydrolysis of cellulose to water-soluble polysaccharide fragments: endo-1,4-β-glucanases and exo-1,4-β-glucanases. A 1,4-β-glucosidase then converts the soluble cellulose fragments into glucose. Oxidative enzymes also can oxidize the cellulose fragments to acids. All these enzymes are produced by different groups of fungi and bacteria.

The white-rot group of fungi is one of the most common groups that attack woody plant tissue in natural systems. This group secretes extracellular enzymes that degrade both polysaccharides and lignins. Eriksson and Wood (1985) have pointed out that the most studied of the white-rot fungi is *Sporotrichum pulverulentum*. This fungus produces five endo-1,4-β-glucanases and one exo-1,4-β-glucanase. The endo-1,4-β-glucanases attack the cellulose chains at random, hydrolyzing β(1→4)glucosidic linkages, producing smaller cellulose fragments. In addition to hydrolysis within the chain itself by the endo-enzymes, the exo-enzymes split off cellobiose or glucose from the nonreducing ends of the cellulose fragments produced by the endo-1,4-β-glucanases. Any one cellulose chain can undergo many attacks by the two types of enzymes, so that at a given time during the degradation of a mass of plant tissue, a wide variety of different-size cellulose polymer fragments will be present. Some of these fragments will be soluble in water and some will not. Further hydrolysis of the soluble polymer fragments is brought about by 1,4-β-glucosidases (Eriksson and Wood, 1985).

Other types of fungi such as brown-rot and soft-rot fungi also attack the crystalline cellulose of plant cells (Eriksson and Wood, 1985). In all these fungi, the mechanism of hydrolysis of the cellulose appears to be similar to that of the white-rot fungi: (1) random attack by endo-1,4-β-glucanases, and (2) attack at the nonreducing ends by exo-1,4-β-glucanases.

In addition to hydrolytic enzymes, the fungi that degrade wood and other plant tissue also produce oxidative enzymes. Thus, the fungus *S. pulverulentum* produces two different types of oxidative enzymes: (1) a cellobiose oxidase that oxidizes cellobiose and higher cellodextrin oligomers to aldonic acids, and (2) a cellobiose:quinone oxidoreductase that degrades both cellulose and lignin (Westermark and Eriksson, 1974a, b). This oxidoreductase enzyme reduces quinones and phenoxyl radicals in the presence of cellobiose; the cellobiose is oxidized to cellobionic acid.

Bacteria also produce cellulase enzymes, but they are much less effective in degrading crystalline cellulose than enzymes from fungi (Eriksson and Wood, 1985). It appears the reason for this lower effectiveness is that most bacterial cellulases contain endo-1,4-β-glucanases but little if any exo-1,4-β-glucanases; however, some evidence exists that the thermophilic anaerobe *Clostridium thermocellum* does produce both endo- and exo-1,4-β-glucanases (Eriksson and Wood, 1985). The presence of both forms of glucanases may explain why this bacterial species is as effective as some fungi in degrading cellulose.

Hemicelluloses

Hemicelluloses, which make up approximately one-third of the dry weight of most higher land plants (Wilkie, 1983), are an extremely important group of naturally occurring polysaccharides. Commonly, a wide variety of different types of plant-cell polysaccharides have been included in the category of hemicellulose. Because of this diversity, hemicelluloses are much more difficult to characterize and define than either cellulose or lignin. In the past, hemicelluloses have been defined operationally as those plant-cell-wall polysaccharides that are soluble either in water or in aqueous alkaline solution and are easily hydrolyzable (Wilkie, 1983).

More recently, Wilkie (1983, p. 307) has proposed the following more specific definition:

> Hemicelluloses are polysaccharides from, or in, higher plants and are abundant in cells that have undergone lignification. In the cell walls they form an aqueous gel in which bundles of cellulose molecules (microfibrils) are embedded in regular or irregular orientations. The hemicelluloses are dissolved by alkali; cellulose is not. They are much more readily hydrolyzed by acid than cellulose and then, depending on the plant give L-arabinose, D-xylose, D-mannose, some D- and less L-L-,galactoses, D-glucose, L-rhamnose, D-glucuronic acid, 4-O-methyl-D-glucuronic acid, D-galacturonic acid and other sugars * * *.

Puls and Poutanen (1989) have pointed out that the hemicelluloses are most commonly heteropolysaccharides in which the backbone of the polymer is made up of one type of saccharide, and the sidechains of other types of sugars. The most abundant hemicelluloses in higher plants are those in which the backbone is made up of 1,4 linked β-D-xylopyranose units. However, backbones made up of other saccharide units such as 1,3 and 1,4 linked D-galactopyranose and 1,4 linked D-mannopyranose also are common. A wide variety of different sidechains can be attached to the hemicellulose backbones including L-arabinose, D-glucose, D-glucuronic acid, D-4-O-methylglucuronic acid, D-galacturonic acid units, and O-acetyl groups; feruloyl and coumaryl esters also may be linked by L-arabinose residues to the backbones.

Enzyme systems capable of degrading the various hemicellulose backbones (hemicellulases) are widely found in nature. Dekker (1985) has pointed out that bacteria from terrestrial and marine environments, phytopathogenic and rot fungi, rumen bacteria and protozoa, cecal bacteria of both ruminants and nonruminants, certain insects and molluscs, crustaceans, marine algae, and germinating seeds of terrestrial plants all produce hemicellulases. The hemicellulases produced by most bacteria, fungi, and yeasts are secreted extracellularly; therefore, any hemicellulose close to these organisms is vulnerable to enzymatic degradation. In addition to enzymes that degrade the backbones of hemicelluloses, specialized enzymes also degrade the side chains.

The mechanisms of enzymatic degradation of the backbones of the hemicellulose polymers are similar to the mechanisms of enzymatic degradation of cellulose. The hemicellulases are hydrolytic enzymes; both endo- and exo-hemicellulase enzymes have been isolated. Endo-hemicellulases, which constitute the most common group of these enzymes, attack the backbone chains in a random manner, causing a progressive decrease in molecular weight and chain length. The exo-hemicellulases then hydrolyze off terminal monomer or oligomer units from the excised backbone fragments (Dekker, 1985; Puls and Poutanen, 1989). Many studies of hemicellulose enzymatic degradation have revealed that a number of different types of specialized hydrolytic enzymes exist that remove the various side chains attached to the hemicellulose backbones (Puls and Poutanen, 1989).

Lignin

The term lignin has been applied to a heterogeneous group of polymers that constitute one of the three major groups of components of the cell walls of vascular plants. Lignin differs from the other two major components, cellulose and hemicellulose, in several different ways. Cellulose and hemicellulose are both polysaccharides that form chain polymers—that is, the polymerization has taken place in a single dimension with a single bond between each of the monomeric units. Lignin, on the other hand, is a group of aromatic polymers composed of three types of phenylpropane monomers linked together by carbon-carbon and ether linkages. The three monomeric units that compose lignin are coniferyl alcohol, sinapyl alcohol, and p-hydroxycinnamyl alcohol (fig. 2). These three monomers are in different proportions to one another depending on the plant source of the lignins.

In the past, most students of lignin chemistry have assumed that lignin consists of a group of

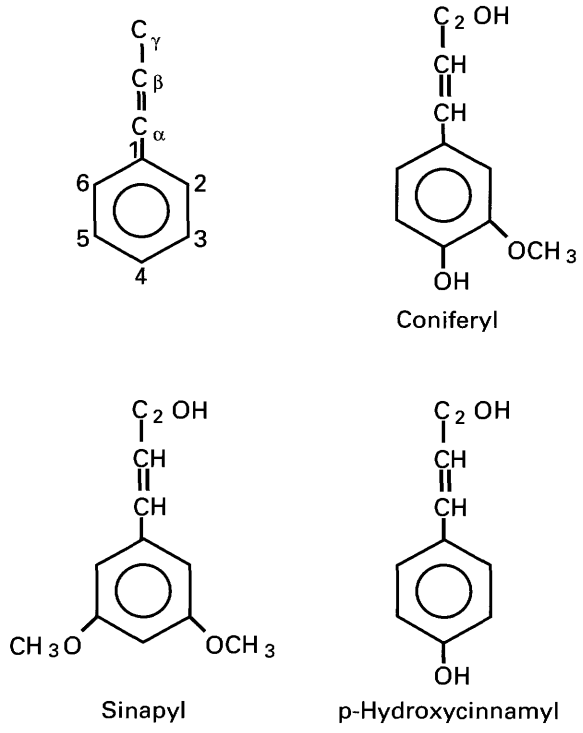

Figure 2. Chemical structures of lignin monomeric units. The standard designations of the various carbon atoms in the units are shown on the structural representation in the upper left-hand diagram.

random, three-dimensional polymers. However, recently it has become increasingly apparent that a new paradigm is necessary for the structure of lignin (Goring, 1989). Goring (1989) has proposed that, although lignin in the middle lamellae of wood may indeed be a random, three-dimensional polymer, the lignin in the secondary walls of wood cells consists of nonrandom, two-dimensional networks that are closely associated with the hemicellulose chains. Goring (1989) has pointed out that middle-lamellae lignin constitutes less than 20 percent of the lignin in a tree, and, therefore, most of the lignin is in the secondary walls.

Some of the lignin in wood is present as lignin-carbohydrate complexes (Koshijima and others, 1989). The phenylpropane groups of the lignin appear to be attached to hemicellulose chains by benzyl-ether linkages. These complexes have number-average molecular weights on the order of 6,000–8,000 daltons. The molecules of the lignin-carbohydrate complexes are amphiphiles that exhibit a strong tendency to form micelle-like aggregates in aqueous solution. The carbohydrate portions of the molecules are hydrophilic and the lignin parts are more hydrophobic.

The chemical stabilities of the lignin and the saccharide polymers cellulose and hemicellulose differ because the bonds binding the monomeric units in these two types of polymers are different. The polysaccharide polymers form in plants by the enzymatic dehydration of monomeric units. Reversal of the polymerization (depolymerization) takes place by rehydration at the glycosidic bonds between the monomeric units mediated either by hydrolytic enzymes or by acid hydrolysis to release the free monomeric units. Lignin, on the other hand, is formed in xylem cells of plants by enzymatic, free-radical dehydrogenation of the phenylpropane monomers. The resulting carbon-carbon and ether linkages are much less susceptible to hydrolysis and other forms of degradation than the glycosidic linkages in polysaccharides (Chen and Chang, 1985). The chemical stability of the major cross-linking bonds in lignin makes it difficult to accept the three-dimensional, cross-linked paradigm for lignin as correct in light of the relative ease with which it is extracted from wood pulp by the kraft or sulfite process. It is more reasonable to assume that most of the lignin molecules are relatively low molecular weight, two-dimensional networks, as has been proposed by Goring (1989) and others.

In the formation of lignin, hydrogen atoms are abstracted from phenylpropane monomers (lignols) by the action of peroxidases and hydrogen peroxide to yield free radicals, which then couple together to form dimeric quinone methides. Subsequent nucleophilic attack by water or adjacent hydroxyl groups causes re-aromatization to dilignols. The dilignols then undergo the same series of reactions over and over again to yield cross-linked lignin polymers. As discussed below, the biodegradation reactions that bring about depolymerization of lignin are very similar to the reactions of lignin formation (Higuchi,1989).

Studies of the biodegradation of lignin have followed a very different pathway from studies of the biodegradation of most other biopolymers such as polysaccharides, proteins, and polynucleotides because its irregular, two- or three-dimensional polymeric structure does not yield well-defined, simple monomeric units after enzymatic attack. In addition, lignin is not used as a sole energy source by any known organism (Leisola and Garcia, 1989). A number of authors have recently reviewed the biodegradation of lignin (Kirk and Shimada, 1985; Higuchi, 1986; Kirk and Farrell, 1987; Leisola and Garcia, 1989; and Schoemaker and others, 1989). Because of the structural complexity of natural lignins, many of

the studies cited in these reviews involve the detailed investigation of the mode of attack of enzyme isolates on model dimers and oligomers. The investigators have then tried to extend their results on model systems to the biodegradation of natural lignin polymers.

The enzymatic reactions that bring about lignin biodegradation are mainly oxidative reactions that cause cleavage of aryl propyl side chains, ether cleavage and demethoxylation, aromatic-ring cleavage, and aromatic hydroxylation and carboxylation. These reactions generally take place extracellularly of the organisms that produce the enzymes because the lignin macromolecules or their aggregates are too large to pass through the cell membranes.

The major lignin-degrading organisms are the white-rot fungi and the related litter-decomposing Basidiomycetes (Kirk and Shimada, 1985). Most of the studies on lignin degradation have been carried out with the white-rot fungus *Phanerochaete chrysosporium*. These organisms secrete extracellular peroxidase and phenoloxidase enzymes; the peroxidase enzymes require hydrogen peroxide for lignin degradation. In addition, Schoemaker and others (1989) have postulated that these organisms also secrete aldehyde and acid reductases.

Most of the oxidation reactions of lignin that have been studied may be described as one-electron oxidations of phenols by laccase or peroxidase to yield phenoxyl radicals, or of nonphenolic moieties to yield radical cations. The hydrogen peroxide necessary for the peroxidase attack on lignin appears to be produced in the presence of added glucose by several different glucose-1-oxidases that have been found in crude lignin-degrading cell extracts (Kirk and Farrell, 1987). Both the oxidation of phenolic moieties and nonphenolic moieties will be discussed below in detail in order to illustrate the types of products that can be expected from lignin biodegradation.

As was pointed out above, the enzymatic degradation of lignins involves one-electron oxidation to yield radical cations. Palmer and others (1987) have reviewed the reaction properties of radical cations. These properties are summarized in table 1. The action of ligninase and hydrogen peroxide on a model compound in which the β carbon of the propane side chain of one lignin monomeric unit (fig. 2) is linked by an ether linkage to the 4 carbon of the aromatic ring of another monomeric unit (β-O-4 model) serves to illustrate radical-cation reactions in lignin degradation (fig. 3). The β-O-4 model has been chosen because it

Table 1. Reaction mechanisms of radical cations
[Modified from table 4 of "The role of peroxidases, radical cations and oxygen in the degradation of lignin" by J.M. Palmer, P.J. Harvey, and H.E. Schoemaker in *Philosophical Transactions of the Royal Society of London A*, 1987, v. 321, p. 499, courtesy of The Royal Society]

Reaction	Result
One-electron oxidation	Oxidation of substrate and reduction of radical cation to ground state
Side-chain cleavage of cation	Cleavage of C_α–C_β bond; Cleavage of C–H bond; Decarboxylation
Addition of water to radical cation	Hydroxylation of styrene; Cleavage of ether bond; Formation of phenol
Reaction with another radical (HOO·)	Opening of ring

appears to be the dominant substructure in lignin (Kirk and Farrell, 1987). Either rings A or B can undergo one-electron oxidation, as illustrated by pathways A and B in this example, depending on the relative oxidation potentials of the two rings.

The oxidation potential of an aromatic-ring compound will be dependent on the type and position of substitution on the ring. Strong electron-withdrawing functionalities, such as C_α-carbonyl groups, will inactivate aromatic nuclei to oxidation, whereas alkoxyl groups will activate the ring. The positions and number of the alkoxyl groups also will have an effect; one would therefore expect that the oxidation rates will be in the order: syringl, greater than guaiacyl (sinapyl), greater than p-hydroxylphenyl.

The reactions illustrated in figure 3 are examples of the types of reactions that take place in lignin degradation. Products 1–3 in figure 3 result from C_α-C_β cleavage, which appears to be the major reaction in intact *P. chrysosporium* cultures. This reaction also has been found to be important in studies of the actual degradation of lignin by white-rot fungi (Chen and Chang, 1985). Product 4 is formed by C_α oxidation; Chen and Chang (1985) point out that these types of products also are found in white-rot fungi degradation products of lignin. Intramolecular nucleophilic attack by the C_γ-hydroxyl at the C_4 of the ring B cation followed by reduction leads to product 5. This product spontaneously decomposes into products 6 and 2. On the other hand, nucleophilic attack by water at the

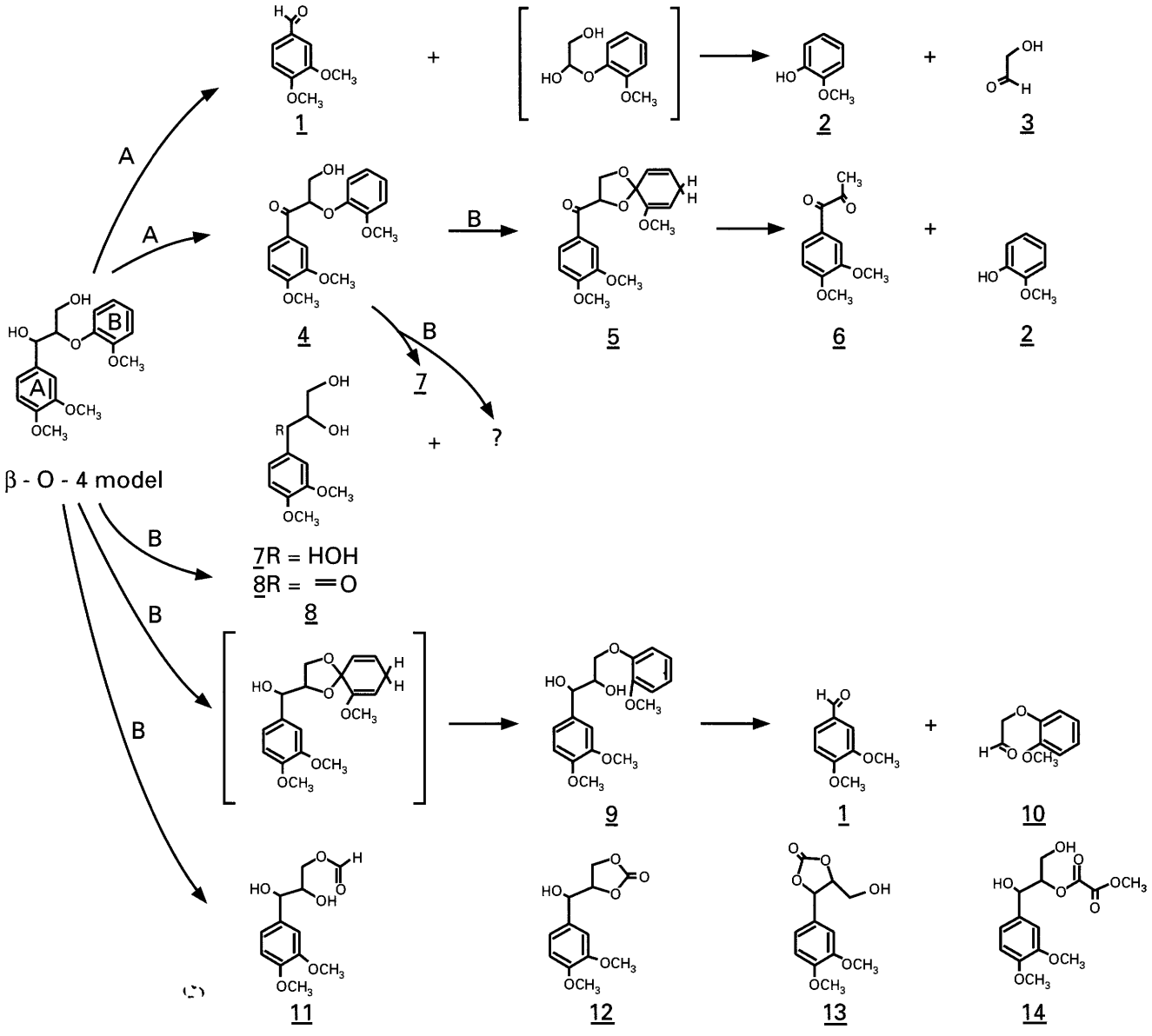

Figure 3. Reaction products of oxidation of β-O-4 lignin model compounds by a ligninase-H_2O_2 system. Oxidation of either the A or B ring may take place as indicated. From Kirk and Farrell (1987, p. 479).

