Products of Chemistry ——————————————

Humic Acids: Marvelous Products of Soil Chemistry

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What soil component is a redox reagent and a pH buffer, retains water, binds metal ions, sorbs organic solutes, photosensitizes soil reactions, stimulates plant growth, and biotransforms toxic pollutants? The answer is the brown-to-black soil biomaterials called humic acids (HAs), which are essential components of healthy, productive soils. The name humic acid is derived from its earthly source, *humus*.

A soil's ability to retain water and stimulate plant growth depends on the soil organic matter (SOM) and especially on its humic substances (HSs) fraction. Arable soils contain up to 10% SOM, and HSs typically account for 80% of the SOM (*1–4*). Figure 1 shows the major precursors of soil organic matter (*5*). Dead leaves, a major soil input, consist of 50–60% cellulose, 15–20% lignins, and 15–20% lipids (*6*). Soil chemical and microbiological oxidation of dead animals and plants (humification) initially is exothermic, but then becomes slow synthesis and degradation of HSs (*7*). Degradation of HSs ultimately leads to coal (mostly aromatic), crude oil (mostly aliphatic), and carbon dioxide. The aromatization of aliphatic soil components or HSs to yield coal is an oxidation (dehydrogenation) process. The CO_2 product of HS respiration completes the carbon cycle, prevents the earth from being covered with HS soup, and reminds us that HSs are long-lived but eventually transient on geological time scales.

Soil has an important role in the air–soil–water cycle. HAs are among the most active components of soil. Consider just a few of the factors that involve humic materials in that cycle.

HSs contain more carbon than all living things.

Soil respiration contributes much more to global CO_2 levels than fossil fuel combustion for heating and transportation (*8, 9*).

Solid HSs act as pH buffers, metal binders, solute sorbents, and redox catalysts, and they are photosensitizers. No other natural materials have so many functions in so many different places (*1–5, 10, 11*).

HSs are more versatile than any other synthetic or natural material and they are biodegradable and non-allergenic if free from harmful metals, xenobiotics and microorganisms.

The deserts are growing, populations are exploding, and huge amounts of soil and humic substances wash away every year by erosion (*12*). HSs lost from soils need to be replenished.

Long-term intensive farming depletes SOM (*13*). Solutions to SOM loss include promoting organic farming, replacing incinerators with waste composting, and seeking alternative natural sources of HSs, the pivotal components of the air–soil–water system.

Small wonder that this class of natural substances has been the object of so much study over the last 100 years. Scientific interest in humic substances is continually expanding, despite inherent natural obstacles. For example, it is important to know which components of HSs are responsible for some of the major processes in soils and waters, and the proportions of these components in a humic source. For meaningful structural studies, it is desirable to deal with pure substances. However, HSs are gross mixtures whose separation into discrete substances is a continuing challenge.

Isolation and Classification of HSs

Figure 2 summarizes a thorough HS isolation protocol that depends on HSs' properties as acids (*14, 15*). Dry soil Soxhlet extraction with benzene–methanol removes trapped lipids, nucleic acids, polysaccharides, proteins, and smaller molecules. The soil then is treated with dilute HCl and washed with water. The residue is repeatedly extracted with dilute NaOH until the supernatants no longer deposit a
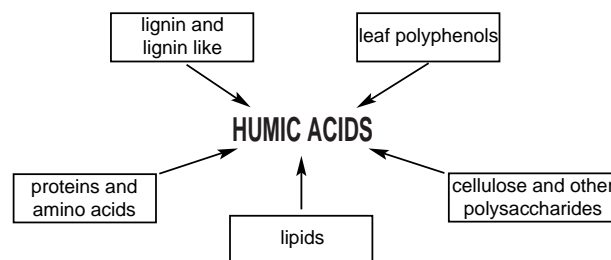
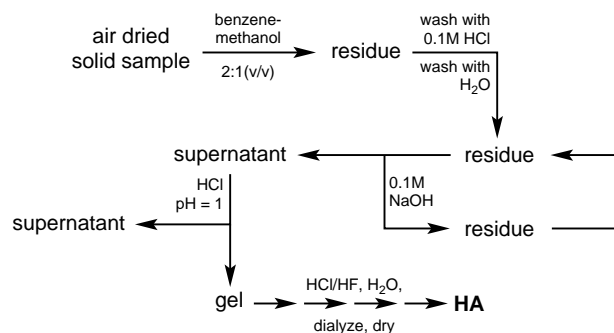

Figure 1. Typical soil inputs.

