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XX

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AND DAMIAN E. SMEULDERS

The Royal Society of New South Wales

Michael Amos Wilson

MICHAEL AMOS WILSON 1946 -

Michael Amos (Mick) **Wilson** was born at Bethnal Green, London (U.K.) on 1 July 1946. After primary education at Debden Primary School and at Highland Primary School in Essex, England, he attended West Hatch Technical High School, Chigwell (Essex) during 1957-1962, and then his family migrated to New Zealand where he completed his secondary education at Christchurch Boys High School in 1963. He then proceeded to the University of Auckland (New Zealand), graduating B.Sc. in 1969, and M.Sc.(Hons.) in 1970. For a period in 1969 he worked as an industrial chemist at May and Baker (UK), and then he returned to the University of Auckland where he was a Junior Lecturer in Chemistry (1970-4); during that time he carried out research on addition reactions to carbon-carbon double bonds under the supervision of Professor Peter B.D. De la Mare, and graduated Ph.D. in 1974. He then proceeded to Lincoln College, University of Canterbury, New Zealand where, in 1975, he obtained tenure as a Lecturer in Chemistry and Soil Science. In 1978 he moved to Australia to take up an appointment as a Research Scientist in the CSIRO Division of Process Technology, North Ryde (formerly the Division of Coal Research). He was promoted to Senior Research Scientist in that Institution in 1981, and then to Principal Research Scientist in 1985, which position he held until 1987. In 1988 he was awarded the degree of D.Sc. at the University of Auckland, New Zealand. For the period 1988-9 he was a Senior Lecturer at the University of New South Wales, then he returned to the CSIRO Division of Coal and Energy Technology as Senior Principal Research Scientist for the period 1989-91. In 1992 he was promoted to Chief Research Scientist, CSIRO Division of Coal and Energy Technology, and during 1993-6 he was Chief Research Scientist and Joint Research and Development Operations Manager, CSIRO Petroleum Resources. In 1996 he was appointed as Professor of Chemistry, and in 1997 as Director of the Centre for Materials Technology at the University of Technology, Sydney; in 1998 he became Head of the Department of Chemistry, Materials and Forensic Science at that Institution.

In 1980 he was a Research Fellow in Organic Geochemistry at the University of Utah. Concurrently with his employment in CSIRO he held a Visiting Professorship at the University of New South Wales for the period 1991-6, and he was a Director of the company Pentapolar Pty Ltd during 1989-93. He has been a Plenary or Invited Speaker at numerous overseas and Australian conferences concerned with various aspects of organic geochemistry, including coal science, and on applications of nuclear magnetic resonance spectroscopy in organic geochemistry.

Mick Wilson's research interests have been in various aspects of environmental chemistry, especially in organic geochemistry, petroleum and coal science. He is also involved with the development of new methods of analysis of illicit drugs; this includes applications of nuclear magnetic resonance spectroscopy and laser ablation mass spectrometry. He holds several patents, and has published a monograph entitled 'The Applications of Nuclear Magnetic Resonance to Geochemistry and Soil Science'; he has also been the author of chapters in several other books.

Honours and Awards (Pre-2001)

1988	Dahelem Lecturer, Dahelem Conference, Berlin
1993	FRACI (Fellow of the Royal Australian Chemical Institute)
1995	Amoco Prize, Chicago, USA
1995	Distinguished Collaborator, Kyushu Research Institute, Japan
1996	Baragwanath award, Australian Institute of Energy
1997	Unsworth Lecturer, University of Technology, Sydney
2000	Liversidge Research Lecturer, Royal Society of New South Wales

Biographical Source

Personal communication

Scientific Publications of M.A. Wilson

Between 1970 and 2000 M.A. Wilson had over 280 publications, including one book, numerous invited book chapters, and four patents.