C_4 of the ring B gives products 7 and 8. It is postulated that product 9 arises from the intermediate one shown in brackets followed by heterocyclic ring opening; cleavage of the C_α-C_β bond of product 9 produces products 10 and 1.

Oxidation of the B ring followed by ring cleavage results in products 11–14. These products are closely related to one another and are substrates for further ligninase oxidation; they have been identified by Higuchi (1986) in studies of the degradation of oligolignols by white-rot fungi. These types of products also have been found in lignins that have been partially degraded by white-rot fungi (Kirk and Farrell, 1987).

Phenolic moieties in lignin undergo one-electron oxidations catalyzed by peroxidase and laccase (a blue copper oxidase) enzymes produced extracellularly by most white-rot fungi. Phenoxyl radicals are produced by disproportionation reactions, as shown in figure 4 (Kirk and Shimada, 1985). In the figure, the phenoxyl radicals also are shown undergoing demethoxylation and a variety of other reactions that can lead to depolymerization.

In all the lignin degradation reactions discussed above, radicals are produced that can undergo coupling reactions and repolymerization. The question then arises as to what limits repolymerization in the

natural degradation of lignin by white-rot fungi and other organisms. Schoemaker and others (1989, p. 469) have postulated that "rapid metabolism of ring opened products and quinone/hydroquinone type intermediates is one possible way of shifting the resulting polymerization-depolymerization equilibrium towards degradation." I would like to suggest that another possible mechanism is the incorporation of lignin degradation products as they are produced into heterogeneous humic membranelike aggregates where they are stabilized; this will be discussed in more detail below.

Other Aromatic Compounds

A wide variety of different types of nonlignin aromatic compounds are found in plants; examples of these include tannins, flavonoids, various phenols, and aromatic acids. Generally, all these types of compounds are present in much lower concentrations in plant tissue than the major components that were dealt with above; nonetheless, they are important in many types of plants and, therefore, deserve some attention here. In addition, as was pointed out above, phenols and aromatic acids are produced by the enzymatic degradation of lignins, and these products of lignin degradation also will undergo the types of reactions discussed here. Because of the diversity of these compounds, I shall attempt only to discuss general types of microbial degradation reactions that ultimately lead to the complete metabolism of the compounds or, at least, to their inactivation and detoxification. Furthermore, the discussion here will be limited to the degradation of the low molecular-weight compounds. The hydrolytic and oxidative degradation reactions of nonlignin polymeric aromatic species are sufficiently similar to the reactions for lignin and polysaccharide degradation that no further discussion is necessary. The mechanisms of microbial degradation will be briefly outlined here; higher organisms also are important in the degradation of some of these compounds, but many of the same types of degradation products are produced as in the microbial degradation reactions.

Barz and Weltring (1985) have summarized the types of reactions involved in the microbial biodegradation of aromatic compounds (fig. 5). Of the reactions listed, the most important for humus formation on the surface of the earth are the oxidation reactions, which are catalyzed by mono-oxygenases and

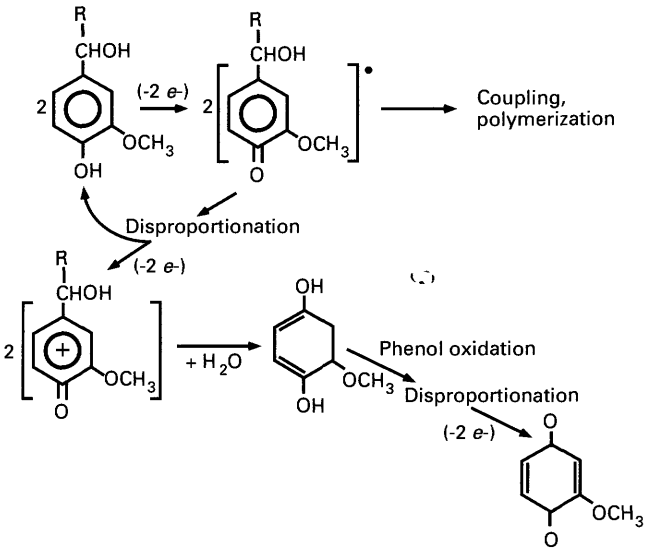

Figure 4. Examples of disproportionation reactions that can take place after enzymatic one-electron oxidation of lignin-derived phenolic compounds.

dioxygenases. Barz and Weltring (1985) have pointed out that the enzymes involved in the initial and intermediate degradation of aromatic compounds are induced enzymes—that is, they are only produced by the micro-organisms in the presence of the substrate.

Mono-oxygenases catalyze aromatic and aliphatic C-hydroxylations, epoxidations, N-, O-, S-dealkylations, and N- and O-oxidations. In these reactions, one oxygen atom of an oxygen molecule is incorporated into the substrate, whereas the other oxygen is reduced to water (R represents any combined substituent group):

$$RH+O=O+NAD(P)H+H^{+}\rightarrow ROH+HOH+NAD(P).$$

In this type of reaction, bacterial mono-oxygenases generally require nicotinamide adenine dinucleotide in the reduced form (NADH), whereas fungal and mammalian mono-oxygenases require reduced nicotinamide adenine dinucleotide phosphate (NADPH). Mono-oxygenases are especially important in adding a second hydroxyl group to a phenol (fig. 6), which subsequently undergoes ring-fission reactions.

Dioxygenases incorporate both oxygen atoms of an oxygen molecule into a single substrate molecule. These enzymes are important in ring-fission reactions of dihydroxyl phenols such as those produced by mono-oxygenases. As shown in figure 6, dicarboxylic acids, aldehydes, and oxohexadienoic acids can be produced in these fission reactions.

The bacterial and fungal enzymatic degradation reactions of the flavonol quercetin provide interesting

OXIDATION

$-CH_3 \rightarrow -CH_2OH \rightarrow -CHO \rightarrow -COOH$

$>CHOH \rightarrow >C=O$

$CH_2CH_2- \rightarrow -CH=CH-$

$>CHCO_2H \rightarrow >CHOH + CO_2$

$-CH_2CH_2NH_2 \rightarrow -CH_2COOH$

HYDROXYLATION

$>CH \rightarrow >COH$

DEMETHYLATION

$-OCH_3 \rightarrow -OH + HCHO$

Figure 5. Examples of aromatic degradation reactions mediated by micro-organisms. From table 1 of "Biodegradation of aromatic extractives of wood" by Wolfgang Barz and Klaus-Michael Weltring in *Biosynthesis and Biodegradation of Wood Components*, Takayoshi Higuchi, ed., 1985, p. 611, courtesy of Academic Press.

examples of the types of products that are produced by oxidative biodegradation. In the degradation of quercetin by the *Aspergillus* fungus (fig. 7) the dioxygenase quercetinase catalyzes the incorporation of two oxygen atoms into the depside ring. Further reaction with water leads to the production of phloroglucinol and protocatechuic acid (Barz and Weltring, 1985). A different mechanism has been found for the degradation of quercetin by *Pseudomonas* bacteria (fig. 8). Monooxygenase-catalyzed hydroxylation first takes place on ring A, followed by dioxygenase-catalyzed metacleavage of the ring. The depside linkage is also cleaved by a dioxygenase-catalyzed oxidation.

Lipids

Lipids are a diverse group of compounds that are defined operationally as those water-insoluble compounds that can be extracted from plant or animal cells by nonpolar organic solvents. The commonly used terms "fats," "oils," and "waxes" refer to groups of such compounds. Those specific chemical groups that constitute the most abundant lipids and their degradation pathways are discussed below.

The most abundant group of lipids are the triesters of glycerol—the triglycerides. The most common of these are the triesters of long-chain carboxylic acids. They serve as the principal energy-storage compounds of living organisms. Those triglycerides that are solid at room temperature are called fats, whereas those that are liquid are called oils. A particular triglyceride may be a triester of a single long-chain acid or of two or three acids. Natural fats and oils extracted from either plants or animals are mixtures of different triesters.

Two other important groups of lipids are the phospholipids and the steroids. The largest group of phospholipids are trigylcerides in which a terminal glycerol hydroxyl group is esterified with phosphoric acid; the other two groups are esterified with fatty acids. All steroids have the tetracyclic ring system (hydrogenated cyclopentanophenanthrene) shown in figure 9 as a common structural feature.

Lipids are used as an energy source by living organisms. For triglycerides, the first step in this utilization involves hydrolysis of the ester linkages between the glycerol moiety and the long-chain fatty acids catalyzed by lipase enzymes. This is followed by oxidation of the β carbon (the second carbon atom from the carboxylate carbon atom) to a ketone. Hydrolytic cleavage then takes place to release acetic acid and a fatty acid two carbon atoms shorter than the starting fatty acid. The β-oxidation reaction will

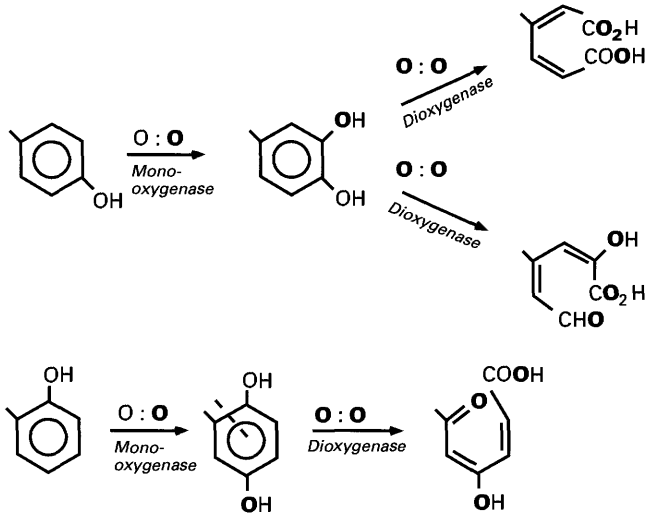

Figure 6. Dioxygenase-induced aromatic-ring fission reactions following oxidation by mono-oxygenase. Oxygen added by the reaction is shown in bold. From figure 2 of "Biodegradation of aromatic extractives of wood" by Wolfgang Barz and Klaus-Michael Weltring in *Biosynthesis and Biodegradation of Wood Components*, Takayoshi Higuchi, ed., 1985, p. 614, courtesy of Academic Press.

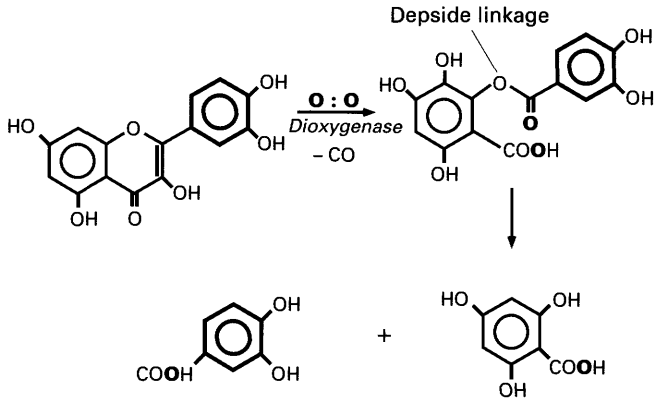

Figure 7. Pathway of fungal degradation of quercetin. Oxygen added by the reaction is shown in bold. From figure 13 of "Biodegradation of aromatic extractives of wood" by Wolfgang Barz and Klaus-Michael Weltring in *Biosynthesis and Biodegradation of Wood Components*, Takayoshi Higuchi, ed., 1985, p. 627, courtesy of Academic Press.

continue to shorten a fatty-acid chain until it is entirely consumed. The lipids that are isolated from soil humus therefore may represent all stages in the biochemical degradation of lipids from plant and microbial remains.

The aerobic degradation of steroidal lipids in soils and sediments has not been studied in detail. Most of the studies in the literature have been concerned with transformations that take place under anaerobic conditions during early diagenesis of sediments (Peakman and Maxwell, 1988). One would expect that the tetracyclic ring systems of steroids would be relatively resistant to oxidation because they generally have few points of unsaturation. For example, cholesterol (fig. 9) has only one point of unsaturation. Enzymatic oxidation of cholesterol in mammalian livers results in the formation of bile acids in which hydroxyl groups have been added to the ring system of cholesterol, the side chain has been partially shortened, and the terminal end oxidized to a carboxylate group. Similar types of reactions would be expected during the enzymatic aerobic degradation of steroids in litters and soils. The resulting products would be amphiphiles, as are the bile acids. The side chains would be susceptible to β-oxidative shortening in the same way as fatty acids. Other alicyclic natural products such as terpenes probably also undergo similar types of degradation reactions in oxidative environments.

A wide variety of intact lipids have been isolated from soils, sediments, peats, and crude oils (Kvenvolden, 1966; Stevenson, 1982). These compounds have been used as biomarkers to provide clues to the identity of the organisms that have given rise to the organic matter in which the lipids are found. Undoubtedly, however, the intact lipids only represent a fraction of the lipids present in the original plants or micro-organisms, the rest of the lipid material having been either completely or partially biodegraded. Lähdesmäki and Piispanen (1988) have found that lipids along with proteins, sugars, starches, amino acids, cellulose, and lignin are present in the decomposed litter of spruce needles and aspen leaves. Their data indicate that lipids degrade more slowly than proteins, simple sugars, or starches.

Baldock and others (1990) have found by studying the conversion of ^{13}C-labeled glucose in soils that many of the lipids in soil are derived from soil micro-organisms. They found that the glucose was converted into carbon dioxide, lipids, new saccharides, and carboxyl groups by soil micro-organisms. Of the organic carbon from the labeled glucose that was converted into soil organic matter, about 25 percent was in polymethylene groups in lipids. At least one-third of these polymethylene groups is highly mobile (liquidlike); the significance of this finding will be discussed in the section on the membrane model of humus.

Cutin and Suberin

Cutin and suberin are closely associated with the waxy material produced by plants. Cutin is the polymeric material that coats the epidermal cells of the leaves and other aerial organs of plants; it serves as a

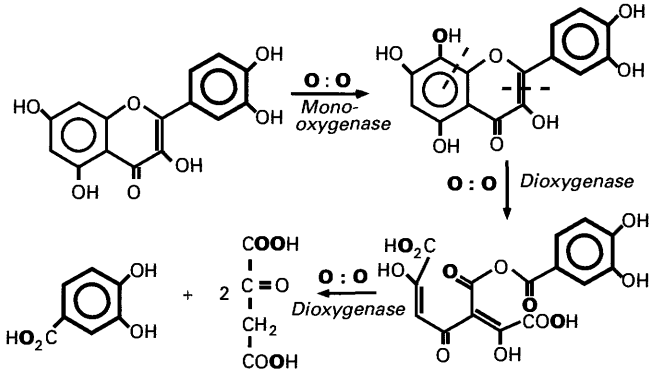

Figure 8. Pathway of bacterial (*Pseudomonas*) degradation of quercetin. Oxygen added by the reaction is shown in bold. From figure 14 of "Biodegradation of aromatic extractives of wood" by Wolfgang Barz and Klaus-Michael Weltring in *Biosynthesis and Biodegradation of Wood Components*, Takayoshi Higuchi, ed., 1985, p. 628, courtesy of Academic Press.

barrier to water loss from the plant tissue. It is a polyester polymer made up of hydroxy and hydroxy-epoxy fatty acids (Kolattukudy and Espelie, 1985) in which the major monomeric units are C_{16} and C_{18} fatty acids. Cutin may be depolymerized by alkaline hydrolysis and other techniques such as transesterification with sodium methoxide and methanol. Suberin is the polymeric material that coats the cork cells located between the inner and outer bark layers. Because of its location, it is much more difficult to isolate than cutin. At the present time, only fractions of cells enriched in suberin have been isolated, and for this reason the composition of suberin is not as well understood as that of cutin (Kolattukudy and Espelie, 1985). Kolattukudy and Espelie (1985, p. 173) hypothesized that "suberin consists of aliphatic polyester domains covalently attached to aromatic domains which are in turn attached to the cell wall * * *." Evidence for this hypothesis comes from depolymerization studies in which fatty acids have been released and chemical oxidation studies in which aromatic moieties similar to those found in lignin have been released.

No controlled studies of the biodegradation of cutin and suberin have been carried out; however, one would expect that hydrolytic and oxidative enzyme systems exist in nature that depolymerize these plant components. The products of partial or complete depolymerization of cutin and suberin would most likely be fatty acids, aliphatic and aromatic esters of fatty acids, and aromatic acids. These possible biodegradation products are similar to those that would be released during the biodegradation of lipids and lignin, and at this stage in our knowledge, it would probably not be possible to distinguish the biodegradation products of cutin and suberin from those of lipids and lignin. In this regard, Zech and others (1990b) have observed that humification in subalpine rendzinas involves a marked enrichment of aliphatic residues, which they state are derived from microbial biomass or from phospholipids, cutins, fats, and waxes of plant origin. They also observed rapid degradation of "lignin subunits bound by aryl-ether linkages and recalcitrance of 'condensed' lignin units" and mineralization of carbohydrates. The carboxylate carbon content increased with increasing degradation due to oxidation of lignin side chains.

Nitrogen Compounds

Nitrogen-containing compounds such as proteins are generally in low concentrations in the structural tissue of plants; however, they are relatively

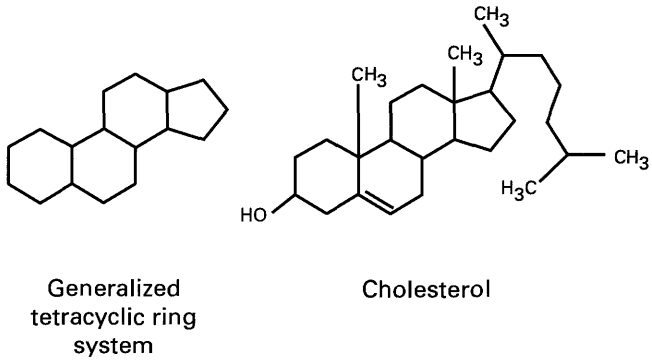

Figure 9. Tetracyclic ring system of steroids, and the structure of cholesterol.

abundant in soil organic matter. This seeming paradox apparently results from the fact that significant organic nitrogen is added to soils by the activity of micro-organisms. Thus, Joergensen and Meyer (1990) determined that the C/N ratio was 45 in fresh leaf litter collected from a beech forest. They observed, however, that this ratio decreased to 10 in the C horizon of the soil beneath the litter. Similarly, they found that the amino-acid-C/amino-sugar-C ratio decreased from 45 in the fresh litter to 3 in the B horizon. They attributed these changes to amino acid and amino sugar contributions from micro-organisms growing in the decomposing plant matter. The C/N ratio of about 10 measured by Joergensen and Meyer (1990) in the C horizon is typical within a factor of 2 of C/N ratios in soils in general (Stewart, 1984; Stevenson, 1986). Plant structural components in general have much higher ratios (60–500 according to Stevenson, 1986). It is therefore reasonable to assume that the process of nitrogen enrichment, caused by contributions from micro-organisms that Joergensen and Meyer (1990) have described, is a universal process that takes places in most soil profiles.