Figure 2. HA extraction scheme.

brown hydrogel when the pH is reduced to 1 with HCl. The washed and dialyzed gel is defined as a humic acid (HA) and contains up to 98% water. It can be vacuum-oven-, freeze-, or supercritical-fluid-CO_2-dried to give solids with different densities, surface areas, and morphologies (16). The supernatants at pH 1 contain the soluble HS subclass called fulvic acids (FAs), which tend to be more aliphatic and have a higher functional group density than HAs. A third HS subclass called humins (HUs) probably are HA–clay–mineral composites (17). HAs stick firmly to clays and other minerals, which prevents them from being washed from soils. They dominate HSs and SOM and are the focus of what follows.

Humic Acid Characteristics

Table 1 indicates that HAs are carbon-rich materials with characteristic and fairly constant 3 mmol/g HA carboxylic acid contents but variable phenol contents and total acidity. Atomic ratios indicate the humification status: the H/C ratio of an HS sample can range from 2 (aliphatic, as in a lipid) to <1 (naphthalene [$C_{10}H_8$] and other polyaromatic hydrocarbons [PAHs]). The O/C ratio could range from 0 to 2 (in CO_2) and will be higher for wet samples. However, the majority of soil HAs have a narrow range of ratios. Note the similar values of the H/C and O/C ratios of HAs from soil sources in Table 1. Despite the different origins of these soil humic acids (forest litter, grasslands, Podsols, Aridisols, Mollisols, etc.), they have remarkably similar empirical formulas (18). HA functional groups also include alcohol, amine, amide, carbonyl, and quinone (1–3). The latter are important in long-lived HA free radicals (5) and in HA redox catalysis (19). Isolated HAs can contain up to 10% each of polysaccharides and proteins, with particular amino acids and carbohydrates predominating (14). Allowance for the amino acid and carbohydrate contents gives the empirical formula $C_{36}H_{30}N_2O_{15}\cdot xH_2O$ (x = 0–15) for HAs isolated from many different sources by many different investigators (18).

HAs characteristically aggregate to give quasi-spherical solid particles (20). There is no clear evidence for the microstructures of these aggregates, although authors are beginning to speculate (Fig. 3) (21). HAs resist separation into fractions
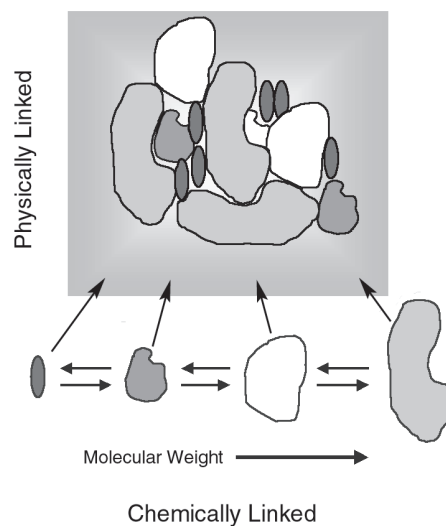

Figure 3. Schematic view of chemical and physical HA aggregation (21).

by capillary electrophoresis (CE) and size exclusion chromatography (SEC). Retention times of HA fractions fall between those of proteins and polysaccharides of similar molecular weight (13–40 kDa, measured by ultracentrifugation [23]). HA aggregation may involve lipid constituents, which restrict sorption of hydrophobic solutes like hexafluorobenzene (24).