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FUNERAL ARRANGEMENTS FOR PLANTS: AN ESSAY IN ORGANIC GEOCHEMISTRY*

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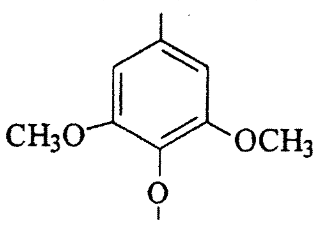
ABSTRACT. Organic geochemistry literally is the dead end of science since it is concerned with the transformation of decaying plant material into humic substances, coal, petroleum and natural gas. The transformation process is primarily controlled by the possible degree of oxidation. Under reducing conditions coal and gas are formed and under oxidising conditions humic material is formed. Not surprisingly, the nature of the input vegetation also has an effect on the type of decomposed organic matter produced. One new finding for oxidising environments reported here, is the concept of a host-guest structures where smaller molecules reside within a framework of a macromolecular host primarily derived from lignin. The guests within the host cannot be removed by physical separation. The structure of the host can be determined by pyrolysis gas chromatography mass spectrometry and nuclear magnetic resonance techniques. Differential thermal analysis, calorimetry, methylation and gas chromatography mass spectrometry and nuclear magnetic resonance data can be used to identify the guests. Some of the guests are probably held by hydrogen bonding but others are true prisoners in that they are alkanes and hence have no binding sites.

KEYWORDS: Humic substances, Host-guest theory, COSY NMR, Differential thermal analysis

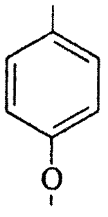
Introduction

The study of a leaf which is physically connected to a plant, belongs to the discipline of botany. For a few moments after death, while a leaf floats to the ground, the study of the mobile object belongs to the aerodynamicist, but thereafter, the leaf is the property of organic geochemists, the undertakers of the scientific world.

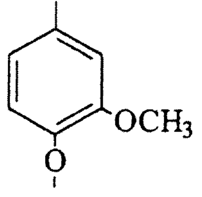
Animals contribute little to decaying matter and it is the major components of plants that are significant. These in order of importance are carbohydrates and lignin, (Figure 1) i.e. the C3 aryl macromolecular skeletal component containing various amounts of syringyl *para*-hydroxyphenyl or guaiacyl groups that keeps plants upright. To a lesser



Syringyl



para-Hydroxyphenyl



Guaiacyl

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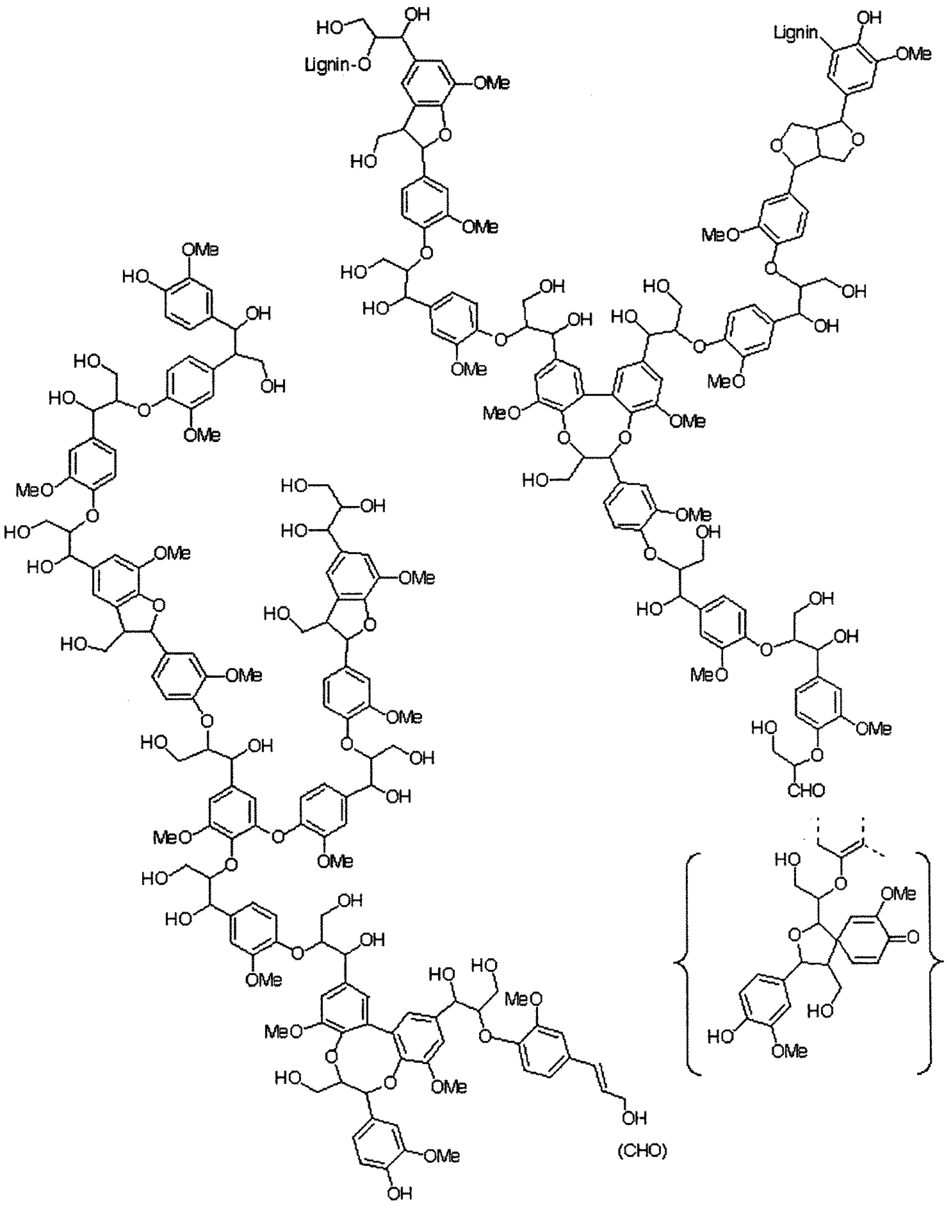