Joergensen and Meyer (1990) pointed out that amino sugars have not been found in the structural tissue of higher plants and are present only in very low concentrations in other plant tissue, but are components of microbial cell walls and extracellular polysaccharides secreted by micro-organisms. Additional evidence for the microbial origin of most nitrogen-bearing compounds found in the forest soil horizons is that fungal tissue isolated from the beech forest studied by Joergensen and Meyer (1990) has an amino-acid-C/amino-sugar-C ratio of 3.5, which is very similar to the ratio of 3 measured in the B horizon of the soil beneath the forest. Joergensen and Meyer (1990)

concluded from the fact that microbial biomass generally accounts for only between 1 and 3 percent of soil organic matter that most of the amino sugars they found are extracellular and present in the humified material in the soil. In addition to the amino sugars, it appears from their data that a majority of the amino acids they isolated in hydrolyzates from the soil layers is also derived from the proteinaceous components of micro-organisms.

Other studies also have shown that many of the nitrogenous compounds in soil humic substances consist of proteins or amino acids and amino sugars. Schnitzer (1985) has reviewed the occurrence of nitrogen-containing compounds in humic substances. Data in this review indicate that approximately 30 percent of the nitrogen in humic acids and fulvic acids from temperate climates consists of amino acid nitrogen. Amino sugars account for between 1 and 2 percent of the nitrogen in these humic acids and between 4 and 5 percent of the nitrogen in the fulvic acids. In tropical soils, approximately 50 percent of the nitrogen in humic acids and humins is present as amino acids; amino sugars account for 3–5 percent of the nitrogen in the humic acids and 8–9 percent of the nitrogen in the humins. Amino acids account for about 40 percent of the nitrogen in fulvic acids from tropical soils and amino sugars about 2–3 percent. In all of the humic substances mentioned by Schnitzer (1985) about 15–20 percent of the nitrogen was present as ammonia.

Data cited by Stevenson (1986) indicate approximately similar nitrogen distributions among amino acids, amino sugars, and ammonia as those mentioned by Schnitzer (1985). In addition, both Stevenson (1986) and Schnitzer (1985) point out that 30–50 percent of the nitrogen in soil humus is unidentified. This nitrogen has been divided into two categories: so-called hydrolyzable unknown nitrogen (HUN) and insoluble nitrogen species. Schnitzer (1985) discussed a number of possibilities for the unidentified nitrogen in humus. These include Schiff bases formed by the reaction of amide groups in proteins or amino acids with carboxyl groups in lignin fragments, phenoxazones formed by the reaction of ammonia with phenols, and polymers that result from Maillard-type condensations of amino acids with reducing sugars. Many other possible reactions have also been suggested; however, no definitive data exist on the actual form of the unidentified nitrogen species in humus.

Recent studies by Thorn and Mikita (1992) on the reaction of ^{15}N-labeled ammonia with humic and fulvic acid isolates indicated that the following nitrogen moieties were formed: indole, pyrrole, pyridine, pyrazine, amide, lactam, and aniline. These were identified by ^{15}N NMR spectroscopy. The most abundant groups were the indole and pyrole groups. The work of Thorn and Mikita (1992) provides important clues about the types of unidentified nitrogen groups that may be present in natural humus. The functional groups that they identified in their model studies could form by the reaction of ammonia produced by microorganisms with humus in natural systems. However, ^{15}N NMR is a very insensitive technique and its use for identification of nitrogen species in unenriched, natural samples has not been possible.

Thorn and Mikita (1992) have proposed a number of possible reaction mechanisms for the formation of the various nitrogren function groups they identified. These include reaction of ammonia with lactones and esters to form lactams and amides and reaction of ammonia with quinones to form aniline. A variety of intramolecular and intermolecular condensation reactions could account for the formation of heterocyclic nitrogen-containing groups; however, additional work will be necessary for definitive results. Because the work of Thorn and Mikita (1992) was carried out using mild reaction conditions, it may also provide clues about the mechanisms of formation of some of the unidentified nitrogen groups in natural systems.

Nitrogen species also may be bound to clay minerals, both at the surfaces of the mineral grains and in interlayer positions. These complexes could account for some of the insoluble nitrogen in soils. Nitrogen species on the surfaces of clay minerals may bind humic substances to the clay surfaces. Thus, Scharpenseel and Kruse (1972) have shown that basic amino acids can function as bridges between clay mineral particles and humic acid molecules. Wershaw and Pinckney (1980) have been able to verify that this type of binding takes place in natural systems by isolating from soils clay-humic complexes in which the humic acids are bound to the clay surfaces by amino acids or peptides.

Nitrogen is an essential nutrient in plant growth and, therefore, information about its cycling through the biosphere and the types of nitrogen species in soils is of great interest. Most of the nitrogen available on the surface of the earth is in the atmosphere. Stevenson (1986) has reviewed the literature on the movement of nitrogen from the atmosphere into the soil and the

transformations of nitrogen species that take place in the soil. Living micro-organisms such as bacteria and blue-green algae in the soil reduce elemental nitrogen to ammonia by means of the enzyme nitrogenase; this process is called nitrogen fixation. This ammonia nitrogen is partially utilized by the organisms to produce amino acids and proteins.

The nitrogen-fixing micro-organisms may either be free-living or associated with higher plants (mostly legumes, but also some nonlegumes). Those micro-organisms that fix nitrogen in association with higher plants are called symbiotic fixers. Both anaerobic and aerobic micro-organisms fix nitrogen.

The free-living nitrogen fixers require a plentiful supply of energy to fix nitrogen. This energy is provided by organic compounds such as carbohydrates in the case of heterotrophic organisms such as the bacterial genus *Azotobacter* (Brock, 1974) and sunlight in the case of autotrophs, such as blue-green algae. The surficial litter layer with its abundant supply of carbohydrates from the degradation of plant remains and its exposure to sunlight, therefore, provides an ideal environment for both heterotrophic and autotrophic nitrogen-fixing organisms. However, autotrophic organisms are generally excluded in environments where there is an abundant source of carbon; thus, Stevenson (1986) has pointed out that in agricultural soils, blue-green algae are probably only important for nitrogen fixation during the initial stages of soil formation.

Nitrogen fixation, however, is not confined to the surface layers of the soil. Aerobic bacteria such as *Azotobacter* and anaerobic bacteria such as *Clostridium* also are active in lower soil layers. Symbiotic nitrogen fixation by the bacteria of the genus *Rhizobium* in association with leguminous plants is another very important source of fixed nitrogen in nonsurficial soil layers. In agricultural soils, much more nitrogen generally is fixed by rhizobia and leguminous plants than by free-living micro-organisms. The fixation of nitrogen by bacteria is favored by low concentrations of available soil nitrogen compounds.

In addition to nitrogen fixation by micro-organisms, ammonia, nitrate, nitrite, and organic nitrogen are added to soils by rainfall and by dry fall. Stevenson (1986) has pointed out that even though the amount of nitrogen added to agricultural soils by precipitation is probably insignificant from the point of view of crop fertilization, it is important in forests and native grasslands where nitrogen loss through harvesting and grazing does not take place.

A wide variety of micro-organisms cause the breakdown (mineralization) of the proteins contributed by nitrogen-fixing micro-organisms and other sources. In the first step of this decomposition, proteinases and peptidases catalyze the hydrolysis of amide linkages to release free amino acids. The amino acids are then attacked by amino acid dehydrogenases and oxidases that convert them to ammonia; this process is called ammonification.

Nonenzymatic Degradation Reactions

In addition to the alteration of the organic precursors of humus by enzymatically catalyzed reactions, nonenzymatic degradation reactions also may be significant in some environments. The most likely nonbiochemical reactions to be important for the formation and alteration of humus are the photochemical reactions. Photochemical reactions are brought about by the absorption of light by a molecular species (chromophore). When the chromophore absorbs light of a particular wave length, electrons in the molecules of the chromophore are excited to a higher energy level. When the energy of an electron in a molecule is higher than the ground-state energy level, the molecule is said to be in an excited state. In order for the molecule to return to the ground state, the difference in energy between the ground energy level and the higher energy level must be dissipated either as heat, radiated energy (light), by energy transfer to another molecule, or by decomposition of the molecule. This decomposition often leads to the formation of a free-radical molecule. Free radicals are very reactive species that can undergo a variety of reactions. Because photochemical reactions require the absorption of light, photochemical reactions are limited to surface waters and the exposed surfaces of plants and litter.

Although a great deal of attention has been paid to photosynthetic reactions in living plants, very little work has been done on the photochemical reactions that take place after a plant or a part of a plant (such as a leaf) dies. Senescent leaves make up the majority of the litter layer in many environments (Benner and others, 1990). Leaves undergo gradual chemical change during senescence even before they fall off the plant. Some of these chemical changes are evidenced by changes in the color of the leaves. These color changes may alter the amount of ultraviolet radiation that is absorbed. If increased absorption of ultraviolet radiation takes place, then formation of free radicals will be enhanced. These free radicals could very well

initiate depolymerization reactions of the components of leaves, including cutins and suberins (M.C. Goldberg, U.S. Geological Survey, oral commun., 1990).

Humic substances, which strongly absorb both ultraviolet and visible radiation, are the most important nonliving absorbers of solar radiation in most natural waters (M.C. Goldberg and K.M. Cunningham, U.S. Geological Survey, written commun., 1990). The radiant energy that is absorbed by humic substances can be dissipated by several different mechanisms. The excited humic-acid molecules themselves can undergo decomposition to form free-radical species, which can combine with oxygen to give $ROO^{\bullet}$ radicals. This type of decomposition probably accounts for the reduction in molecular weight observed during the photolysis of aquatic humic substances (Geller, 1985). It also could account for some of the increase in acidity observed in aquatic humic substances as compared to soil humic substances. Excited humic substances also can transfer energy to other dissolved species, which will then participate in chemical reactions or undergo decomposition. For example, an excited humic molecule can transfer energy to an oxygen molecule, which will be transformed to singlet oxygen, a highly reactive species. Another possibility is for the humic molecule to undergo oxidation by transferring an electron to an oxygen molecule, producing a superoxide radical which, in turn, can produce hydrogen peroxide.

The products that result from the degradation reactions discussed above will be mainly oxidized fragments of plant polymers. It is proposed that these fragments consist generally of relatively unaltered plant polymer segments that have been oxidized to carboxylic acid groups at one or more ends of the segments. The unaltered portions of the segments will be more hydrophobic than the carboxylic acid groups on the ends of the segments. Molecules that consist of separate hydrophobic (nonpolar) parts and hydrophilic (polar) parts are called amphiphiles (see fig. 10 for a generalized diagram of an amphiphile). The phosphoglycerides, which are major constituents of the membranes of living cells, are amphiphiles. In these compounds, two of the glycerol hydroxyl groups are esterified to fatty acids and one is esterified to a phosphate group (left-hand diagram, fig. 10). Amphiphilic molecules display surfactant behavior in aqueous solutions because the hydrophobic parts of the molecules are repelled from the water molecules and, therefore, forced out of the water phase at the interface between water and a less polar phase.

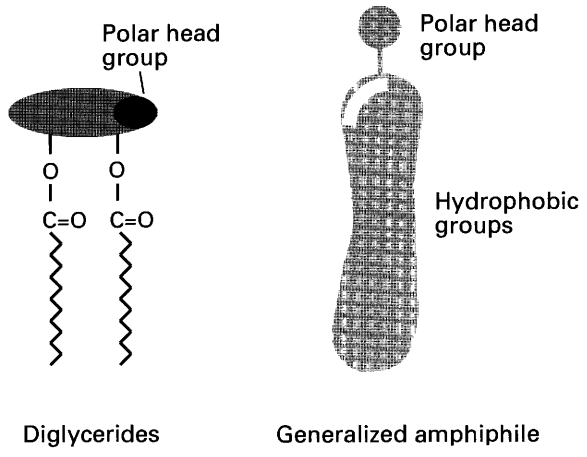

Figure 10. Representations of amphiphilic molecules.

MEMBRANE-MICELLE MODEL OF HUMUS

Description of Model

The leachate from the litter layer containing the amphiphilic molecules of plant degradation products will percolate down into the soil and the amphiphilic molecules will interact with the soil mineral grains. Wershaw (1986) has proposed that interactions between the amphiphilic molecules and the soil grains result in the formation of membranelike coatings of amphiphiles on soil mineral grains. It is these coatings that constitute the humus in soils. In this model, humus in soils and sediments consists mainly of the types of amphiphilic oligomers and simple compounds, described above, that arise from the partial degradation of plant tissue. Fungal melanins and compounds that arise from soil enzymatic polymerization reactions may also contribute to the formation of humus.

The most thermodynamically stable configuration for amphiphilic molecules in an aqueous system (except at very low concentrations) is in ordered aggregates in which the hydrophobic parts of the molecules are in the interiors of the aggregates isolated from the aqueous phase, and the polar, hydrophilic parts of the molecules are on the surfaces of the aggregates in contact with the aqueous phase (Witten, 1990). The aggregates commonly exist in three different geometrical configurations: membranes, micelles, and vesicles (Fendler, 1984). A membrane is a sheetlike structure of amphiphilic molecules arranged in a bilayer. The membrane is shown in figure 11 as being

composed of only diglyceride molecules; actual cell membranes are generally more complex, containing other types of molecular species such as proteins. In aqueous systems, the amphiphile molecules are oriented such that the hydrophobic parts of the molecules form the interior of the membrane, and the hydrophilic parts of the molecules constitute the two exterior surfaces. A micelle is a globular aggregate of amphiphile molecules, which in aqueous solutions has a hydrophobic interior and a hydrophilic exterior surface. A vesicle is a closed, membranelike structure (fig. 12).

Much of our understanding of the characteristics of membranes, micelles, and other ordered structures of amphiphilic molecules comes from studies on biological membranes. All living organisms are made up of lipid membranes, and all their life processes are membrane processes. Tanford (1980) has shown that lipid bilayer membranes form spontaneously in aqueous systems when certain types of lipids are present. The formation of an ordered membrane structure of lipid molecules in which the hydrophobic parts of the molecules are isolated as much as possible from the water molecules results in an increase in entropy of the system. It is this increase in entropy that drives the process.

The amphiphilic component molecules of humus also will arrange themselves into aggregates that, for simplicity, will be referred to as membranes; however, at the present time, the precise geometrical configurations of the structures cannot be specified. In some instances, the humus molecules will be present as membranes around mineral grains; in other instances they will be present as vesicles or micellelike aggregates. Humus membranes are thermodynamically stable in soils and sediments, but

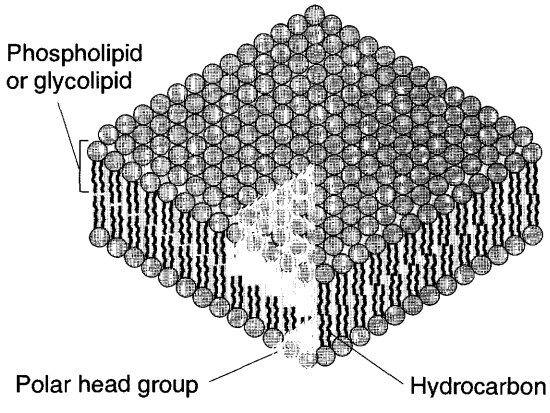

Figure 11. Representation of a bilayer lipid membrane.

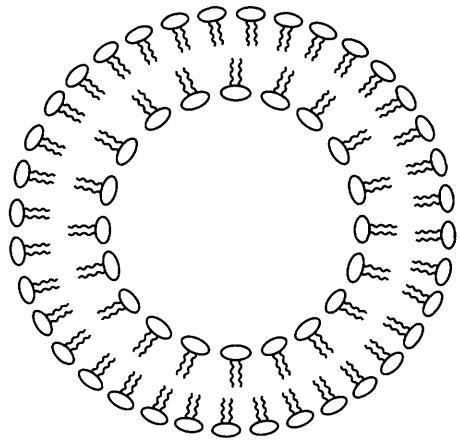

Figure 12. Representations of the cross sections of a vesicle and a micelle composed of diglyceride molecules.

they can be partially disrupted by basic solvents such as those used to extract humic and fulvic acids from soils (Hayes, 1985).

The molecules that make up the hydrophobic interior regions of the humic membranes and micelles will possess some freedom of motion; that is, the hydrophobic interiors of the humic aggregates will be liquidlike. In this regard, humic aggregates will be similar to biological membranes (Quinn, 1981). As Wershaw (1990) has pointed out, this hydrophobic region can be considered as a separate, liquidlike hydrophobic phase. Quinn (1981) has reviewed in great detail the concept of fluidity in membranes and the types of interactions that take place between the various components of membrane bilayers. At the present time, not enough is known about the components of humic aggregates to discuss in detail their interactions; however, some general statements can be made about the major types of molecules that make up the humic aggregates.

The bulk of the evidence discussed above indicates that the humic aggregates consist mainly of lignin, carbohydrate, and lipid fragments derived from enzymatic degradation of plant tissue. The ^{13}C NMR characterizations of humic acid fractions isolated from Sephadex gels (Wershaw, 1990) suggest that lignin-hemicellulose complexes that occur naturally in higher

plants may be important components of the humic aggregates. Some of the Sephadex fractions also appear to contain well-defined carbohydrates and some lipid fragments. Much stronger evidence for the presence of lipids in humus has been obtained by Rice and MacCarthy (1989), who actually isolated a lipid mixture from humin. Lesser amounts of proteins, amino acids, and nonlignin-derived phenolic compounds from plants and soil micro-organisms are probably also present in the humus membranelike and micellelike aggregates.

The humic membranelike aggregates are similar to biological lipoprotein membranes in that they have hydrophobic interiors and hydrophilic exterior surfaces. The major components of biological membranes are lipids, proteins, and oligosaccharides (Kamo, 1990). The simplest type of biological membrane is one composed mainly of lipids. In general, each of these lipid molecules consists of one or more hydrophobic, hydrocarbon tails and a hydrophilic, polar head group. In most biological membranes, the lipid molecules are arranged in two parallel sheets in which the hydrophobic tails of the molecules form the interiors of the membranes and the polar groups constitute the exterior surfaces. Imbedded in the lipid bilayer membranes are proteins and carbohydrates.

Membranes and Micelles in Well-Defined Systems

A discussion of the behavior of amphiphiles in well-studied aggregating systems will provide insight into the types of interactions that can be expected in humic ordered aggregates. Fendler (1984) has pointed out that lipids are not the only molecules that form bilayer membranes. Any amphiphilic molecular species can aggregate to form membranes, and, indeed, a wide variety of different types of amphiphiles have been used to prepare models of biological membranes (Araki and Tsukube, 1990).

