

Understanding Humic Substances: Advanced Methods, Properties and Applications

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Understanding Humic Substances

Advanced Methods, Properties and Applications

Edited by

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“...at moments of very intense creativity, chemistry, art and religion join.”

Morris Schnitzer

Foreword

1 OVERVIEW

I recently had the opportunity to write an account of progress made during the second half of this century on the chemistry of humic substances. As I was writing the article I noticed that advances in this field were directly related to how well we could adapt newly developed methods and instruments. For example, in the late 1940s wet chemistry done with beakers, flasks, test tubes, burettes, pipettes and balances was predominant. The major instruments available at that time were pH meters powered by batteries and colorimeters requiring filters for changing wavelengths.

In the early 1950s, recording UV and visible spectrophotometers became available and, in the mid 1950s, IR spectrophotometers. The arrival of the latter held out high hopes that with the aid of IR spectrophotometry it would be possible to obtain significant new information on the chemical structure of humic substances. Unfortunately, these hopes were not fulfilled, as researchers began to realize that the IR beam was unable to penetrate the matrices of the humic acid, fulvic acid and humin molecules, and only registered the presence of OH and CO₂H groups on the outside surfaces of the samples.

The early 1960s saw the arrival of gas chromatographs, which allowed researchers to separate complex mixtures of humic acid and fulvic acid oxidation products, and also of organic soil extracts containing alkanes, alkenes, fatty acids and esters. In the mid-1960s mass spectrometers, which could be attached to gas chromatographs, came on the market. This made it possible to not only separate complex mixtures of organics but also to identify the separated compounds. At about the same time, electron-spin resonance (ESR) spectrometers became available, which enabled researchers to measure concentrations of free radicals in humic materials and to obtain information on the nature of the free radicals. ESR also made it possible to study the symmetry and coordination of complexes formed by humic substances with paramagnetic metals such as Fe³⁺, Cu²⁺ and Mn²⁺.

In the early 1980s first liquid-state and then solid-state ¹³C NMR became available. This was an important addition to our arsenal of equipment. It showed, for the first time, that aliphatic C in humic substances was as important as aromatic C, and that the older theories that humic substances were almost completely aromatic were no longer valid. The mid-1980s saw the arrival of pyrolysis-soft ionization mass spectrometry, which could be applied to the *in situ* analysis of humic substances in soils.

Other types of mass spectrometry, such as Curie-point pyrolysis-gas chromatography-mass spectrometry, provided important structural information on humic substances. From the chemical, spectroscopic, and mass spectrometric data, two- and three-dimensional model structures were proposed for humic acid, soil organic matter, and a whole soil with the aid of computational chemistry. During the past 50 years, I was fortunate to have participated, along with other scientists, in the developments described in this paragraph. I

was a witness to the evolution of the chemistry of humic substances from wet chemistry to computational chemistry.

2 NOMENCLATURE

The current nomenclature of humic substances (humic acid, fulvic acid and humin) originated over 200 years ago.¹ Early workers² believed that the three humic fractions were different substances, well separated by the 'classical' extraction procedure. The latter consists of extraction of the soil with dilute base, acidification of the alkaline extract to produce a coagulate that is humic acid, while the material remaining in solution is fulvic acid. Thus, humic acid is soluble in base but not in acid, while fulvic acid is soluble in both base and acid. The humic material that cannot be extracted from the soil by base is referred to as humin.

We know now from chemical, ¹³C NMR and mass spectrometric analyses that the main structural features of the three humic fractions are similar so that the assumptions of earlier workers are incorrect. Thus, the terms humic acid, fulvic acid and humin do not stand for distinct chemical substances. Each of these fractions consists of hundreds of compounds, which appear to be associated at the molecular levels by mechanisms not yet well understood.

While it is true that the terms humic acid, fulvic acid and humin have no chemical meanings, these designations have been used over the past 200 years in thousands of scientific papers. What are we to do now? Change these terms, and start to express ourselves in the language of chemistry, but lose the connection with the voluminous literature and with current researchers?