CE and SEC runs give typical HA "humps" with little hint of separated fractions. However, recent SEC work (25) suggests that HAs can be disaggregated by mobile-phase additives into longer-retained, smaller molecules (like those found in FAs and dissolved organic matter) (26). These entities can reaggregate to give apparent $M_w \geq 5$ kDa (25). This order of magnitude of M_w has been measured for HAs by flow field-flow fractionation (27). Taken together, these results suggest building blocks of HAs with $M_w \approx 1$ kDa. Light-scattering data show that HAs behave as fractals. The fractal dimensions D = 2.3–2.5 correspond to three-dimensional entities with regions of different density, a bit like Swiss cheese

Table 1. Analytical Data for Purified HAs

Humic Acid		Elemental Analysis[a] (%)				H/C	O/C	Ash[b] (%)	Total Acidity/ (mmol/ g HA)[c]	R–COOH/ (mmol/ g HA)[c]	Phenolic OH/ (mmol/ g HA)[c,d]
HA	Source	C	H	N	O						
PHA	*Pilayella littoralis*	46.60	5.68	5.68	42.10	1.46	0.68	1.1	10.5	3.1	7.4
WHL	Water hyacinth leaf	52.58	5.81	8.10	33.15	1.33	0.47	0.9	6.4	1.6	4.8
WHS	Water hyacinth stem	45.22	5.20	3.40	46.02	1.11	0.76	0.7	8.2	2.4	5.8
WHR	Water hyacinth root	48.46	5.35	4.33	41.68	1.32	0.65	0.6	9.0	2.8	6.2
GHA	German peat	50.50	5.32	1.71	42.47	1.26	0.63	1.5	7.9	3.2	4.7
IHA	Irish peat	50.50	5.56	2.06	41.88	1.32	0.63	0.9	12.4	3.3	9.1
O	Forest litter, 0–7 cm[e]	55.10	5.50	2.70	36.70	1.20	0.50	1.0	5.8	2.9	2.9
AE	Forest litter, 7–9 cm[e]	56.70	4.80	2.80	35.70	1.01	0.47	1.9	7.2	3.9	3.4
Bs	Forest litter, 9–16 cm[e]	56.00	4.80	3.30	36.30	1.03	0.49	3.6	7.3	4.1	3.2
NHA	New Hampshire soil	52.90	5.40	2.00	37.00	1.22	0.52	0.25	8.4	2.7	5.7
NYHA	New York organic farm soil	49.80	4.70	3.60	41.90	1.13	0.63	1.20	8.4	3.0	5.4

[a]On a dry, ash-free basis. [b]Determined by combustion of 100.0-mg HA samples in air at 850 °C for 2 h. [c]For methods see ref 2. [d]Taken as the difference between total acidity and R–COOH. [e]Data from Paramonova, T. A.; Zech, W. (28, pp 469–474).

Figure 4. a: β-O-4 lignin building blocks (*31*). b: Proposed "TNB" HA building block (*32*). c: Hollow elliptical helix obtained by linking lowest-energy TNB HA building blocks (*32*). d: Proposed 3-dimensional HA molecule. Note the predicted cavities for solute and water binding (reproduced by permission from ref *34*).
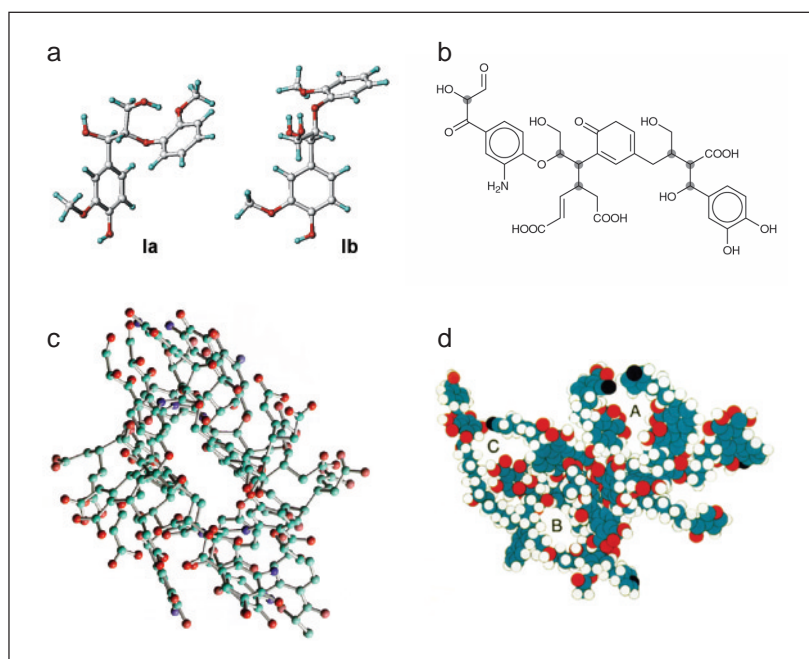
(*28*). Highly aromatic HAs are more compact. At higher pH, time-dependent disaggregation and molecular stretching on dissociation of acidic groups decreases D owing to negative charge repulsion (*29*).