Micelle and vesicle formation is a manifestation of the same phenomenon as membrane formation. At very low concentrations, amphiphiles exist in solution as monomeric species; micelles form at concentrations higher than the so-called critical micelle concentration (CMC); and at still higher concentrations, vesicles, membranes, or liquid crystals form. To a large extent, membranes and liquid crystals can be looked upon simply as large micelles or micelles with different geometrical configurations. Indeed, in some systems it is possible to observe a smooth transition from micelles to membranes or liquid-crystal phases. In these systems, the size of the micelles that are formed increases with increasing concentration until, at some high concentration, an insoluble liquid crystal or membrane phase precipitates. If particles of another insoluble phase, such as clay mineral grains, are present in the system, then the membranes will form on the charged surfaces of the particles and coat them (Okahata, 1990).

The hydrophobic interior of a membrane or micelle is not always composed of nonionic hydrocarbon groups. It also may consist of moieties that contain polar functional groups that are bound by weak interactions, such as hydrogen bonding, to other polar groups to form more or less hydrophobic aggregates (Fendler, 1984). For example, the sterol portions of bile salts can hydrogen-bond to one another to form aggregates that can enter into the hydrophobic interiors of bile salt-lipid micelles (Mazer and others, 1982).

Ordered aggregates such as micelles and membranes also form in systems in which more than one type of amphiphile is present. An example of a multicomponent system that has been studied extensively is lecithin-sodium cholate-water (Small, 1971). In this system, both the bile salt (sodium cholate) and the lecithin are amphiphiles, and they will each form micelles if present alone in solution. When the two are present together, cylindrical mixed micelles are formed with the sodium-cholate molecules forming the peripheries and the lecithin molecules forming the interiors of the cylinders (fig. 13). Increasing the ratio of lecithin to bile salt causes an increase in the size of the micelles. In these mixed micelles, as in pure bile-salt micelles, the polar groups of the bile-salt molecules are in contact with water molecules both along the sides and ends of the micelles. The lipoprotein particles that transport cholesterol and other lipids in the blood of animals also are examples of mixed micelles that are found in nature. These particles are quite complex in structure and composition, but they still have the basic micellar structure of a liquidlike hydrophobic interior and a polar hydrophilic surface (Stryer, 1988).

The carbohydrate components of plant tissue exist in plants as long polymeric chains of monomeric units. Even after degradation, chains several monomeric units long are preserved. Polar groups (hydroxyls) are more or less evenly spaced along the length of these elongated threadlike molecules; therefore, they are not amphiphiles. However, they can

still form aggregates by the hydrogen bonding of one chain to another. Examples of these aggregates are the gels formed by plant pectins and gums. Rees and Welsh (1977) have reviewed the formation of polysaccharide aggregates and the structural features that enhance aggregations. Other plant components also form hydrogen-bonded complexes; thus, tannins hydrogen-bond with proteins, uronic acids, pectin, hemicellulose, and cellulose (Benoit and others, 1968; McManus and others, 1981). The formation of such hydrogen-bonded complexes can reduce the polarity of molecules and allow them to enter into the hydrophobic portion of a membrane or micelle. It is likely also that some of the larger polysaccharide chains are present in soils as a separate gel phase that binds the soil grains together into crumbs.

Humus Membranes and Micelles

By using the properties of membranes discussed above, a generalized model of a humus membrane can be developed. A diagrammatic representation of such a humus membrane coating a positively charged hydrous oxide mineral grain is shown in figure 14 with the hydrous-oxide surface pictured as being positively charged. The humus membrane is composed of amphiphiles (see figure 15 for a generalized, schematic representation of a humus amphiphile) derived from partially decomposed components of plant tissue. The humus amphiphilic molecules may contain one or more carboxylate groups. For simplicity, only one carboxylate group and one nonionized group are shown in figure 15; in some instances, more of these groups may be present in the same molecule. In the case of multiple carboxylate groups on a molecule, the amount of amphiphilic character that the molecule exhibits will be a function of the spatial distribution of the polar carboxylate groups. If the polar groups are mainly at one end of the molecule, then it will be an amphiphile whereas if the polar groups are more or less evenly distributed on the molecule, then it will not be an amphiphile. Humus membranes will consist of amphiphiles that have been produced by enzymatic oxidation reactions that attach carboxylic-acid groups to the ends of partially degraded plant polymers and compounds, such as some lipids, that occur naturally as amphiphiles. In addition to amphiphiles, nonamphiphilic species can be incorporated into the humic membranes, as will be discussed below.

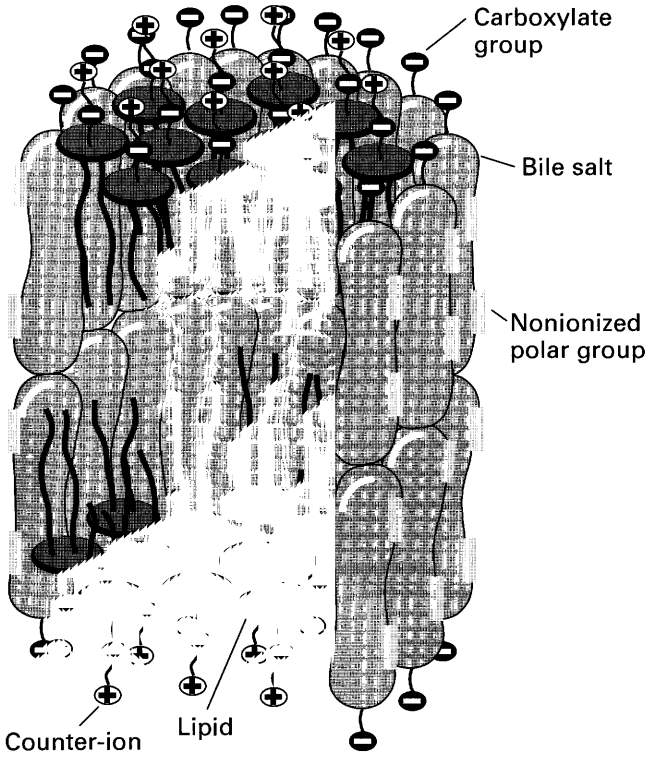

Figure 13. A mixed micelle of bile salt and lecithin.

The composition of the humus membranes in a given soil or sediment is apparently not completely uniform because the humin fraction of humus is more resistant to extraction than are the humic-acid or fulvic-acid fractions. The relative resistance of the humin component of humus to extraction is undoubtedly due to more than one factor; however, one reason is probably the higher lipid content of humin compared to humic and fulvic acid. The higher lipid content of the humin apparently increases the hydrophobicity of the humin aggregates and renders them less susceptible to disruption by strong base. However, competitive hydrophobic interactions between the more hydrophobic components of the humin and a relatively hydrophobic solvent, such as the methyl isobutyl ketone used in the Rice and MacCarthy (1989) procedure discussed above, apparently can bring about disruption of the humin aggregates. Another possible reason for the resistance of the humin aggregates to extraction will be discussed in the "Membrane Model and Extraction of Humic Substances" section below.

The differences between humic acid and humin are depicted in figure 14 by showing lipids (depicted as diglycerides with two long, narrow hydrophobic tails) as more abundant in some regions of the membrane structure than others. However, at

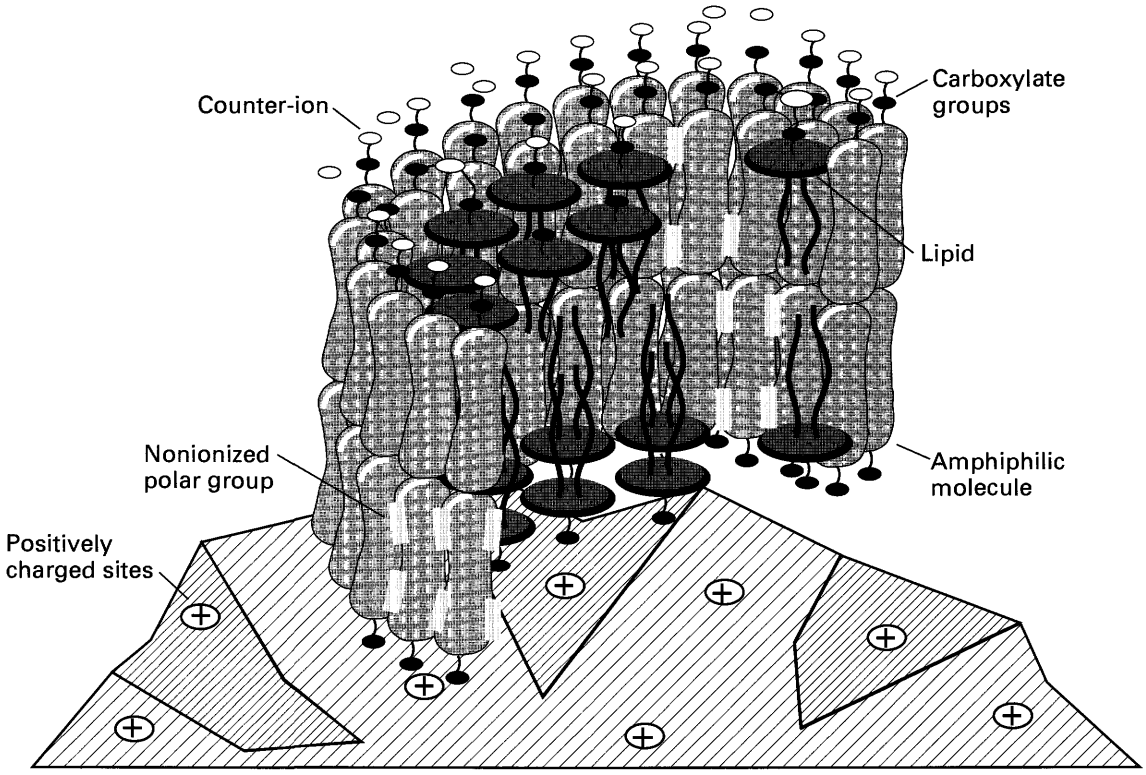

Figure 14. Representation of a humus membranelike structure (upper part of diagram) attached to a hydrous-oxide surface (lower part of diagram).

this stage in the development of the model, the actual geometrical configurations of the humic membranes are unknown.

In the simplest type of lipid membrane, the interior of the membrane is uniformly hydrophobic. However, in the humic membrane, it is proposed that portions of the interior are also composed of molecules that have polar functional groups that are rendered more hydrophobic by hydrogen bonding to other polar groups. This phenomenon is well known in bile-salt systems (Mazer and others, 1982).

Formation of Humus Membranes on Mineral Surfaces

Insight into the possible mechanisms of formation of humus membranes on soil and sediment mineral surfaces can be obtained from studies on the interactions of well-defined surfactants with mineral surfaces. Gaudin and Fuerstenau (1955a and 1955b) studied the interaction of anionic and cationic surfactants with quartz in aqueous systems. In pure water, quartz has a negatively charged surface; the amount of charge on the quartz surface increases with increasing pH. Both cationic and anionic surfactant molecules can be adsorbed by quartz; however, anionic surfactant molecules are only adsorbed in the

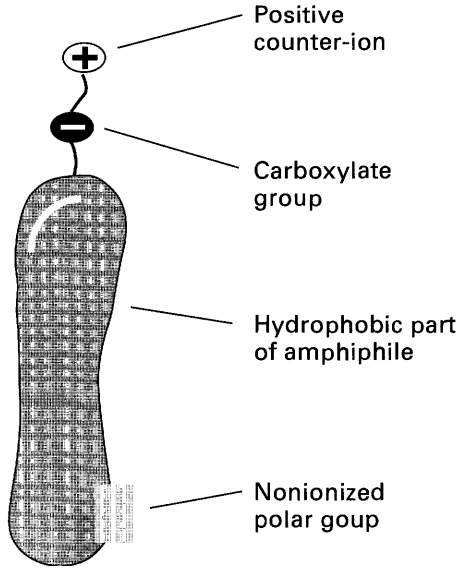

Figure 15. Representation of a generalized humus amphiphilic molecule.

presence of divalent cations such as barium ions, which are chemisorbed at the Stern layer. The anionic surfactant molecules are then bound to the surface by the sorbed barium ions.

Cationic surfactant molecules, on the other hand, are directly adsorbed by the negatively charged quartz surface. Gaudin and Fuerstenau (1955b) determined that the adsorption of dodecylammonium ions on quartz is a stepwise process. At low concentrations of surfactant molecules (less than the critical micelle concentration), the molecules are randomly distributed on the mineral surface (fig. 16*A*). At higher concentrations, aggregates Gaudin and Fuerstenau called "hemi-micelles" form (fig. 16*B*). Gaudin and Fuerstenau (1955b, p. 960) introduced the hemi-micelle hypothesis to explain the rapid increase in adsorption of surfactant molecules that they observed above "a concentration at the solid-liquid interface which is similar to the bulk critical micelle concentration * * *." These hemi-micelles are pictured as patches of closely packed surfactant molecules that form on the surface of the sorbent because of the tendency of the nonpolar hydrocarbon chains to aggregate. The hydrocarbon chains are exposed to the water in the hemi-micelles. At still higher concentrations, however, bilayer structures form in which the polar ends of the surfactant molecules are exposed at the surface (fig. 16*C*).

Somasundaran and Fuerstenau (1966) also found evidence for hemi-micelle formation when anionic surfactants are adsorbed on positively charged alumina surfaces. They pointed out that at low surfactant concentrations, adsorption takes place by electrostatic interactions between the negatively charged surfactant ions and the positively charged alumina surfaces. At concentrations higher than the critical hemi-micelle concentration, both electrostatic forces between the surfactant ions and the oppositely charged surfaces and hydrophobic interactions between the nonpolar chains of the surfactant ions act in concert to bring about the formation of hemi-micelles. After all the surface charge has been neutralized, further adsorption of surfactant ions takes place through hydrophobic interactions, which result in the formation of bilayers.

From the few data available, it appears that a mechanism similar to that outlined by Somasundaran and Fuerstenau (1966) may be proposed for the adsorption from aqueous solution of humic and fulvic acid anions onto positively charged mineral surfaces.

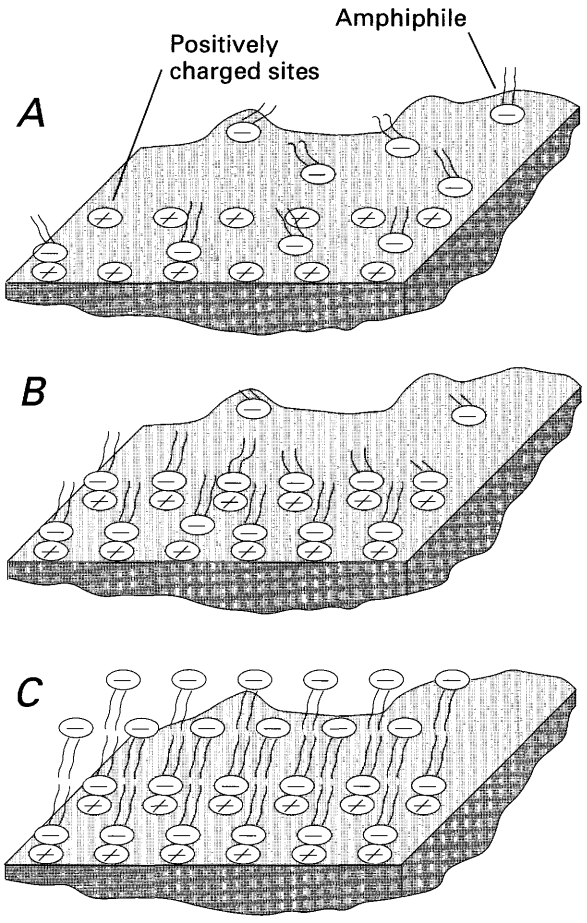

Figure 16. Representation of hemi-micelle and membrane formation by surfactant molecules on a positively charged mineral surface. *A*, random molecule distribution at low concentrations. *B*, hemi-micelle formation at critical micelle concentrations. *C*, formation of bilayer membranes at still higher concentrations of molecules.

Murphy and others (1990) interpreted results that they obtained on the adsorption of four humic and fulvic acids onto kaolinite, hematite, and a clay fraction from a subsurface soil horizon as indicating that fulvic and humic acids at low solution concentrations form monolayers on the charged mineral surfaces; at higher concentrations, the data for some of the humic substances they studied may be interpreted as indicating that multilayer coatings form. Carboxylate groups are the active functional groups in binding humic- and fulvic-acid ions to the mineral surfaces (Nayak and others, 1990).

Chander and others (1983) have reviewed the thermodynamics of adsorption of surfactant ions on charged surfaces. They pointed out that the interaction between adsorbed ions may be treated thermodynamically by introducing a term, ϕ, into the expression for standard Gibbs free energy of adsorption, ΔG°_{ad}, to

account for the van der Waals energy of interaction between adjacent nonpolar chains of surfactant ions. For long aliphatic chains, $\phi = n\phi'$, where ϕ' is the van der Waals energy of interaction per CH_2 group, and n is the number of CH_2 groups per chain. The van der Waals interaction between nonpolar groups may be considered as a component of the so-called hydrophobic effect, and as Chander and others (1983) pointed out, "Correspondence between the hemi-micelle formation and micelles in bulk solution is taken as indicative of the 'hydrophobic effect' in both cases." Recently, more elaborate thermodynamic models have been developed incorporating hemi-micelle formation into the mechanism of adsorption of surfactants on mineral surfaces (Woodbury and Noll, 1988 and 1989). These models should be useful in helping us gain further insight into the mechanisms of adsorption of humus onto mineral surfaces.

POSSIBLE POLYMERIZATION REACTIONS OF DEGRADATION PRODUCTS

In addition to the aggregation of the plant degradation products into humic membranes and micelles, it is possible that molecules of the degradation products may participate in repolymerization reactions. These polymerized products are probably also incorporated into the humic membranes and micelles. It is also possible that molecules in the humic aggregates could undergo some polymerization. Previous models of the chemical structure of humic substances in soils have assumed that the humic substances arise from the polymerization of plant-degradation products (Stevenson, 1982; Vandenbroucke and others, 1985). However, the preponderance of evidence now appears to support a model in which the molecules of plant-degradation products are held together by weak interactions in ordered membranelike or micellelike structures. Nevertheless, it is possible that some polymerization of the fragments of plant polymers may take place, and for that reason the more likely polymerization mechanisms are discussed.

Enzymatic Polymerization Reactions

Several possible types of enzymatic polymerization reactions may be involved in the formation of components that are incorporated in soil humus. Bollag and others (1979) and Bollag (1983) have shown that the soil fungus *Rhizoctonia practicola* produces an extracellular phenol oxidase that can catalyze phenolic coupling reactions. They also have shown that xenobiotic compounds such as phenolic pesticides also can undergo phenol-oxidase-catalyzed coupling reactions. In addition, Suflita and Bollag (1980) have been able to isolate from a number of different soils an extract that catalyzes oxidative coupling reactions of phenolic compounds. They extracted the active component by shaking a soil sample with pH–6 citrate buffer and measured the activity of each extract by the amount of 3,3′,5,5′-tetramethoxydiphenoquinone produced from 2,6-dimethoxyphenol. Suflita and Bollag (1980) concluded after heating the soil extracts and treating them with enzyme inhibitors that one or more extracellular enzymes were responsible for the catalytic activity they observed in their soil extracts. They further noted that "the observed oxidative coupling ability of soil extracts was remarkably similar to a phenoloxidase isolated from the fungus *R. practicola* * * *."