For an answer to this very complex problem we may want to turn to philosophy. The medieval Jewish philosopher Maimonides, who lived in Spain, Morocco, and Egypt, was concerned with the relationship between man and God. He proposed that the first thing to do in this discussion was to define God. "How can we define God?" he asked. His answer was that we can define God by His characteristics. For example, we can say that God is merciful. He heals the sick, He feeds the hungry, He lifts up those who have fallen, *etc.* It seems to me that we can apply the same approach to the definition of humic substances. Each humic fraction can be defined by its ¹³C NMR spectrum, its IR spectrum, its mass spectrum, its elemental analysis, and so on. In this manner we can retain the designations humic acid, fulvic acid and humin without having to substitute them by other terms, and so avoid turmoil and confusion in our field of research.

3 CHEMISTRY AS ART

In 1961 I was offered the opportunity by Agriculture Canada to do post-doctoral studies at an English University. I decided to spend a year in the Organic Chemistry Department of the Imperial College of Science and Technology in London, England. After some correspondence, I was invited by Sir Derek Barton, Nobel Laureate, who was at that time Professor of Organic Chemistry at Imperial College. While most of the students and researchers at the College worked on natural products (steroids, alkaloids, and so on), I arrived there equipped with a glass bottle containing 200 g of purified fulvic acid, which I had extracted in Ottawa from a Spodosol Bh horizon. I had decided to work on elucidating

the chemical structure of fulvic acid because I thought at that time that fulvic acid had a simpler molecular structure than humic acid. The work that Sir Derek and I did is described in the literature³ and I will not discuss it here. During my stay at Imperial College, Sir Derek and I had many discussions not only on my immediate objectives but also on more general topics such as science and philosophy. One morning, Sir Derek approached me saying: "Morris, chemistry is not a science, chemistry is an art." I was somewhat surprised by what he said. In the 1960s, science was the greatest endeavor one could pursue, it was pure and virginal, how could one compare it to art? Nonetheless, I never forgot what he said to me. In the 1970s and 1980s, my co-workers and I made some, at least what I thought important discoveries on the chemical structure of humic substances. Each time we made such discoveries I got very excited, euphoric at having caught a glimpse of something that had never been seen before. At such moments I felt that chemistry and art had joined, so that chemistry had become art as Sir Derek had told me many years earlier. I also had another feeling at such moments: I felt that I was closer to God. So, at moments of very intense creativity, chemistry, art and religion join. Most chemists refrain from writing or speaking about the spiritual aspects of chemistry. But as a chemist who has worked for 50 years on materials that originate from the outer crust of the earth and as a retired person, I feel that these experiences deserve to be discussed.

Advances in the chemistry of humic substances depend on developments in analytical, physical and organic chemistry and how effectively we can apply the newly developed methods and instruments to our own research. It is very likely that these trends will continue in the future. We can also look forward to increasing applications of computational chemistry to elucidate the arrangements of molecular constituents in humic substances and the nature of interactions of humic substances with metals, minerals, pesticides and herbicides. It is important that the computational chemistry be based on data generated by 'classical' analytical, organic and physical chemistry.

Ottawa
March, 1999

Morris Schnitzer

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Preface

This book complements the volume *Humic Substances: Structures, Properties and Uses* published by the Royal Society of Chemistry in 1998. Both books describe front-line research on humic substances (HSs), the remarkable brown biomaterials in animals, coals, plants, sediments, soils and waters. HSs' functions include water retention, pH buffering, photochemistry, redox catalysis, solute sorption and metal binding. No other natural materials have so many functions in so many different places.

HSs are highly functionalized, carbon-rich molecules with a strong tendency to aggregate. This helps HSs to hide their identities as macromolecules or self-assembling systems. HSs are chameleons that can behave like lipids, polysaccharides or proteins depending on the circumstances. We need to know HSs' molecular structures in order to understand their properties.