HA Formation and Structures

As revealed by their ^{13}C NMR spectra, HAs isolated from plants tend to be more aliphatic than those from soils. This suggests that HA formation from plants is completed in the soil (*14*). Humification is an oxidative process that includes aromatization and condensation reactions in the soil environment. Major pathways of HA synthesis in soils involve enzymatic and abiotically catalyzed free radical and condensation reactions, phenolic oxidative coupling, demethylation, and functional group oxidation of plant polyphenols, which occurs in leaves primarily in the fall (*6, 30*). Humification probably is catalyzed by microorganisms on clay and mineral surfaces, where life may have begun (*2*).

The HA products are heterogeneous in three respects. First, they are made from different feedstocks. Second, they may be contaminated with sorbed lipids, nucleic acids, polysaccharides, proteins, steroids, clays, minerals, and metals. The pre-extraction with benzene–methanol in Figure 2 lessens this possibility. Third, they contain functionalized aliphatic–aromatic backbones that may be neutral, acidic, or basic and have low or high molecular weight.

Consistent analytical and other properties encourage modeling of HAs. The first kind of model envisions the "average" HA building block and the second models the whole SOM. One HA building block (Fig. 4a [*31*]) comes from conversion of lignins and tannins; the other pathway is biosynthesis from amino acids (*32*). Lignin also is biosynthesized from amino acids. Actually, the so-called Temple–Northeastern–Birmingham (TNB) building block (Fig. 4b [*14, 18, 32*]) can be generated in known reactions from

known lignin components as suggested in Figure 5 (*33*). The proposed building blocks all have chiral centers and link together to give hollow helical structures that normally are filled with water (Fig. 4c). Random-coil HA behavior was deduced from frictional ratios in ultracentrifugation data (*23*). Thus, the helical model accounts for water retention by HAs in terms of their hollow centers and many hydrophilic functional groups on the outside of the secondary structure.

The whole SOM model in Figure 4d (*34*) consists of substituted benzene rings linked by long aliphatic chains in a molecule with $M_w \approx 5$ kDa. The structure has cavities large enough to accommodate peptides, small polysaccharides, water, and other solutes. The building blocks and whole-molecule HA models are mass fractals because of their "holes".

Whatever their structures, the fact is that HAs are excellent sorbents because they are hydrophilic, hydrophobic, and functionalized. This qualifies them as amphiphiles (*6*) that lower the surface tension of water and form micelles that can sequester hydrophobic molecules such as pyrene, adsorb more polar compounds, and bind metals. Another great gift of their versatility is that solid HAs behave as free-energy buffers (*35*).
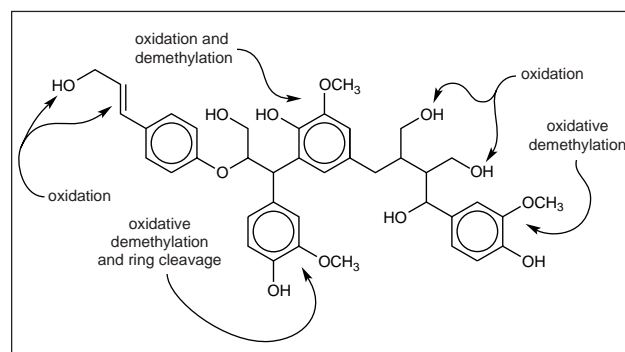

Figure 5. Conversion of a lignin unit toward a TNB HA building block.

Supramolecular models of HS structures are being put forward (*21, 25*) and the supramolecular chemistry of HSs is a new and exciting field (*25*). Instead of single monomeric species, HSs are being described as micelles, colloids, aggregates, vesicles, fractals, clathrates, and surfactants. Macroscopic surface areas are being measured and their morphological features described. Advanced computer modeling is predicting shapes, folding patterns, and metal chelation sites (*32*).