Another possible type of enzymatic polymerization in litter layers and soils involves the repolymerization of lignin-degradation fragments. Because lignin degradation involves some of the same types of enzymatic reaction pathways as lignin biosynthesis (Higuchi, 1985), one might expect that some enzymatic polymerization could take place during lignin biodegradation. During lignin biosynthesis in plants, monolignol free radicals are formed by dehydrogenation mediated by laccase/O_2 or peroxidase/H_2O_2; these radicals then couple to give dilignols and higher oligomers. These reactions result in the formation of the types of linkages shown for the β-O-4 dilignol in figure 3. Similar types of reactions can take place during lignin degradation, but as was pointed out above, depolymerization reactions apparently are favored during degradation, possibly because the degradation products are incorporated into heterogeneous humic aggregates before they can polymerize. Undoubtedly, some repolymerization does take place, but the bonds formed are most likely of the same types as those already present in the lignin. Therefore, it has not been possible to distinguish repolymerization products from the original lignin.

Fungal synthesis is a possible source of non-lignin-derived phenolic and quinoid constituents of humic substances. The production by soil fungi of dark-colored pigments called fungal melanins that have properties similar to soil humic acids has been

studied by Martin and Haider (1969), Haider and Martin (1970), Haider and others (1972), and Haider (1976). These authors have shown that the fungi *Aspergillus sydowi*, *Stachybotrys atra*, *Stachybotrys chartarum*, *Epicoccum nigrum*, and *Hendersonula toruloidea* produce fungal melanins. Haider and others (1972) have proposed that the fungal melanins are formed by secondary metabolic processes using either the acetate-malonate or shikimic acid pathways (fig. 17). The work of Haider and Martin (1970) provides examples of the types of phenolic compounds that have been detected in fungal cultures. Within 2 to 4 days of culturing *Aspergillus sydowi* in a glucose-asparagine medium, orsellinic acid, 2,4-dimethylresorcinol, orcinol, p-hydroxycinnamic acid, p-hydroxybenzoic acid, and 6-methylsalicylic acid were detected in the culture. Soon afterward, they detected protocatechuic acid and 2,3,4-trihydroxybenzoic acid. Still later, caffeic acid, 2,6-dihydroxytoluene, 2,6-dihydroxybenzoic acid, 5,5-dihydroxybenzoic acid, resorcinol, 5-methypyrogallol, methylphloroglucinol, 2,4,6-trihydroxybenzoic acid, and 2,5-dihydroxybenzoic acid were detected. The types of transformations that Haider and Martin (1970) have proposed to account for the products they have detected fit into the categories proposed by Barz and Weltring (1985), which are summarized in figure 5. These types of transformations are illustrated in figures 18, 19, and 20. After 5 weeks of culturing, they were able to isolate about 4 g of humic-acidlike material from 10 L of culture solution. Haider and Martin (1970) have proposed that the humic-acidlike polymers arise from the polymerization of the phenols with amino acids and peptides. Martin and others (1972) have pointed out the possibility that the phenolic compounds formed by the fungi also may undergo autoxidative coupling or oxidative coupling catalyzed by phenolase enzymes.

Saiz-Jimenez and others (1975) showed that the fungus *Eurotium echinulatum* isolated from a Spanish soil produced humic-acidlike, dark-colored pigments after growing for 2–3 months on a glucose culture medium. During the first 6–7 days of growth, they detected orsellinic acid, p-hydroxycinnamic acid, p-hydroxybenzoic acid, and three different anthraquinones. After 4–6 weeks, they detected more than 50 different phenols and anthraquinones in the ether extracts of the fungal cultures. Saiz-Jimenez and others (1975) have proposed that the phenolic acids they detected were synthesized by either the shikimate or acetate-malonate pathway. The shikimate pathway also has been proposed for the biosynthesis of p-coumaric acid, which is a precursor in the biosynthesis of lignin in plants (Robinson, 1980).

It is not clear from the work of Saiz-Jimenez and others (1975) how the humic-acidlike pigments arise from the precursors that they detected in their experiments. No phenols or anthraquinones could be extracted from the dark-colored, presumably polymeric pigments by ether or alcohol extraction, but reduction with sodium-amalgam yielded phenols, anthraquinones, anthrones, anthranols, and probably some anthracene derivatives. Saiz-Jimenez and others (1975) concluded that the anthrones, anthranols, and anthracenes were probably reduction products and were not present in the original polymer.

The dark-colored pigments produced by *Eurotium echinulatum* also contained about 1 percent nitrogen when the fungus was grown in a glucose-sodium nitrate medium and about 4.5 percent nitrogen when grown in a glucose-asparagine medium. Some of this nitrogen was probably present as peptides because acid hydrolysis released a significant amount of amino acids. Some of the nitrogen also could have been present as amino acids linked to phenyl rings.

As Haider and others (1972) have pointed out, fungal melanins are secondary metabolites—that is, products that are not produced by the primary biosynthetic processes of fermentation or oxidation (Brock, 1974, p. 271–272). Secondary metabolites are generally produced in relatively small quantities at the end of the growth cycle of an organism and during the onset of the stationary phase. Because fungal melanins are secondary products, it is not at all clear how important they are as actual contributors to humic substances in natural systems. In systems where large amounts of lignin are present, one would expect that lignin degradation products would be in much higher concentrations than fungal melanins in humus. However, in environments where lignins are absent or present only in small quantities, fungal melanins may be important in the formation of humus.

Soil micro-organisms not only degrade polysaccharides but also synthesize polysaccharides. One of the most common groups of polysaccharides produced by soil micro-organisms is the dextrans. Dextrans are branched 1,6-α-glucosides that form gels in water solutions. In addition, the enzymes that degrade carbohydrates can also catalyze transglycosylation reactions. The hydrolytic enzymes that attack the glycoside linkages of polysaccharides act by transferring a glycosyl group to a water molecule. If instead of water as an acceptor, another glycosyl group functions

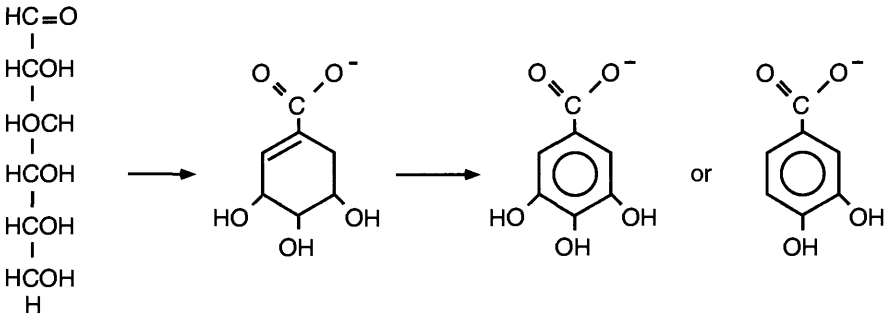

Figure 17. Simplified reaction scheme for the formation of aromatic compounds from sugars in plants by the shikimate pathway.

as an acceptor, then transglycolation takes place. Biely and others (1981) have shown that the yeast *Cryptococcus albidus* produces a xylanase that catalyzes both hydrolysis of xylan chains and synthesis of oligosaccharides from the hydrolysis products. Other fungal xylanases also have been shown to possess transglycosylation activity (Takenishi and Tsujisaka, 1975; Gorbacheva and Rodionova, 1977). Vrsanska and others (1982) found that transglycosylic reactions are catalyzed by an *Aspergillus niger* xylanase only at relatively low oligosaccharide concentrations; at higher concentrations, hydrolysis is favored. It is not clear at this time, however, if all enzyme-catalyzed transglycosylic reactions only take place at low oligosaccharide concentrations. If this is the case, then one would not expect to find high concentrations of transglycosylic products in natural systems.

Nonenzymatic Polymerization Reactions

Nonenzymatic oxidative coupling reactions of phenolic compounds have been extensively studied because of the wide use of some phenolic compounds as antioxidants (Hardy, 1968). For example, alkoxyphenols such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) are important antioxidants in packaged foods. The antioxidant activity of these compounds is a result of the fact that many phenols are susceptible to oxidation by mild oxidizing agents such as molecular oxygen; the antioxidant protects the product by being preferentially oxidized. In the first step of the oxidation, a hydrogen atom is abstracted from a phenolic hydroxyl group with the production of a phenolic free radical. In alkaline or neutral solutions, the free radicals produced can then undergo coupling with one another to form dimers and higher oligomers (Hewgill and Lee, 1968).

No definitive studies have been published on the importance of nonenzymatic oxidative coupling reactions in solution in the formation of humic substances. However, Lindqvist and Lindqvist (1969) found that humic-acidlike materials were formed in solutions of pyrocatechol and hydroquinone (pH 8 in bicarbonate buffer) exposed to air. They further found that the reactions were accelerated in the presence of fine quartz sand at pH 7 in a phosphate buffer. In acidic soils, it is unlikely that nonenzymatically catalyzed oxidative coupling reactions take place; however, in basic or neutral soils, some oxidative coupling of phenolic compounds may take place. Lindqvist and Lindqvist (1969) determined that laccase and radish peroxidase will catalyze oxidative coupling reactions under acidic conditions. Van der Linden (1979) has proposed that autoxidative reactions of polyphenolic groups in humic substances at basic pH leads to the formation of hydrogen peroxide, which can oxidize amino acids.

Oxidative reactions also may alter humic substances during extraction with basic solutions. Studies of the physical chemical properties of humic substances before and after treatment with strong base provide evidence of the possibility for alteration of humic substances during alkaline extraction. Swift and Posner (1972) have shown that humic acid in a 1 N solution of sodium hydroxide will consume oxygen. They determined that after 30 days in the alkaline solution under oxygen, the higher molecular weight humic-acid fractions were markedly decreased in concentration, the ultraviolet (UV) absorbance and visible absorbance were decreased, and the cation exchange capacity increased. When oxygen was excluded from the solution, changes in molecular weight, UV-visible spectra, and cation exchange capacity were much less than when oxygen was present.

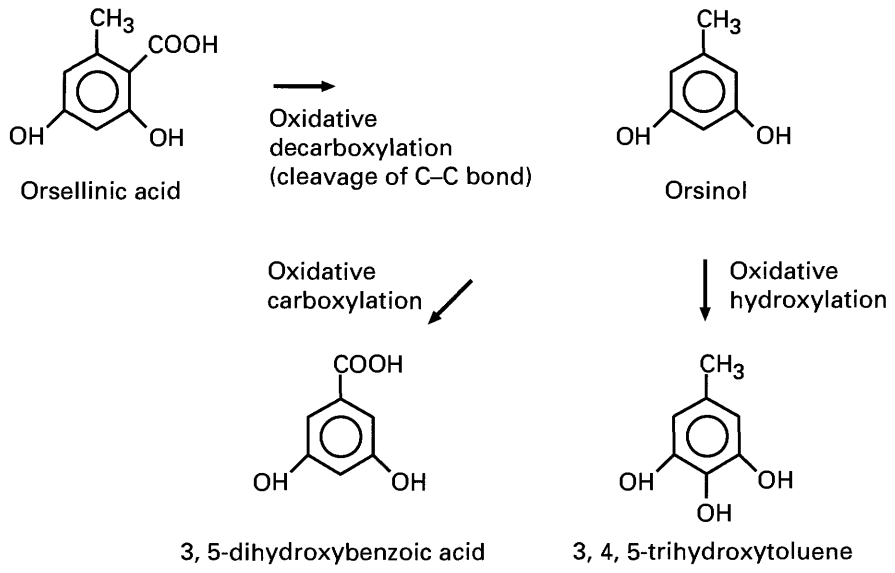

Figure 18. Transformations of orsellinic acid mediated by *Aspergillas sydowi*. Modified from Haider and Martin (1970, p. 147).

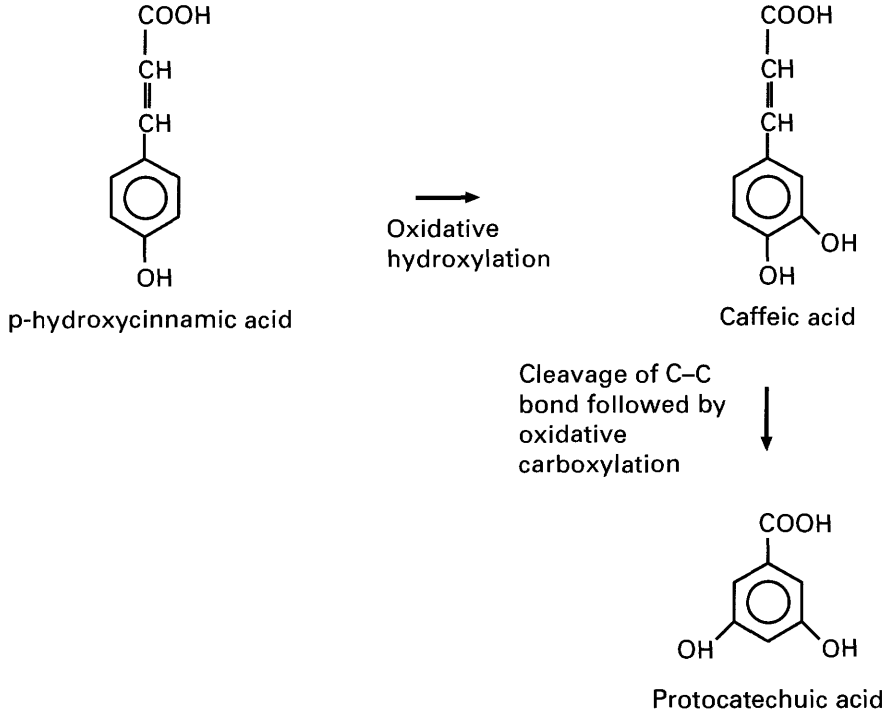

Figure 19. Transformations of p-hydroxycinnamic acid mediated by *Aspergillas sydowi*. Modified from Haider and Martin (1970, p. 148).

Electron spin resonance (ESR) data compiled by Steelink (1987) indicate that free radical concentrations in humic substance solutions markedly increase with increasing pH. These free radicals have long lifetimes in basic solutions. The long lifetimes of the free radicals in alkaline solutions and the reductions in molecular weight observed by Swift and Posner (1972) suggest that coupling of humic-acid-radical species is not taking place under highly basic conditions, or at least that degradative reactions are dominant.

The most likely possibility for polymerization of organic molecules in soils and sediments is catalytic polymerization on clay-mineral surfaces. Studies by Wang and his coworkers (Wang and others, 1978a, b; Wang and others, 1980) indicate that the polymerization of phenolic compounds is catalyzed by clay minerals. In the experiments conducted by Wang and his coworkers, simple phenols such as orcinol, syringic acid, and vanillic acid in solution were added to dried clay minerals and soils. The samples were then allowed to react for approximately 40 days; at the end of this time they were able to extract humic-acidlike and fulvic-acidlike substances from the samples. It is not clear from these studies what the mechanism or mechanisms of the reactions are; however, the formation of phenolic carbonium ions may be involved. Helsen (1982) has pointed out that there are high concentrations of protons on the surfaces of montmorillonite grains and that these protons react with organic compounds to form carbonium ions. These carbonium ions can then participate in addition reactions. It is therefore possible that the constituent molecules of humus membranes on some clay surfaces could undergo repolymerization reactions. These reactions would be most likely to occur between the organic molecules that are in contact with clay surfaces in the humus bilayer membranes; the organic molecules on the outsides of the bilayers would probably be less susceptible to carbonium ion formation.

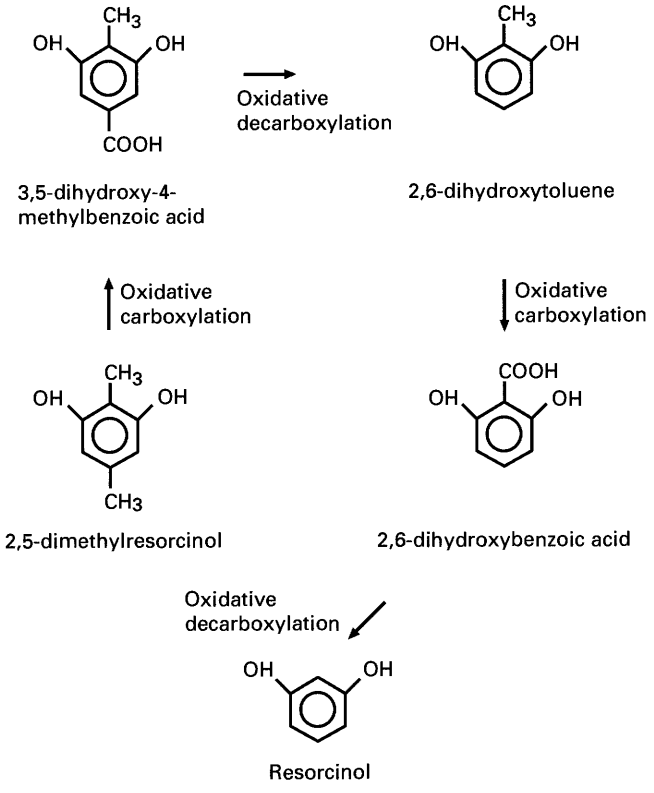

Figure 20. Transformations of 3,5-dihydroxy-4-methylbenzoic acid mediated by *Aspergillas sydowi*. Modified from Haider and Martin (1970, p. 148).

Maillard Reaction

In the Maillard reaction, an aldose reacts with an amine to form an aldosylamine. The aldosylamines spontaneously rearrange to 1-amino-1-deoxy-2-ketoses by the so-called Amadori rearrangement (Danehy, 1986). These Amadori products are commonly encountered in food browning; they can form in foods both at room temperatures and during cooking.

A number of workers have proposed that humic acids are similar to melanoidins formed by the Maillard reaction of sugars and amino acids (Stevenson, 1982; Rubinsztain and others, 1986). Rubinsztain and others (1986) were able to produce humic-acidlike melanoidins by reacting mixtures of glycine with galactose in alkaline solutions at 100°C for 170 hours. Although the temperatures that Rubinsztain and others (1986) used to form humic-acidlike compounds are unrealistically high to simulate what happens in soils, some Maillard-type reactions may still take place during humus formation. Stevenson (1982) has speculated that, although Maillard reactions are generally slow at soil temperatures, frequent freezing and thawing and wetting and drying cycles may accelerate the reactions. In addition, active mineral surfaces may catalyze Maillard reactions.