FIGURE 1. Typical molecular structure of gymnosperm lignin.

extent but still important, are proteins and pigments e.g. chlorophyll (Figure 2), and also tannins and alkadiene polymers. These materials may be transposed by microorganisms and the detritus from microorganisms should not be underestimated; they too have to die.

Once on the ground two things can happen to the leaf. It may be transformed by oxidation or by reduction. These are the great overriding chemical reactions on the planet primarily brought about by the two redox half couples:

$$1/2\ O_2 + 2H^+ \text{ -------> } H_2O + 2e \quad \text{and} \quad 2H^+ + 2e \text{ ------> } H_2$$

either directly or through biological activity.

Sometimes cyclic processes, involving both oxidation and reduction in sequence, can ensure that transformations can occur via both routes.

The limiting factor in our understanding of both oxidation and reduction processes has been the lack of methodology available for understanding the molecular structure of amorphous, non-crystalline solvent-insoluble organic solids. It has only been over the last twenty years with advances in appropriate instrumentation that progress has been made.

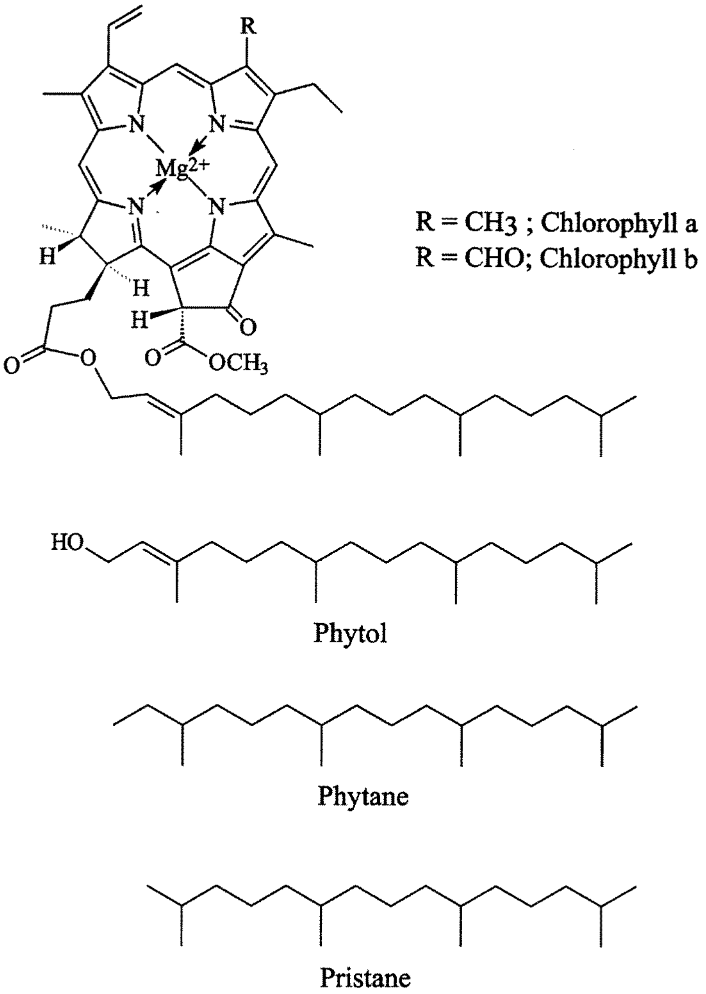

FIGURE 2. Structure of chlorophyll illustrating the phytol chain. On cleavage during reducing conditions phytane is formed but on oxidation one carbon is lost to form pristane.