The importance of HSs in the environment and human health is encouraging the best minds with the best tools in a concerted effort to solve one of nature's greatest mysteries. This book benefits from the wisdom of Drs. Morris Schnitzer and Cornelius Steelink, two of this century's greatest contributors to humic substances research. They remind us of the history and importance of research into HSs and ask us not to forget that many questions about HSs have been answered. Drs. Schnitzer and Steelink make it plain that advances in HS research have always followed the proper application of some analytical technique. Some of the most fundamental questions about HSs are being addressed with the sophisticated tools and methods described in this book. New experimental work and well-known data are stimulating sophisticated molecular modeling that keeps our sights set on molecular structures.

This book is derived from Humic Substances Seminar III, which was held at Northeastern University, Boston, Massachusetts, USA on March 22–23, 1999. We were honored by the presence of Drs. James Alberts (President), Michael Hayes (Immediate Past President), Nicola Senesi (Past President) and C. Edward Clapp (Treasurer) of the International Humic Substances Society, together with other eminent authors from 15 countries. It was a memorable meeting, as you will discover from reading the excellent papers that follow.

ACKNOWLEDGEMENTS

We thank the authors and reviewers for their contributions and unwavering co-operation. Financial support from Arctech, Inc. and our other sponsors is gratefully acknowledged. We thank Northeastern University for providing facilities for the Humic Substances Seminar series. Michael Feeney ably managed the Seminar III presentations. Beth Rushton and the staff of the Barnett Institute handled many of the organizational details with

efficiency and charm. The talented undergraduates of the Humic Acid Group were excellent hosts and we thank them for honest effort gladly given. Last but not least, we thank Janet Freshwater and her staff at the Royal Society of Chemistry for ensuring timely publication of the authors' fine work.

Boston, Massachusetts
August, 1999

Elham A. Ghabbour
Geoffrey Davies
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WHAT IS HUMIC ACID? A PERSPECTIVE OF THE PAST FORTY YEARS

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1 THE PERENNIAL QUESTION

"What is humic acid?" A college newspaper reporter asked me that question shortly after Humic Substances Seminar III in March 1999. Her question reminded me of the identical question posed by Jon Jakob Berzelius 190 years ago in one of his publications.

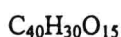
Do we have an answer to that question today? For science newspaper reporters there is a simple answer: 'Humic Acid is a complex polyelectrolyte of variable composition found in nearly every scoop of dirt'.¹ But for working scientists in the field there are many answers to that question. I have thought of all the conceptual and operational descriptions of humic substances in the past 40 years of my involvement in this field. I decided to put together an historical perspective of changes I have seen in humic substances (HSs) science over the past forty years. This will not be a formal history of the subject. It will be one man's thoughts on his favorite field of research.

Forty years ago I attended the First International Symposium on Humic Acid at University College in Dublin, Ireland.² It was a meeting dominated by soil scientists, agronomists and coal scientists. The majority of the talks focused on structure, biosynthesis, isolation and reactions. Everyone at the Symposium was awaiting the forthcoming book by the leading humic acid authority in the world, Maria Kononova, of the Dokuchaev Institute of Soil Science in Moscow. She did not disappoint us.³

Forty years later, in 1999, I listened to the papers at Seminar III at Northeastern University. Once again I was struck by the fact that certain questions in the HS field have never changed. The newcomers in the field today are asking the same fundamental questions as the newcomers did 40 years ago. Nevertheless, these questions continue to provide incentives for research in the field.

1.1 Molecular Structure

A number of investigators are still asking the same question today as Berzelius did 190 years ago: what is the chemical (or molecular) structure of soil humic acid? Berzelius' student Mulder published an empirical formula of



for soil HA in 1840.⁴ Since that time, countless investigators have viewed HA as a monomolecular species with a specific structure. These investigators have proposed numerous empirical and structural formulas for HA in attempts to fit a bewildering array of experimental data to a universal model. I, too, have been guilty of publishing HA model structures.

On the other hand, a number of investigators in recent years have concluded that the search for a universal structural formula for HA is meaningless. In their view, humic substances must be viewed as macromolecular species with fractal, micellar or other supramolecular properties. They say HSs are complex systems whose properties are not fully explained by understanding its component parts.