PHYSICAL AND CHEMICAL EVIDENCE FOR THE MEMBRANE MODEL OF HUMUS

The physical and chemical properties discussed herein are mainly those of extracted humic substances; relatively few data are available for the physical and chemical properties of unextracted humus. The discussion of the properties of humic substances given here is not intended to be an exhaustive compendium but rather an outline of those properties that provide insight into the structure of unextracted humus. The reader is referred to the books by Stevenson (1982), Aiken and others (1985), and Hayes and others (1989) for more comprehensive coverage.

Surface Activity

One of the most important clues to the structure of humus comes from measurements of the surface activity of extracted humic substances. Wershaw and others (1969), Tschapek and Wasowski (1976), Rochus and Sipos (1978), Tombacz and others (1981), Hayano and others (1982), and Tschapek and Wasowski (1984) have found that humic acids significantly lower the surface tension of water. Using surface-tension measurements, Rochus and Sipos (1978) and Tschapek and Wasowski (1984) determined that the slopes of plots of the logarithm of surface tension versus humic-acid concentration decrease to almost zero at concentrations above about 1 percent. They interpreted this decrease in slope as indicating that a humic acid in solution has a critical micelle concentration above which the humic acid forms micelles. The changes in slope that they observed were not as sharp as seen in solutions of pure surfactants, but this is to be expected in humic acids that are mixtures of molecular species. Because a humic acid is a mixture of many components, one would predict from the phase rule that more than one micelle phase is possible and, therefore, a single critical micelle concentration would not be expected. The reader is referred to Cistola and Small (1990) for a more complete discussion of the effect of added degrees of freedom on detection of critical micelle concentrations.

The work of Hayase and Tsubota (1983) supports the hypothesis that more than one critical micelle concentration should be expected in humic-acid solutions. They measured the surface tension of solutions of humic and fulvic acids extracted from a Tokyo Bay sediment. The humic acid was fractionated into four molecular-weight fractions by ultrafiltration. The surface tensions of the solutions of all the humic acid fractions steeply decreased with increasing concentration until a critical concentration was reached; very little change was then observed in surface tension with increasing concentration. However, the critical micelle concentrations for the different fractions were not the same but varied from about 0.1–1.0 percent. In general, Hayase and Tsubota (1983) found that the higher the molecular weight, the lower the critical micelle concentration. From these data, one would predict that in an unfractionated humic acid, the larger molecules will tend to aggregate before the smaller molecules—that is to say, aggregation will take place over a range of concentration, as was observed in the studies cited above.

Hayase and Tsubota (1983) also were able to obtain information on the size and shape of the humic acid and fulvic acid molecules. From measurements of change in surface tension with concentration below the critical micelle concentration, the authors were able to calculate the surface area occupied by a single humic-acid or fulvic-acid molecule. The range of areas they measured was from about 0.3–0.7 nm^2 per molecule. Since the area occupied by a carboxylate group is about 0.2 nm^2 they inferred that the humic-acid and fulvic-acid molecules are long and narrow, rather than globular. Further support for this conclusion is provided by the data obtained by Hayase and Tsubota (1986) that indicated that all their humic-acid fractions formed monolayers at air-water interfaces, and that the thicknesses of these monolayers were eqivalent to between 23 and 67 methylene units.

Far fewer studies have been conducted on the surface activity of fulvic acids than of humic acids. In addition to Hayase and Tsubota (1983 and 1986), Chen and Schnitzer (1978) also found that fulvic acids are surface active. The fulvic acids that Chen and Schnitzer (1978) studied, however, were from soils rather than sediments. Both the soil fulvic acids of Chen and Schnitzer (1978) and the sediment fulvic acid of Hayase and Tsubota (1986) were more surface active than equal concentrations of humic acids from the same sources.

Molecular Aggregation of Humic Substances

In addition to the evidence from surface-tension measurements that humic substances form molecular aggregates, other types of measurements also have

provided evidence of aggregation of humic substances. These measurements give further insight into the mechanisms of aggregation. For example, evidence of the importance of hydrogen bonding in aggregate formation in humic acids has been provided by small-angle x-ray scattering measurements and electrophoritic studies. Polarographic studies have provided evidence of the formation of mixed fulvic-humic-acid aggregates in natural waters.

Small-angle x-ray scattering measurements have been used to study the effect of pH on the molecular aggregate of humic substances in solution (Wershaw, 1989c). Wershaw and Pinckney (1971, 1973a, and 1973b) found that humic-acid fractions isolated by adsorption chromatography on Sephadex gel form molecular aggregates in solution and that the degree of aggregation is a function of both pH and concentration. The different fractions displayed three different types of aggregation behavior, to wit: (1) Increased aggregation below pH 3.5 with little disaggregation above pH 3.5; (2) disaggregation up to pH 7 with some reaggregation above pH 7; and (3) a continuous decrease in aggregation with increasing pH. Wershaw and Pinckney (1973a) concluded from these observations that the differences in aggregation behavior were due to chemical differences between the fractions, and that the changes in aggregation of the fractions in solution probably reflect the interaction of several different bonding mechanisms, including hydrogen bonding, π-bonding, and charge-transfer complexation.

Wershaw and Pinckney (1977) compared the results of x-ray scattering measurements of fractions of a humic acid with results from the unfractionated humic acid. They observed an apparent overall reduction in the size of the particles after fractionation, which they attributed to the breaking up of mixed aggregates by the fractionation process. They further observed that oxidation by molecular oxygen also altered the degree of aggregation of humic acids in solution.

Castagnola and others (1978) obtained further evidence of the importance of hydrogen bonding in humic-acid aggregate formation from electrophoretic studies of solutions of humic acid and urea. Urea has been widely used for the disruption of intermolecular and intramolecular hydrogen bonds. They found that the gel electrophoretic separation of humic-acid fractions was altered by the presence of urea. Two new bands were detected when urea was added to the humic-acid solutions. With increasing urea concentration, the intensity of the new bands increased, but the intensity of one of the original bands that had been observed in the absence of urea decreased. Castagnola and others (1978) interpreted these results as indicating that urea was causing disaggregation of the original band into two lower molecular-weight fractions. A detailed discussion of the evidence for aggregation from electrophoresis of humic substances in solution has been given by Duxbury (1989).

The existence of hydrogen-bonded aggregates in unextracted humic substances may be inferred from studies of extraction efficiencies of humic substances by different solvent systems. Beckwith and Nayyar (1984) found that 8 M urea greatly enhances the effectiveness of sodium bicarbonate, sodium orthophosphate, and sodium pyrophosphate solutions in extracting humic substances from soils. They attributed this increased extraction efficiency to disruption of hydrogen bonds by urea. Urea also interferes with some hydrophobic interactions, and it is commonly used to disrupt biological membranes. Hayes (1985) also has found that addition of urea increases the amount of humic substances that can be extracted with organic solvents such as dimethylsulfoxide (DMSO).

The extraction of humin from soils and sediments by the technique described by Rice and MacCarthy (1989) provides additional evidence that humic substances exist as aggregates in soils and sediments. As Rice and MacCarthy (1990) have pointed out, humin has been operationally defined as that fraction of humus which is insoluble in both acidic and basic solutions. Because of this insolubility, the published literature on humin is very limited. However, Rice and MacCarthy (1989) recently have demonstrated that humin may be disaggregated by a relatively mild procedure into a lipid fraction, a humic-acid fraction, a fulvic-acid fraction, and an inorganic-mineral fraction. The first step in the Rice and MacCarthy (1989) extraction procedure is the same as the ordinary extraction of humic and fulvic acids from soil—the slurrying of the soil with strong base. This step is followed by the addition of a water-immiscible solvent such as methyl isobutyl ketone (MIBK) and the acidification of the mixture to pH 1. At this stage in the extraction, the humin passes as a suspension into the MIBK phase; only a fulvic-acid fraction of the humin remains in the aqueous phase. A humic-acid fraction of the humin can be isolated by back extraction of the MIBK phase with 0.5 N NaOH. A lipid fraction

remains in the MIBK. During the extraction procedure, the mineral matter associated with the humin settles out. The mechanism of the extraction of humin apparently involves disruption of hydrogen-bonding interactions by the strong base and disruption of hydrophobic interactions by the MIBK. The humic and fulvic acids isolated from the humins studied by Rice and MacCarthy (1989) were very similar to the humic and fulvic acids that they isolated from the soils and sediments by basic extraction. These results demonstrate that humin consists of humic-acid and fulvic-acid components and lipids. The resistance of humin to extraction with aqueous NaOH solution is possibly due to the presence of the lipids. Another possible explanation for this resistance is that the initial extraction with aqueous NaOH strips off only the outer layer of the humus bilayer, leaving an interior layer similar to a hemi-micelle (fig. 16); this interior layer is protected from extraction by its hydrophobic surface. The presence of a hydrophobic solvent such as MIBK, which will wet the hydrophobic interior layer, allows the NaOH to interact with the components of the inner layer and disrupt the layer.

In a very innovative study, Hunter and Lee (1986) demonstrated that humic substances in natural waters also are present as aggregates. They used polarography to measure the surface activity of dissolved organics in natural waters. They found that isolated humic acid was four times more surface active than any of the other organic components present in the water. When the humic acid was mixed at natural concentrations with the other organic components (including the fulvic-acid component), the surface activity of the mixture was only about 50 percent of the sum of the surface activities of the individual components. They interpreted this result as indicating that the humic-acid molecules interact with the other organic components in the water. Hunter and Lee (1986) also found evidence that humic acid undergoes similar weak interactions with synthetic surfactants and gelatine (a mixture of proteins). The most likely types of interactions that would yield the results they observed are hydrophobic interactions and hydrogen bonding leading to the formation of molecular aggregates.

Polyvalent metal ions, which often are strongly complexed by humic substances, also cause aggregation of humic substances (Ghosh and Schnitzer, 1981; Wershaw and others, 1983; Gamble and others, 1984; Underdown and others, 1985). In some instances, the mechanism of this aggregation appears to be the binding of more than one humic molecule by a single metal ion so that the metal ion acts as a bridge between molecules. Evidence for the bridging mechanism is given by Jouary and Chassin (1987), who found that humic-acid aggregates precipitated by excess iron or protons were hydrophobic, whereas those precipitated by calcium ions were hydrophilic. In the case of the iron, the most likely explanation is that the iron ions bridge between the polar groups of the humic molecules, causing the more hydrophobic parts of the molecules to be on the exterior surfaces of the aggregate. In a somewhat similar fashion, neutralization of the acid groups by protons will allow acid groups to hydrogen bond with one another, leading to aggregate formation. Apparently the more hydrophobic parts of the molecules are exposed on the surfaces of these aggregates also. Aggregates in which the exterior surfaces are hydrophobic and the interiors are hydrophilic are similar to reverse micelles that form in amphiphile solutions in nonpolar solvents. It is not clear why the calcium aggregates are hydrophilic; perhaps the calcium ions only neutralize the surface charges of humic particles but do not bridge between them.

Extracted lignins have many of the same aggregation properties as humic acids. Rudatin and others (1989) have reviewed the literature on the association of kraft lignins in solution. They point out that a number of workers have found evidence for hydrogen bonding between kraft lignin molecules. The degree of association of the lignin molecules is a function of concentration, ionic strength, and pH. In addition to hydrogen bonding, it appears that nonbonding orbital interactions between regular structural units also takes place (Dutta and others, 1989). Dutta and others (1989) have concluded from their observations of the association behavior of kraft lignins that "the macromolecular kraft lignin complexes embody a well-defined regular structure derived, presumably, from the original configuration of the native biopolymer."

Sorption of Humic-Substance Molecules by Mineral Surfaces

The strong complexation of metal ions by humic substances leads to the formation of humic coatings on hydrous-metal-oxide surfaces. Davis (1982), Murphy and others (1990), and Ranville and others (1991) have shown that the surfaces of hydrous-aluminum oxides, hydrous-iron oxides, and the edge sites of

aluminosilicates are covered by humic substances in natural systems. Davis (1982) pointed out that the adsorption of humic substances changes normally positively charged mineral surfaces to negatively charged surfaces. This change is brought about by the binding of humic substances by relatively basic hydroxyl groups exposed on the surfaces of the mineral particles.

Clay-humic-substance complexes have been isolated from a variety of different types of soils (see Anderson and others, 1974, Wershaw and Pinckney, 1980, and references cited in Wershaw and Pinckney, 1980). Wershaw and Pinckney (1980) found that humic acids are bound to clay-mineral surfaces by amino acids or proteins. The most likely mechanism for this binding is bridging by positively charged amide or amino groups between negatively charged carboxylate groups on humic acids and negatively charged clay-mineral surfaces. Evidence for this mechanism is provided by the observation of Wershaw and Pinckney (1980) that deamination of their clay-humic complexes with nitrous acid released the humic acids from the clays. The participation of carboxylate groups in the binding of humic substances to mineral surfaces is supported by the observation of Duxbury (1989) that the total charge on soils is generally much less than the total acidity of the humic substances in the soil; some of the carboxylates must, therefore, be neutralized by the mineral surfaces.

Hydrophobic Properties of Humus

The participation of humus in hydrophobic interactions is very closely related to surface activity of humic substances. Wershaw (1989a; 1990) has pointed out that humic-acid isolates and in-place soil humus behave as separate, liquidlike hydrophobic phases that can serve as solvents for hydrophobic organic compounds. Evidence for this conclusion comes from the work of Wershaw and others (1969) and Chiou and others (1983). Chiou and others (1983) pointed out that the sorption of hydrophobic pesticides by soils is analogous to the partitioning of pesticides between water and an immiscible organic-solvent phase. They have shown that the same equations hold for solvent-water partitioning and soil-water partitioning. The model used by Chiou and others (1983) for calculation of the thermodynamic activities of nonionic organic compounds in soil humus is an extension of the theory of Flory (1942) for calculation of the entropy of mixing of high molecular-weight polymers in low molecular-weight solvents. In the development of his equations, Flory (1942) assumed that a polymer molecule is a long chain composed of segments that are about the same size as the solvent molecules. He also assumed that these segments exist as kinetic units that are more or less independent of each other. Thus, implicit in the model of Chiou and others (1983) is the assumption that at least part of the humus behaves as a hydrophobic phase in which the segments of the molecular constituents of the phase are free to move more or less independently of each other—that is, like the molecules in a liquid.

Functional Groups in Humic Substances from Nuclear Magnetic Resonance Analysis

NMR spectroscopy, and especially ^{13}C NMR spectroscopy, has provided more specific chemical structural information about humic substances than any other technique. Examination of published ^{13}C NMR spectra of humic acids shows that the ^{13}C NMR spectra of unfractionated humic acids generally consist of broad bands that occur in well-defined spectral regions. The remarkable similarity in the positions of the major spectral bands of most of the spectra is shown in table 2. In this table, the spectral regions in which the major bands occur are given on the first line of the table, and in the following lines, the actual measured positions of the bands for the various examples are recorded. Wershaw and others (1990) propose that this similarity in positions of the spectral bands is due to all humic acids being composed of very similar functional groups that are derived mainly from the partial degradation of the structural components of plants.

Comparison of the spectra of the unfractionated humic acids with the spectra of forest litter yields some very interesting relationships that give insight into the origin of humic acids. The major bands of the humic acids (table 2) are in the same positions as the bands that have been observed by Zech and others (1987) in the solid-state ^{13}C NMR spectra of fresh spruce and pine litter (table 3). Zech and others (1987) have shown that all the major peaks in the solid-state ^{13}C NMR spectra in fresh spruce and pine litter are retained in the decomposed litter, but the relative intensities of peaks change. They also extracted the Klason lignin fractions of the fresh and decomposed spruce litter and measured their solid-state ^{13}C NMR

Table 2. Major bands of representative ^{13}C nuclear magnetic resonance spectra of humic acids from different soils and sediments

[Leaders (--), band is absent]

Source	Spectral regions of major bands (ppm)									
	230–220	180–170	155–145	140–130	120–110	105–95	78–70	65–50	40–29	25–10
Estuarine sediment[1]	--	174	--	130	--	100	71	61	30	22
Indonesian inceptisol[2]	--	178	154	132	--	--	75	59	33	20
Onday spodosol[2]	--	176	--	130	--	--	72	59	30	--
Australian clay soil[3]	--	175	--	130	120	--	72	57	30	--
Bainsville clay loam[4, 5]	--	172	--	129	--	--	70	56	29	--
Four Spanish soils[6, 7]	--	178–175	150	135–130	--	78–72	60	30	--	--
Three Saskatchewan soils[7, 8]	230–229	178	152	130	105–102	75	58	32	25	--
Alberta chernozem[9]	--	180	--	132	--	--	73	63–58	40, 31	25
Scottish lake sediment[10]	--	173	--	131	--	--	72	55	30	--
Agricultural soil[11]	--	175	--	129	--	--	103	72	60, 55	25–18
Laguna Lake sediment[11]	--	174	--	130	119	--	70	62, 54	38, 30	25–10

[1]Kalinowski and Blondeau (1988).
[2]Lobartini and Tan (1988).
[3]Skjemstad and Dalal (1987).
[4]Preston (1987).
[5]In dimethyl sulphoxide.
[6]Saiz-Jimenez and others (1986).
[7]Solid-state spectra.
[8]Schnitzer and Preston (1986).
[9]Schnitzer and Preston (1983).
[10]Wilson (1987).
[11]Wershaw and others (1990).

spectra. The lignin spectra contained all the peaks that were observed in the litter except for the carbohydrate peaks at 70 and 105 ppm. Nordén and Berg (1990) also studied the decomposition of pine-needle litter. The solid-state ^{13}C NMR spectra that they obtained were very similar to those obtained by Zech and others (1987). In addition, Nordén and Berg (1990) showed by multivariant analysis that the intensities of certain bands of the ^{13}C NMR spectra of their samples were very closely correlated with the Klason lignin contents of the samples. Thus, the lignin content of a sample could be calculated from its solid-state ^{13}C NMR spectrum.

Similar results to those from forest litter have been obtained from composting studies. Almendros and others (1987) have measured the ^{13}C NMR spectra of tree leaves and needles, grape marc, and wheat straw used as sources for compost; no new bands were observed during the composting process, and the spectral bands that were observed were in the same positions as those given in table 2. The ^{13}C NMR spectra of separated cow manure composts measured during different stages of decomposition also were composed of the same spectral bands shown in table 2 (Inbar and others, 1989).

The presence of the same bands in the NMR spectra of extracted humic acids, fresh and decomposed litters, and composts strongly suggests that during the humification process, the major chemical structural components of the precursor plant material are retained in the humic substances that were derived from them. In order to identify these structural components, spectra of higher resolution than those obtained from unfractionated humic acids are necessary.

Wershaw (1986) and Wershaw and others (1990) have shown that liquid-state ^{13}C NMR spectra of increased resolution can be obtained from humic-acid fractions isolated by fractionation of humic acids on Sephadex gels. The increased resolution apparently is the result of an increase in homogeneity of the fractions. The sharp bands observed in the spectra of the fractions arise from residues of plant structural components such as carbohydrates and cutins. The broad bands that remain in the spectra of the humic-acid fractions and those in the unfractionated humic-acid spectra are probably envelopes of overlapping or broadened lines (Wershaw, 1986).