Major advances have been made because of the development of three techniques. These are:

(1) the development of separation methodology for high molecular weight solids (Swift, 1989),
(2) the development of solid state high resolution nuclear magnetic resonance spectroscopy (NMR),
(3) the development of pyrolysis gas chromatography mass spectrometry (py GC/MS).

Gel permeation chromatography and dialysis have allowed the complex macromolecules that arise from the decomposition of plants to be separated so that they can be studied. They have also allowed assessment of molecular weight and size to be determined which, with structural and elemental data, has allowed constraints on molecular formulae (Sihombing *et al.*, 1996). Laser desorption mass spectrometry can also be useful to obtain molecular weights directly (Wilson *et al.*, 1992). The development of nuclear magnetic resonance spectroscopy (NMR) in geochemistry (Wilson, 1987), particularly solid state NMR (for which myself, Pat Hatcher (now at Ohio State) and Caroline Preston (Forestry Canada) can claim to have contributed) has allowed some understanding of structural group composition and the nature of transformation processes. While the detail obtained on proteins is not present in ^{1}H or ^{13}C NMR spectra of these materials it is possible to estimate structural group content including the fraction of carbon which is aromatic f_a, the fraction of carbon which is carboxylic (ester or amide have usually been hydrolysed during preparation), and the fraction of carbon which is alcoholic (primarily carbohydrate). Using selected spectral editing techniques it is possible to estimate the fraction of carbon which is aromatic and protonated $f_a^{a,H}$, and the length of the aliphatic chains. The amounts of methoxy, phenolic anchimeric carbohydrate and ketonic and aldehyde groups are also measurable. Two "typical" analyses are shown in Table 1 for base soluble fractions from the same soil. When combined with elemental data additional constraints on structure are imposed.

Table 1

Fractions of Carbon Types in Geo-Organic Matter Produced under Oxidising Conditions.

	f_{COOH}	f_a	$f_a^{a,H}$	f_{OH}	$f_{phenolic}$	f_{OMe}	f_{CH_2}	$f_{aliphatic}$
Fraction 1	0.15	0.35	0.30	0.20	0.05	0.10	0.10	0.10
Fraction 2	0.30	0.15	0.30	0.20	0.05	0.20	0.05	0.10

Pyrolysis gas chromatography mass spectrometry (Saiz-Jimenez and de Leeuw, 1986) is essential for structural studies because many plant decomposition substances have such high molecular weights that conventional GC/MS is almost useless and only gives us information on a very small amount of organic matter present. Pyrolysis is a method of determining structures that are components of the macromolecules. Structures that are of interest are those which can be recognised as being devised from plant building blocks. The furans derive from carbohydrates while guaiacyl and syringyl groups from lignin structures. By measuring the amounts of these in different molecular weight fractions sequencing information can be obtained.

Reduction Products

The loose term kerogen is used to identify products under reducing conditions (Tissot and Welte, 1984). Kerogens are further divided into three groups classified on the basis of the type of organic matter involved in transformation. Type I kerogens are mainly algal derived and are the precursors to petroleum which is formed by further geochemical processing. Type II kerogens are from lakes and rivers. Type III kerogens are known as coal (Van Krevelan, 1993).

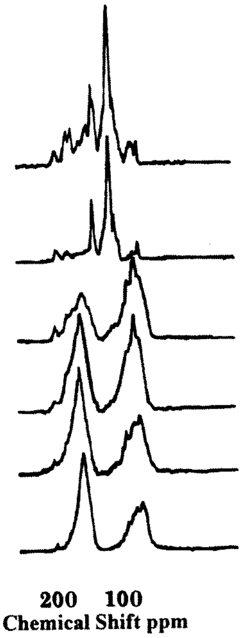

FIGURE 3. ^{13}C solid state nuclear magnetic resonance spectra of fossil woods as a function of rank. The uppermost spectrum shows carbohydrate resonances at 76 and 102 ppm or thereabouts as the largest two peaks. With increasing rank these are removed. The sub-bituminous wood (third spectrum) shows only trace amounts of carbohydrate. The lowest three spectra are from wood that is of bituminous coal rank. Note the high aliphatic carbon content centering around 30-40 ppm.