Macromolecular architecture and surface features can be measured by atomic force microscopy (AFM) and other microprobe techniques (*20, 36*). The shapes and properties of HSs can be dramatically altered in response to changes in pH or ionic strength and exposure to minerals (*20, 36*). The actual behavior of HSs in natural soil and water environments is a function of the molecular structure, which has important implications for contaminant mobility, aggregation, and adhesion to clay surfaces.

Humic Substances in Drinking Water

There is an old saying in the Wild West: "Whiskey is for drinking, but water is for fighting." Battles over water rights are not only a characteristic of the western USA but also a worldwide phenomenon. In recent years, however, the battle lines have focused on drinking water quality as well as ownership.

Surface waters contain a range of inorganic salts and organic solutes. These waters are required to be treated and disinfected by the Environmental Protection Agency (EPA) as well as local governmental agencies, thus adding more ingredients to the water. Odor and taste properties have been affected as a result. These treatments have caused a change in the public perception of municipal tap water. In the past 10 years there has been a sharp increase in the consumption of bottled water as a result of the public suspicion of tap water.

Possibly the most influential group of organic solutes in surface waters are HSs, specifically HAs and FAs. Although benign and even therapeutic in themselves, these humates react with disinfectants to produce disinfection by-products (DBPs), which are toxic and regulated by the EPA. The most common disinfectant in municipal treatment plants is chlorine. It reacts with HSs to produce chloroform and other haloforms, which are classified by the EPA as trihalomethanes (THMs). This reaction, the haloform reaction (eq 1), is a common topic in sophomore organic chemistry.

$$OH^- + RCOCH_3 + 3Cl_2 \rightarrow RCOO^- + CHCl_3 + 3HCl \quad (1)$$

The yield of chloroform strongly depends on the apparent molecular weight of the humic acid species (*37*). Many other halogenated aromatic and aliphatic products (DBPs) are also produced, but all of them in much smaller quantities than the THMs. The THMs are potential carcinogens: approximately 10,000 people a year in the USA contract bladder or colon cancer from drinking chlorinated waters (*38*). At present, municipal water suppliers must limit the concentration of THMs to less than 80 µg/kg (80 µg/L, or ppb for dilute aqueous solutions) in the potable water distribution system.

Faced with the EPA restrictions, municipal water companies have used technologies to reduce the production of THMs. Two methods commonly used in water treatment plants are (i) alternate disinfectants to chlorine, such as ozone, chloramine, and chlorine dioxide, and (ii) removal of humic acids from the source (raw) water.

The first method is most widely used. The second is more expensive; it employs filters such as activated charcoal, anthracite, or treated sand. None of the filtration systems remove all the humic substances, but they can reduce chloroform levels to meet EPA guidelines.

One other method of removing humic acid from surface waters relies on nature itself. It is known as soil aquifer treatment (SAT) and utilizes soil as the filter. By percolating raw water into large basins, water managers can recharge the aquifer while removing much of the humic acid (*39*). The treated, stored water can then be pumped to the municipal water delivery system when needed.

Redox Properties of Soil Humic Acids

Soil humic acids are redox polymers. Although they can act as oxidants and reductants, their most common behavior under natural conditions is as reducing agents. The standard reduction potential of a typical soil HA is 700 mV (*40*). In other words, humic acids are capable of reducing Fe(III), Sn(IV), V(V), and Cr(VI). In return, certain functional groups in HA are oxidized. Some possible candidates are catechol moieties (oxidized to quinones), phenolic groups (to free radicals or quinhydrones), aldehydes (to carboxylic acids), and alcohols (to aldehydes or carboxylic acids).

Oxidation and reduction of HAs is a reversible, repeatable process (*41*) that takes place in one-electron steps. The intermediate species is a semiquinone free radical. The phenolic and quinone groups in the HA polymer are the functional groups responsible for this redox behavior (*5, 19*).