The ^{13}C NMR spectra of humic-acid fractions from three different types of environments in the Philippines—a lake sediment, a peat soil, and an agricultural soil (Wershaw and others, 1990)—are representative of the spectra of humic-acid fractions isolated by Sephadex chromatography (Wershaw and others, 1988). These spectra will be discussed in some detail here in order to illustrate the type of information that can be obtained from the ^{13}C NMR spectra of

humic substances. The NMR spectra of the Philippine humic-acid fractions (figs. 21, 22, 23, and 24) fall into two of the three general categories or groups reported by Wershaw and others (1988). These groups are carbohydratelike (fraction 1), ligninlike or melaninlike (fractions 2, 3, and 4), and aromatic (absent in the Philippine samples). Within the carbohydrate group, the most prominent structural features of the spectra are sharp, well-defined carbohydrate bands in the region between about 65 ppm and 105 ppm. The spectra in the second group display bands that are similar to those seen for lignins or melanins whereas in the aromatic group, the only prominent feature observed is a broad band centered at about 130 ppm.

The bands in the ^{13}C NMR spectrum of a humic isolate are representative of the various functional groups that are present in the isolate. The sharper a particular band is, the more specific is the information about the functional group that the band represents. As was pointed out above, there is some increase in sharpness of some of the bands observed in humic-acid fractions compared to those of the unfractionated humic acid; therefore, we can expect to obtain somewhat more specific structural information from the spectra of humic-acid fractions. The spectral region that is least affected by the fractionation is that between 170 and 180 ppm. In this region, the carbonyl carbon resonances of carboxylic acids, esters, or amides are found. Aliphatic acids generally have bands in the region above 175 ppm, whereas bands for aromatic acids are generally below 175 ppm. In addition to the band at 175 ppm, fractions 1, 2, and 3 of the peat soil have a broad band between 190 and 220 ppm (figs. 21, 22, and 23) that also is present in the unfractionated peat-soil humic acid (fig. 25). This band most likely represents carbonyl carbon atoms in ketone and aldehyde groups. We have previously detected this band in other fulvic and humic acids (Thorn and others, 1987), and Zech and others (1987) observed this band in a Klason lignin fraction isolated from forest litter.

Another region that is only slightly affected by fractionation is that between 110 and 140 ppm. This region contains resonances from various types of aromatic carbon atoms, as indicated in table 3. Many of the major bands in lignins occur in this region. The variations that are seen in this region are well represented by the differences in the spectra of the three Philippines humic acids and their fractions. The spectrum of the unfractionated Laguna Lake humic acid is

Table 3. Positions of major bands in the solid-state ^{13}C nuclear magnetic resonance spectra of spruce and pine litter
[Data from Zech and others (1987)]

Position of bands (ppm)	Chemical structural units
30	Long-chain aliphatic structures with polymethylene groups
70	Polysaccharides
105	Anomeric carbon atoms in polysaccharides
110–120	C–1, C–5, C–6 in coniferyl units
130–140	Primary carbon atoms at position 1 in aromatic rings
145–155	C–3 and C–4 coniferyl units
175	Carbonyl carbon atoms in aliphatic esters, carboxylic-acid groups, and amides

the best resolved in this region and is very similar to the spectrum of Bainsville humic acid measured by Preston (1987), with a large, well-resolved line at 130 ppm and a smaller, well-resolved line at 119 or 120 ppm. These lines are also prominent in lignin spectra, where a line at approximately 130 ppm is often due to the C–1 (see fig. 2 for numbering of phenylpropane units) in substituted phenylpropane units, and a line at approximately 120 ppm often is due to the C–6 (Kringstad and Mörck, 1983).

Perhaps the most interesting fractions are those whose spectra display sharp, well-resolved carbohydrate lines (fig. 26). These lines are most prominent in fraction 1 of the agricultural soil humic acid. Fraction 1 of the lake-sediment humic acid also contains well-resolved carbohydrate lines, but the relative intensities of these lines compared to the other bands in the spectra are much less than in fraction 1 of the agricultural soil humic acid. In contrast, the carbohydrate lines in the spectrum of fraction 1 of the peat-soil humic acid are not resolved at all; only a single broad band is present in the spectral region of the nonanomeric carbon atoms (65–80 ppm). The relative intensities of the carbohydrate lines compared to the other lines in the spectrum are higher in fraction 1 of the Philippines agricultural soil humic acid than in any other fraction that we have ever measured. However, Fründ and others (1988) have measured similar 13C NMR spectra from low molecular-weight humic-substance fractions that passed through dialysis casing.

Yamaoka (1983) used a very similar Sephadex fractionation technique to that of Wershaw and others (1990) to isolate a carbohydrate-rich fraction from a humic acid isolated from the sediments in Hiroshima

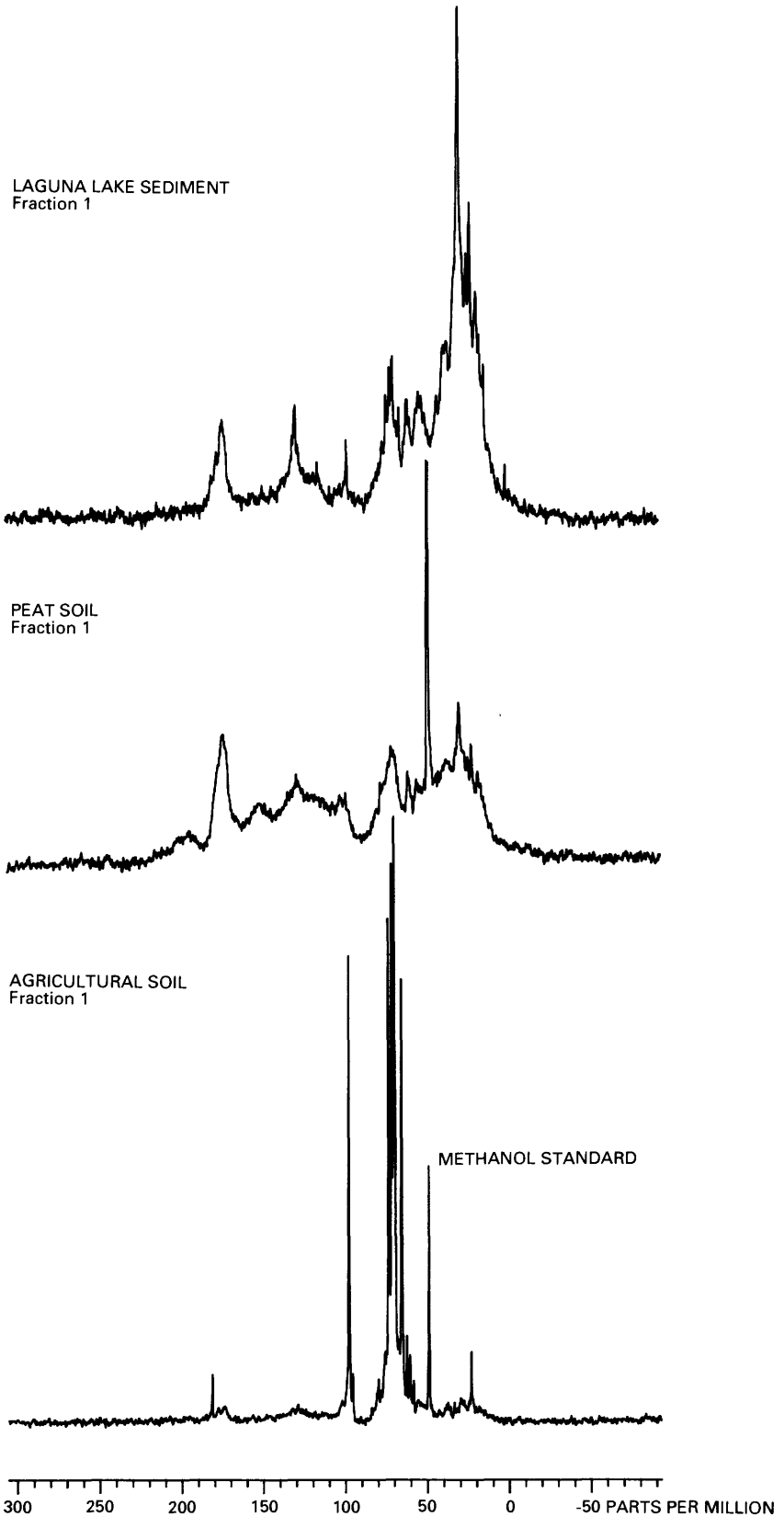

Figure 21. ^{13}C nuclear magnetic resonance spectra of fraction 1 isolates from three Philippine humic acids.

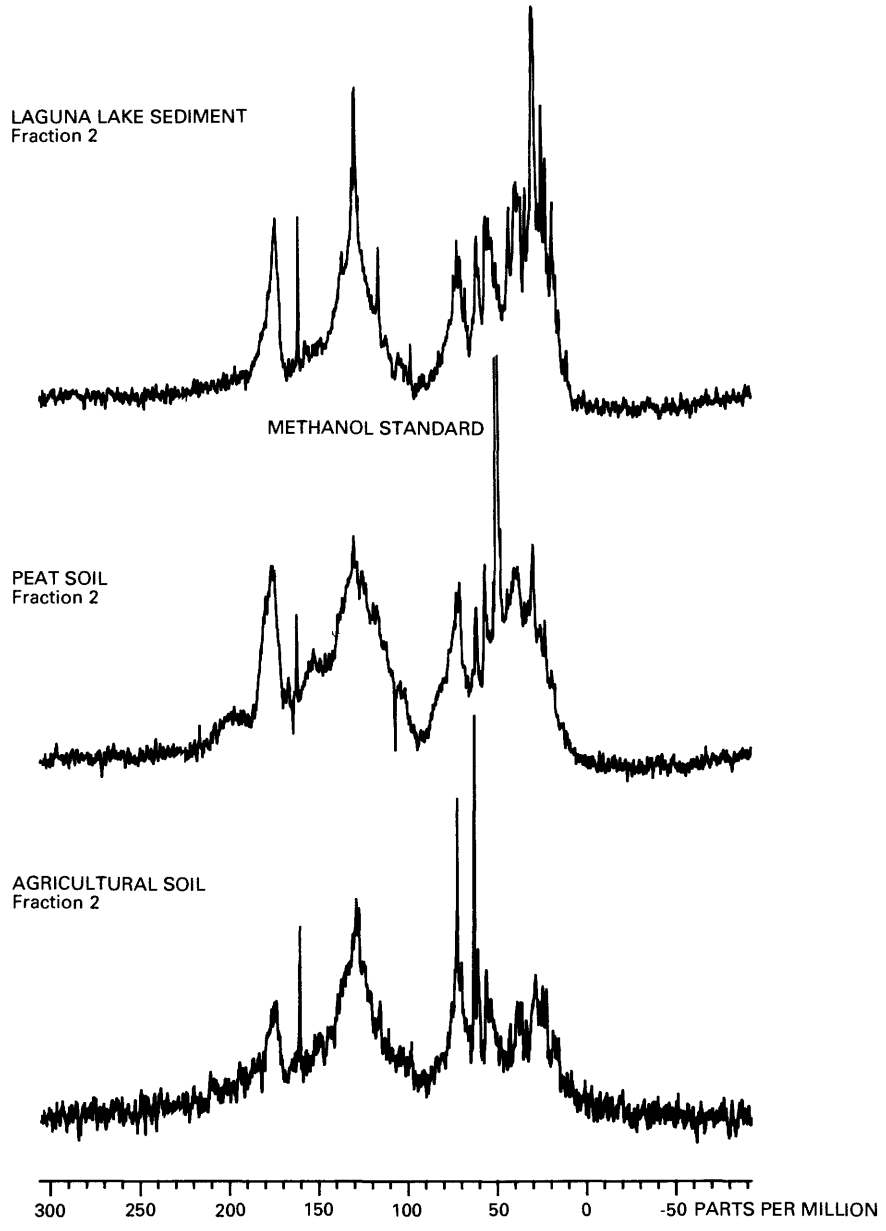

Figure 22. ^{13}C nuclear magnetic resonance spectra of fraction 2 isolates from three Philippine humic acids.

Bay. As in the study of Wershaw and others (1990), the carbohydrate fraction was the first to elute from the Sephadex gel column. Hydrolysis of the carbohydrate isolate followed by gas chromatographic analysis of the alditol acetates of the monosaccharides in the hydrolyzate allowed Yamaoka (1983) to identify the monosaccharides. Glucose was the most abundant monosaccharide, followed in order of abundance by xylose, galactose, rhamnose, arabinose, and fucose. Yamaoka (1983) used the relative concentration of xylose as an indication of the amount of hemicellulose in the sample.

Broader carbohydrate bands are present in the spectra of the other fractions. The presence of carbohydrate bands and bands characteristic of lignins in the spectra of these fractions strongly suggest that they may be lignin-carbohydrate complexes of the type discussed by Koshijima and others (1989).

The lines in the region between 50 and 65 ppm may be divided into two groups: (1) those in the region from 50 to about 52 ppm, which are due generally to the methyl carbon atom in aliphatic and aromatic methyl esters, and (2) those in the region from about 54 to 65 ppm, which in lignin structures often are due

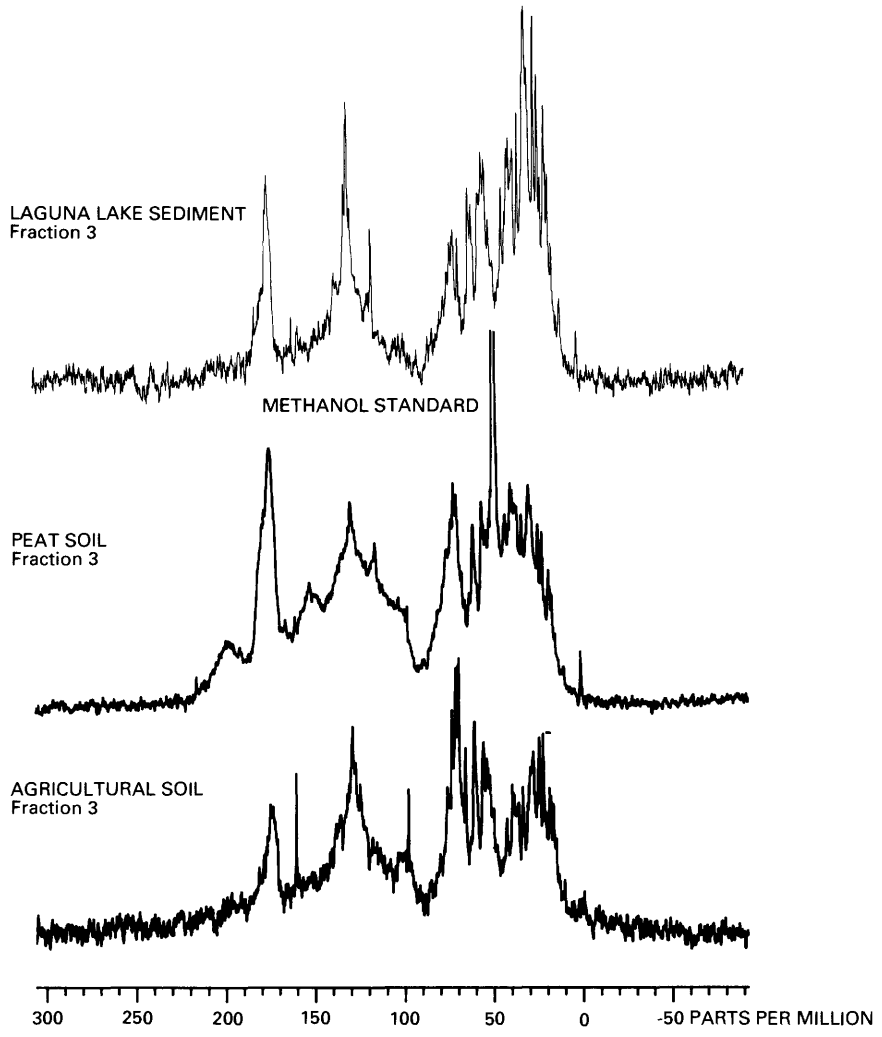

Figure 23. ^{13}C nuclear magnetic resonance spectra of fraction 3 isolates from three Philippine humic acids.

to a methyl carbon atom or other aliphatic carbon atoms adjacent to an oxygen in an ether. In general, most of the lines in the spectra of both the unfractionated and fractionated humic acids occur in the region between 54 and 65 ppm and, therefore, represent ether structures. Ether structures, and especially methyl ethers, are characteristic of lignins (Higuchi, 1985), and the presence of these lines in the spectra of humic acids provides additional evidence for the presence of lignin fragments in humic acids. Lignins contain few, if any, ester groups.

The methyl ether band has been very useful in structural studies of lignins. For example, in a quantitative ^{13}C NMR spectrum of a hardwood lignin, the ratio of the methoxyl band to the integrated area of the aromatic carbons may be used to calculate the ratio of syringlpropane to guaiacylpropane units in the polymer (Obst and Landucci, 1986).

The major band in the region between 29 and 40 ppm in the ^{13}C NMR spectra of unfractionated humic acids occurs at about 30 ppm (table 2 and fig. 25). This peak has been ascribed generally to methylene carbon atoms in long aliphatic chains such as those in lipids and waxes (Hammond and others, 1985). In plants, one of the most common groups of compounds that have long methylene chains is the cuticle waxes that coat the leaves of higher plants. Slight differences in the chemical shifts of the methylene-chain carbon atoms in a wax generally broaden the band at about 30 ppm with respect to the other lines in the NMR spectrum of the wax. The positions of the other spectral lines are dependent upon the pattern of substitution on the chain. For example, the presence of β-diketone groups on the chain can perceptibly alter the chemical shifts of carbon atoms as much as 6 or 7 carbon atoms away from one of the ketone groups (Tulloch, 1985).

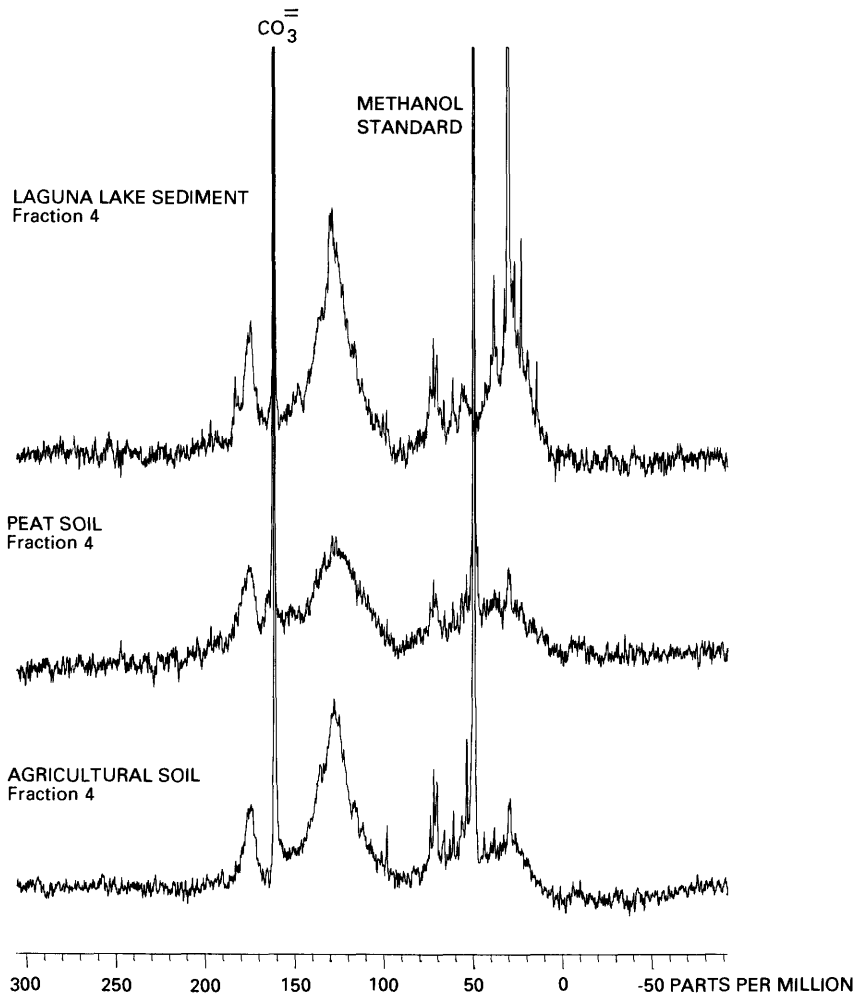

Figure 24. ^{13}C nuclear magnetic resonance spectra of fraction 4 isolates from three Philippine humic acids.