Still, the desire for a classic structural formula remains strong among many of us. It is a continuing search by many HA scientists. Therefore, I was astounded to see Mulder's empirical formula reappear in a journal this year. It was proposed as a general formula for soil humic acids at Seminar II.⁵ After normalizing the empirical formula to reflect a multiple of the C-9 lignin building block and adding nitrogen, Jansen et al. put forth the structural formulas shown in Figure 1.

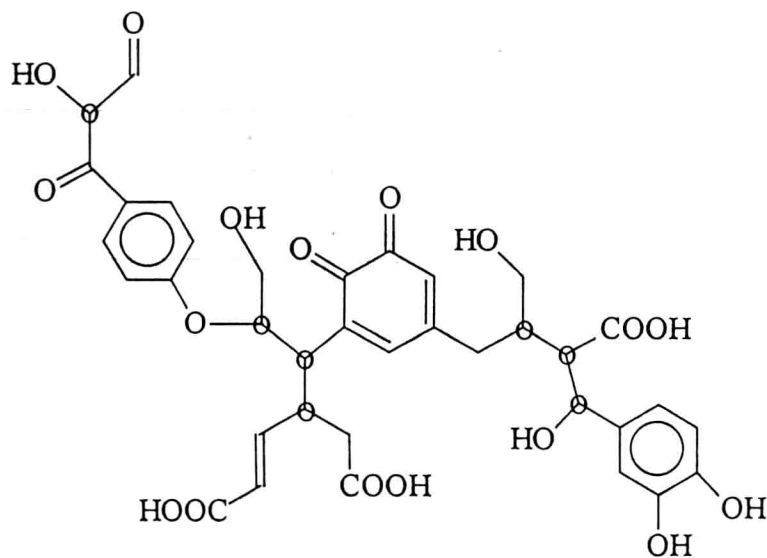
Experimental evidence to support this model was derived from all sorts of advanced spectroscopic and analytical measurements that lend credence to the formula. Only three weeks after Seminar III I was further astounded to see a study of binding sites of humic acid. Those sites were identical to the structural features of this TNB formula and were being examined as scavengers for uranium by a University of California, Berkeley scientist Heino Nitsche.¹ Both newcomers to this field as well as old hands are still intrigued with the goal of finding a discrete chemical structure for these elusive substances.

For many investigators, the answer to the question: WHAT IS HUMIC ACID? still remains A DISCRETE MOLECULAR STRUCTURE, at least for soil humic acid.