Recently, a number of commercial products derived from HAs have capitalized on these remarkable reducing properties (*42*). Humic acids derived from natural sources are cross-linked with polymers to make them insoluble in water. These products are then able to detoxify soils and surface waters contaminated with toxic organic and inorganic chemicals. Some metals are reduced from toxic valence states to a nontoxic state—for example, Cr(VI) to Cr(III). More striking is the reductive cleavage of halogenated hydrocarbons. Substances like trichloroethylene (TCE), a very common pollutant in soils and ground water, are dehydrohalogenated to ethylene and HCl (*43, 44*). This reduction corresponds to the reduction of TCE by elemental iron! A very bright future is envisioned for the detoxification applications of humic substances.

Solid HAs as Sorbents and Metal Binders

Solid humic acids have large sorption capacities for hydrophilic and hydrophobic solutes. ^{13}C NMR spectra show that soil HAs consist of aliphatic and aromatic backbones with the aromatic fraction rarely exceeding 50% of the total carbon (*45, 46*). Functional groups such as –COOH give hydrophilic character to HAs. Aliphatic chains give HAs flexibility, while the aromatic units are rigid. This is why HAs can interact with so many different solutes.

Sorption of hydrophobic organic compounds occurs by partitioning/dissolution into an amorphous phase (*47*) or interaction with rigid units such as stacked aromatic rings (*48*). One piece of evidence for specific interactions is to have

$N < 1$ in the Freundlich equation (eq 2)

$$A = K_f c^N \qquad (2)$$

where A is the mass of sorbed solute per unit mass of HA, K_f is the sorption coefficient, and c is the equilibrium solute concentration. For solute distribution between water and a structureless "liquid" HA phase, N would be 1. Another clue comes from competition between solutes for adsorption (48). Data for adsorption of hydrophilic solutes such as uracil fit the Langmuir equation (eq 3) for specific adsorption in sequential steps A, B, and C. Here, K is the equilibrium constant and v is the site capacity. As expected, adsorption of hydrophilic solutes is competitive. Physical adsorption results in higher solute desorption rates than for chemically sorbed solutes (e.g., by condensation of amines with HA carboxylic acid groups) (34).

$$A = Kvc/(1 + Kc) \qquad (3)$$

Solid HAs have an unusual ability to "create" additional adsorption sites by disaggregation as a result of primary organic solute adsorption. Linear correlation of the thermodynamic adsorption parameters ΔH_i and ΔS_i for every solute and adsorption step indicates that HAs selectively bind solutes with small, fairly constant, negative free-energy changes. That is, they are free-energy buffers, which is made possible by compensating enthalpy and entropy changes for adsorption. Water and bound metals evidently play major roles in HA–solute interactions and in HA aggregation/disaggregation (34, 49).

Humic acids have higher metal-binding capacities than most commercial ion exchangers and they selectively bind, store, and release metals. Humic acids can be used in soil remediation to trap contaminants. They also can transport metals to other sites. Metals principally are bound by HA carboxylate groups (50), which means that HAs act at least in part as weak-acid cation exchangers. Bound metals and minerals reduce HA flexibility and cause HA aggregation (49), or they block the sorption sites and reduce the sorption capacity (51). HAs' molecular flexibility enables them to wrap around a metal center. Metals also can bridge functional groups on different HA molecules (34, 50). X-ray absorption fine-structure spectroscopy (XAFS) and X-ray absorption near-edge spectroscopy (XANES) are helping us to understand how HAs bind metals. They identify the metals and their oxidation states as well as their binding sites (14, 52, 53).

Other HA Sources

Humus and potting soil sales are big business, but it makes little sense to replenish large amounts of desert with soil from another location. Polyphenols and lignins have only part of soil HAs' attributes because they are more aromatic and need to be modified, for example as in Figure 5. One solution to soil shortages is accelerated large-scale composting, which is of worldwide interest (7). Plants that contain HAs are good composting feedstock. Another HA source is soft brown coals and Leonardite, which contain up to 80% extractable HAs that resemble soil HAs but are more aromatic (46). These economical coal-derived HAs are useful for soil and water remediation. They promote plant growth by improving the availability of Fe, Zn, and other micronutrients (54, 55).

Conclusions

Humic acids are essential and remarkable products of soil chemistry. Current HA models help to explain their origin and behavior as flexible, aliphatic-aromatic, and highly functionalized molecules. Composting and economical HA extraction from coal will help to combat water and soil pollution, fight soil erosion, and lessen our dependence on chemical fertilizers.

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