Examination of the bands at 30 ppm in the spectra of the humic-acid fractions and of the unfractionated humic acids reported here clearly show that these bands are broader than many of the other bands in aliphatic regions of the spectra. The presence of ketone bands between 190 and 220 ppm in the spectra of some of the humic-acid fractions studied here indicate that ketonic structures may well be present in the aliphatic chains of these fractions.

Quantitative NMR spectra of the four fractions of the Laguna Lake humic acid show that there is an increase in aromaticity from fraction 1 to fraction 4 as indicated by the integrated area of the bands between 100 and 155 ppm (Wershaw and others, 1990). As Wershaw and Pinckney (1973b) have pointed out, one would expect the more aromatic fractions to be more strongly retained on the Sephadex column than the less aromatic fractions, and these results confirm this expectation. There is probably also a size contribution to the fractionation of humic acids on Sephadex (Wershaw and Pinckney, 1973a).

The ^{13}C NMR spectra of whole soils consist of the same bands that are present in the ^{13}C NMR spectra of extracted humic and fulvic acids; however, the relative intensities of the bands are much different. Wilson (1987) and Baldock and others (1990) have found that the carbohydrate band (between 65 and 80 ppm) and the polymethylene band (centered near 30 ppm) are much stronger in whole soils than they are in humic acids extracted from the soils; in fact, these two bands are the most intense bands in the soils they examined. Baldock and others (1990) concluded from their data that the most abundant constituents of humus are saccharides and fatty acids (lipids). Studies by Baldock and others (1990) with ^{13}C-labeled glucose indicate that the

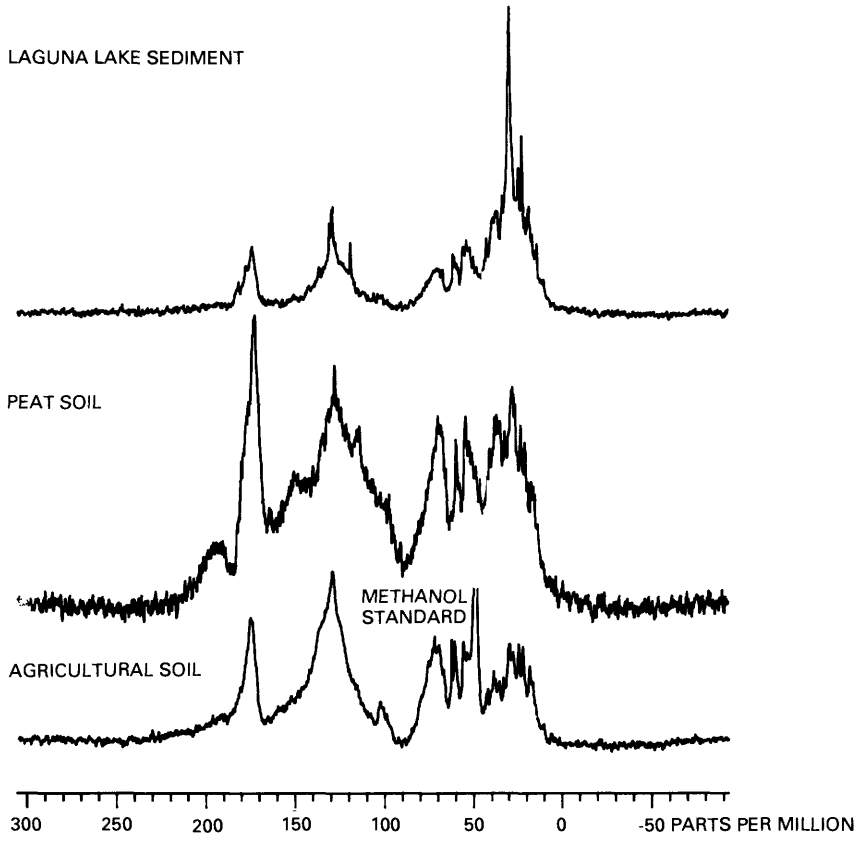

Figure 25. ^{13}C nuclear magnetic resonance spectra of unfractionated Philippine humic acids.

lipids are derived mainly from micro-organisms that convert glucose to lipids.

The higher concentrations of lipid and polysaccharide in whole soils than in humic or fulvic acids are indicative of higher lipid and polysaccharide concentrations in humins than in humic or fulvic acids. ^{13}C NMR studies indicate that lipids and carbohydrates are generally the most abundant components of humins (Wilson, 1987; Hatcher and others, 1985). In addition, Rice and MacCarthy (1990) have isolated high lipid concentrations from humins.

Functional Groups in Humic Substances From Degradation Reactions

A wide variety of degradation techniques have been used for the elucidation of the chemical structure of humic substances. These techniques have been thoroughly reviewed by different authors in the symposium volume edited by Hayes and others (1989). The main conclusions from each of these reviews are discussed briefly here.

Griffith and Schnitzer (1989) have reviewed the application of oxidative degradation to soil humic substances. They pointed out that the different oxidative degradation techniques all yield alkanes, fatty acids, aliphatic carboxylic acids, phenolic acids, and benzene-carboxylic acids. Oxidation of soil humic and fulvic acids yield similar quantities of aliphatic compounds; however, oxidation of fulvic acids yield more phenolic acids but less benzenecarboxylic acids than oxidation of humic acids. Griffith and Schnitzer (1989, p. 95) concluded that:

> Applications of oxidative degradations to soil humic substances suggest that substantial portions of the structures are composed of compounds which oxidize to aromatic polycarboxylic and hydroxypolycarboxylic acids, and to aliphatic di- and polycarboxylic acids. The products in the digests suggest also the presence of unsaturated aliphatic hydrocarbons, and these might be involved in linking the aromatic structures.

These conclusions are consistent with the presence of lignin structures in humic substances, but they

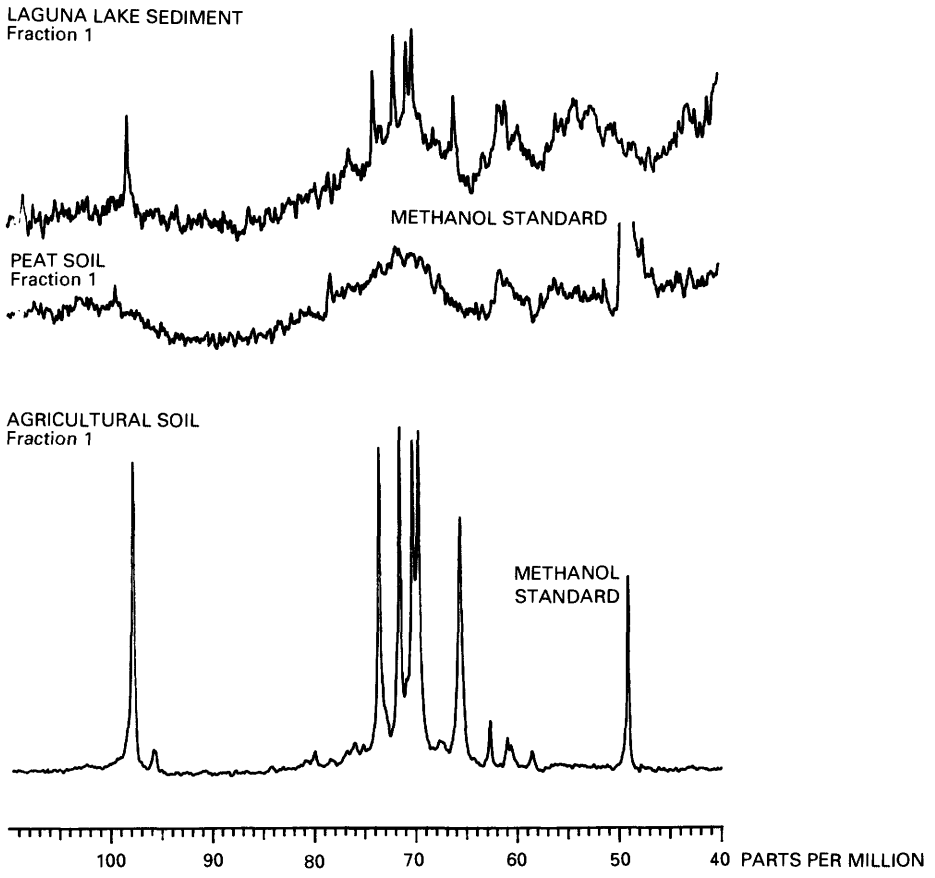

Figure 26. ^{13}C nuclear magnetic resonance expanded spectra of fraction 1 isolates from three Philippine humic acids.

do not prove it. It is interesting to note that Griffith and Schnitzer (1989) do not mention anything about carbohydrates in their conclusions, although there is abundant evidence that carbohydrate groups are present in humic substances. It may be that the evidence of carbohydrates is destroyed by the oxidizing reagents that are used in the degradation procedures. Oxidation of freshwater humic substances has yielded similar information to that from oxidation of soil humic substances (Christman and others, 1989).

Evidence for the presence of carbohydrates and proteins in humic substances is obtained from hydrolytic degradation studies (Parsons, 1989). In addition to identification of the individual carbohydrates and proteins in the hydrolyzates of humic substances, the carbohydrates may be subjected to further analysis by the standard techniques that are available to yield detailed information about the glycoside linkages that existed prior to hydrolysis.

Although some evidence for the presence of ligninlike groups in humic substances has been found by hydrolysis, hydrolysis has yielded less information about aromatic functional groups in humic substances than about carbohydrates and proteins. Some additional information about aromatic linkages can be obtained from reductive-degradation studies (Stevenson, 1989) and from degradations with sodium sulfide or phenols (Hayes and O'Callaghan, 1989). The most informative results to date have been obtained from sodium sulfite degradation. These results suggest that lignin groups are present in humic acids.

The most definitive chemical structural data on humic substances have come from pyrolysis studies. Bracewell and others (1989) have reviewed the progress of pyrolysis studies of humic substances. Bracewell and others (1989, p. 216) stated that:

> Whatever the origins of humic substances, the minimum structures are now known which produce the pyrolysis products observed, whether these are fundamental or peripheral. The structures consist of aliphatic chains, saturated or unsaturated, with OH, CO, and COOH substituents, linked to lignin, polysaccharide, and polypeptide residues.

The main products of pyrolysis of humic acid from composted cattle manure are lignin derivatives (Saiz-Jimenez and others, 1989). Apparently, the cattle metabolized the carbohydrates and proteins in their feed and excreted the lignaceous components. The chemical structures that Bracewell and others (1989) have enumerated are the major chemical structural components of plants. They are the chemical structural groups that have been incorporated into the humus membranes and micelles.

Membrane Model and Physical-Chemical Interactions of Humus

The study of the physical-chemical properties of highly complex systems such as soil-water or sediment-water systems requires that, at least initially, simplifying assumptions be made to render the systems tractable to investigation. Therefore, Wershaw (1989b) has proposed that the physical-chemical properties of humus may be most easily understood by considering humus as consisting of a number of different hydrophobic and hydrophilic phases. In a given natural water system, the humic substances will be present in several different phases: (1) the liquidlike interior regions of the humic membranes coating mineral grains, (2) the charged surface regions of these membranes, (3) the liquidlike interiors of the humic micelles in solution or colloidal suspension in the water, and (4) the charged surface regions of the micelles. For simplicity, one may assume that, although there is a very large number of discrete humic membranes or micelles in a given natural system, the humic substances in the system are uniform enough so that each of the four regions enumerated above may be considered as a single phase.

In addition to the above four phases, the components of the humic phases also will be present as dissolved monomeric units in the aqueous-solution phase. These monomeric units and the other components of the solution phase are free to interact with the aggregated humic phases. Examples of the types of interactions possible are the movement of metal ions in and out of the charged surface phases of the humic membranes and micelles, partitioning of hydrophobic molecules in and out of the liquidlike interior phases, the association of monomeric units into micelles, and the dissociation of micelles to monomers. In all of these examples, dynamic equilibria are set up that can be characterized by appropriate equilibrium constants. Evaluation of these equilibrium constants will allow one to characterize and model the interactions between humic substances and the rest of the components of natural water systems.

The most common type of interaction of metal ions with humus is ion exchange, in which protons on carboxylic-acid groups are replaced by metal ions. There are no published data on ion-exchange reactions between in-place humus and metal ions; all of the measurements have been made on isolated humic and fulvic acids. Most workers have attempted to model their data assuming that the humic isolates behave as dissolved, polyfunctional acids (Purdue, 1985). However, only a very small fraction of the humus in a soil or in sediment-water system is actually dissolved; the rest of the humus is present as membranes coating mineral grains or as micellar aggregates.

One can expect that metal ions will be bound to humus membranes or micelles in two different ways. In the vicinity of charged humic surfaces, there will be nonspecific condensation of metal ions (Manning, 1979). In addition, metal ions also will be bound by specific binding sites embedded within the charged surface phases of the humic membranes or micelles. The binding constants of these sites will be markedly influenced by the surrounding charge densities. This picture of the interaction of metal ions with humus is very similar to the interaction of metal ions with synthetic, insoluble ion-exchange resins. Marinsky (1985) has developed a model for ion-exchange reactions for weakly acidic polymeric gels, which at the present time appears to be the best model for humus ion-exchange reactions. Marinsky and Ephraim (1986), Ephraim and others (1986), and Ephraim and Marinsky (1986) have extended this model to dissolved humic substances, but they have not yet applied it to systems in which insoluble or colloidal humic phases are present.

Organic species may interact with humic micelles or membranes in a number of different ways (Wershaw, 1989a). Two of the most important of these are partitioning into the liquidlike phase in the interiors of the humic micelles or membranes, and ion binding at the surfaces of these structures similar to that which takes place when metal ions are bound to humus. Chiou and others (1983) have shown that the sorption of nonionic organic compounds by wet soils may be treated as a partitioning of the organic compounds from an aqueous phase into a hydrophobic, liquidlike phase. In the membrane model of humus, this phase is the hydrophobic interior of the humic membrane. The hydrophobic parts of the molecules in the interiors of membranes

have considerable freedom of motion, similar to the molecules in a liquid.

Solubilization of hydrophobic organic compounds by dissolved or colloidal humic substances is an analogous process to sorption by partitioning that takes place in soils. Wershaw and others (1969) found that the sodium salt of humic acid enhances the solubility of DDT in water. This increase in solubility apparently is brought about by the partitioning of the DDT into the hydrophobic interiors of humic-acid micelles. A number of other workers have extended this work to include a variety of other hydrophobic compounds, and they have observed similar solubilization (Wershaw, 1989a).

Membrane Model and Extraction of Humic Substances

The extraction of humic substances from soils and sediments is dependent on the disruption of the humus aggregates that coat the mineral grains. As has been pointed out above, both bilayer (membranes) and monolayer (hemi-micelles) coatings may form on the mineral grains. In general, basic solutions have been used to extract humic substances. These solutions will cause ionization of the ionic groups on the humus-membrane surfaces and thereby increase the electrostatic repulsion between the molecules. In addition, basic solutions also will tend to disrupt hydrogen bonding; further disruption of hydrogen bonding can be accomplished by agents such as urea or guanadine. The increased electrostatic repulsion and the disruption of hydrogen bonds apparently brings about release of some of the constituent molecules of the humus aggregates. The molecules that are released are probably mainly from the outer layers of the humic membranes. The inner layers adjacent to the mineral surfaces are, most likely, more resistant to extraction. These molecules probably constitute the humin fraction. In addition, humin probably is more resistant to extraction because it contains more hydrophobic constituents (for example, lipids) than do humic and fulvic acids.

Humic monolayers that are present naturally on mineral grains or that result from the stripping of the outer layers by basic extractants are probably more resistant to extraction because they are hydrophobic and are not readily wetted by aqueous extractants. However, in the presence of a more hydrophobic solvent, such as MIBK in the Rice and MacCarthy (1989) procedure, these coatings can also be disaggregated. Once the humic constituents are freed from the mineral grains, they will form micellelike aggregates in the solvent. If the solvent is water, then the micelles will have the so-called normal configuration with the polar groups on the outsides of the micelles and the nonpolar groups in the interiors of the micelles. However, in a hydrophobic solvent, reverse micelles will form in which the polar parts of the constituent molecules make up the interiors of the micelles and the exterior surfaces are composed of the nonpolar parts of the molecules.

In peats, other highly organic soils, and composts, much of the humus is probably present as micelles and large, multilayer, vesiclelike aggregates simply because there are not enough mineral surfaces to accommodate all the organic molecules. The micelles are probably relatively easy to extract, but the vesicles may well behave like membranes in which the outer layers can be stripped off by basic solutions, but the inner layers, being more hydrophobic, require the presence of hydrophobic solvents for extraction.

SUMMARY

Humus results from the partial degradation of the molecules that make up plants and, to a much lesser extent, animals. The degradation reactions that produce humus consist mainly of enzymatic depolymerization reactions and enzymatic oxidation reactions. These reactions give rise to molecules that are amphiphiles, that is, molecules that have a hydrophobic part and a hydrophilic, polar part. Exudates from plants and micro-organisms—especially proteins and lipids derived from micro-organisms—also are included in the pool of molecules that constitute humus. All these amphiphilic molecules are stabilized in soils, sediments, peats, and composts by incorporation into membrane structures that coat mineral grains, or by incorporation into micelles and vesicles that are dispersed in solution or are present as free organic aggregates in peats and composts of low mineral content.

Even though humus generally constitutes only a small percentage of the total mass of soils and sediments, the physical and chemical properties of soils and sediments are, to a large extent, controlled by the humus that coats the more reactive mineral surfaces. Other organic and inorganic components in natural water systems will interact with humus. Ionic species will interact with the hydrophilic surfaces of humic membranes and micelles, whereas hydrophobic species will partition into the hydrophobic interiors of the humic membranes and micelles. Humus, therefore, may be modeled as consisting of separate hydrophilic and hydrophobic phases.

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