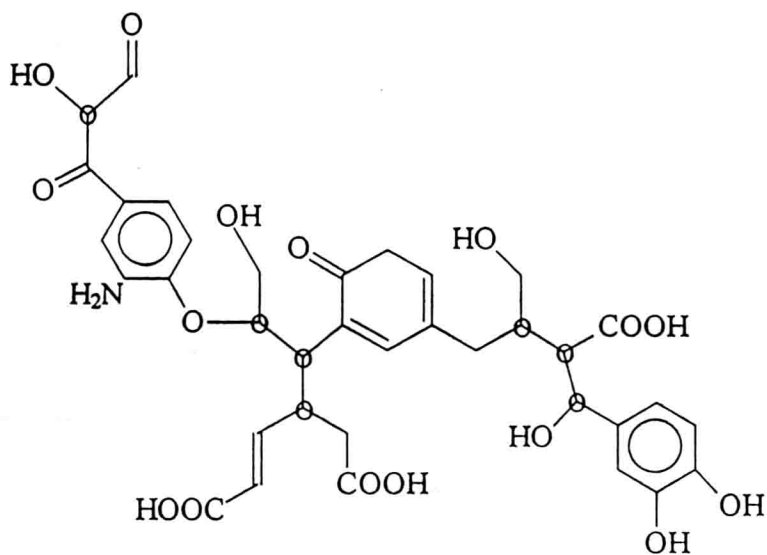
1.2 Biosynthesis of Humic Acid

Humic acid has also been defined as a product of a specific biosynthetic pathway. One of the 1959 Dublin Symposium papers described the origins of humic acids from leachates of plant leaves. Analytical methodology was crude in those days: paper chromatography, ultraviolet absorption spectroscopy and titration were the common tools to identify natural products. Yet remarkable deductions were made from the analytical measurements of these leachates and their interaction with the soil. Forty years later I listened to a presentation on the spectra of humic substances in leaf leachates. Once again I was struck by the fact that certain questions in the HSs field have never changed. Where do humic substances come from? How are they biosynthesized? Today there are sophisticated analytical tools to illuminate these processes. We can monitor processes over periods of time with a variety of non-intrusive spectroscopic probes. Compelling evidence can now be presented for the origin of soil humic acid from leaf leachates.

Another proposal for the origin of soil humic acid is the Maillard reaction. Over the past 80 years this proposal has been buried and resurrected many times. When a reducing sugar reacts with amino compounds (amino acids, peptides, proteins), brown polymeric substances result.⁶ These Maillard products appear to have the properties of humic acid. The biosynthetic pools of amino acids and sugars certainly are available in plant cells and soil. Once again, this proposal is being examined, because now we have spectroscopic



(a)



(b)

Figure 1 Molecular structure of (a) Steelink and (b) TNB humic acid monomers showing chiral centers as open circles.^{5,10}

probes (^{15}N and ^{13}C NMR)⁷ that can monitor the formation of these Maillard products.

Forty years ago,⁸ a hypothesis was put forth that humic acid actually was an *in situ* plant cell product. It claimed that humic acid formation began in the leaf as a result of cell senescence. In the intervening years, not much could be found in the literature about this theory. This proposal has been revived, examined, tested and found to be alive and healthy.⁹

For some scientists, therefore, the answer to the question WHAT IS HUMIC ACID? would be: AN END PRODUCT OF A SPECIFIC BIOSYNTHETIC SEQUENCE.

1.3 Isolation, Fractionation, and Purification

For many years, humic acid has been operationally defined. That is, it is defined by the method of its removal from its original source. Forty years ago the most common source was soil. Humic acid was defined as a base-soluble, acid-insoluble fraction of soil leachate. There were many modifications of this procedure including pre-treatments and post-treatments, but the base extraction definition was the same in 1959 as it was in 1859.

Since 1959 sophisticated separation methods have dramatically changed this definition. The most fundamental change has been achieved with resin chromatography, including XAD, GPC and HPSEC. Nowadays, I am impressed with the different ways in which humic substances were characterized by their chromatographic treatment. It reminded me of my previous work with lignin chemistry in which a specific lignin substrate was identified by its extraction procedure. Thus, lignins were classified as Native lignin, Bjorkman lignin, Klason lignin, etc. depending on the solvent methodology used to remove them from wood.

Today, for some the answer to the question WHAT IS HUMIC ACID? would be THE FINAL FRACTION OF A SPECIFIC EXTRACTION PROCEDURE.

1.4 Supramolecular Concepts and Structures

In the past decade there has been a growing interest in non-traditional concepts of molecular architecture for humic substances. Instead of single monomeric species, humic substances are being described as micelles, colloids, aggregates, vesicles, fractals, clathrates and surfactants, just to name a few. Molecular surface areas are being measured and their morphological features described. Advanced computer modelling is predicting shapes, folding patterns and chelation sites, much as was done with proteins, polysaccharides, nucleic acids and lignins.¹⁰ Humic Substances Seminar III gave maximum exposure to these developing concepts. Certainly, these new ideas will be thought provoking and may be considered heresy by some of us. But it will provide challenge and dialogue, as all new ideas should in a robust and healthy scientific gathering.

For some scientists, the answer to the question WHAT IS HUMIC ACID? would be A SUPRAMOLECULAR SPECIES DERIVED FROM TERRESTRIAL PLANTS.

1.5 Structure and Instrumentation

Most scientific research follows new advances in instrumentation. Since the 1959 Dublin Symposium on Humic Acid there have been dramatic changes in analytical and spectroscopic technology. With each change has come a new insight into the nature of HSs.