If the decomposition of organic matter is slowed so that macromolecules build up in the environment rather than being transformed to gas, then reducing reactions occur. Under reducing conditions the organic matter does not decay to carbon dioxide but forms peat, and, given sufficient time and pressure, brown and black coals. As expected, the carbohydrate carbon is first to go and this can be followed by high resolution solid state NMR (Figure 3).

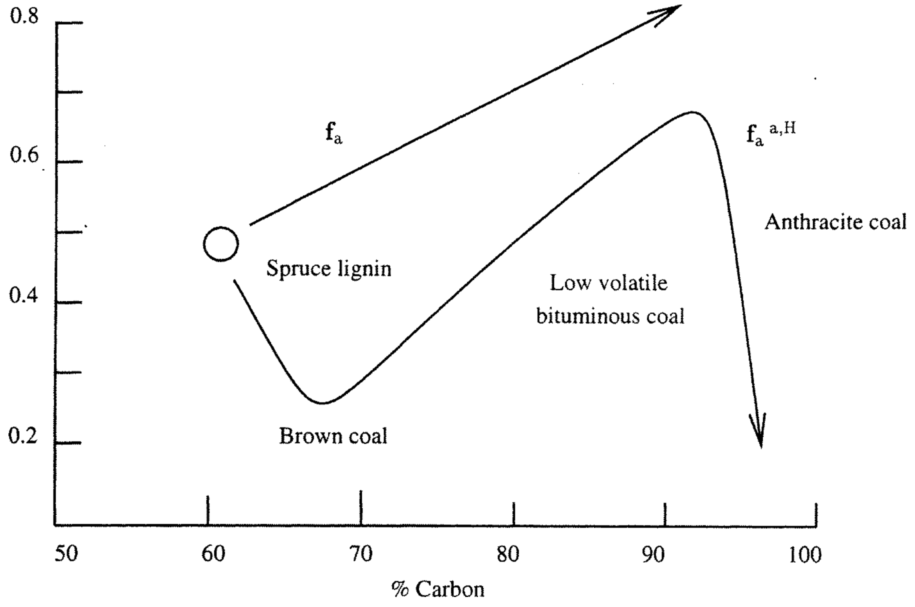

Figure 4. Diagram to illustrate the effects that occur during coalification. Aromaticity f_a increases but the ring substitution parameter f_a^{aH} first decreases and then increases before decreasing again.

The reductive transformation of carbohydrates is complex and is not understood but a strong possibility exists that furans are formed. On reduction carbon and methane alone are not formed but coals. Coals are not carbon, nor are they unmodified plant matter. During reductive transformation of lignin, bridging groups between aromatic rings are formed. On further geological transformation from brown to black coal these bridging groups are lost (Figure 4) and the aromatic rings become less substituted until the product has about 89% carbon content. On further reduction, substantial cross linking occurs to form anthracite and then graphite (Wilson *et al.*, 1984).

Oxidation Products

The loose term "humic material" is used to identify products formed under oxidising conditions. Humic materials have a number of beneficial roles in nature. They contain considerable amounts of carboxylic acid functionality, and aquatic humic materials being water soluble, have particularly high carboxylic acid contents. Some aquatic humic materials contain as much as 33% of their carbon in carboxylic acid groups (Wilson, 1987). Such structures are little more than zipped-up carbon dioxide units and it is not surprising that they are such vociferous chelating agents and can be responsible for the transport of a wide range of metal ions in soils and waters. Humic materials affect the availability of a wide range of cations and hence fertility. Their chelating role is significant in preventing run off of cations. Hence humic materials allow soils to store useful cations. (Stevenson, 1982). Their nutrient value however is restricted to slow release of nitrogen and phosphorus.

Nevertheless, humic materials are not always beneficial in industrial processing. In water they generate colour and affect taste and hence are removed during purification. The removal process can generate chloroform, which is known to be a liver carcinogen and can be present in processed waters in small amounts (Hanna *et al.*, 1991). Furthermore, because of their chelating ability any industrial process that involves surface activity can be inhibited by humic materials. Thus they are very important in alumina processing from bauxite with sodium hydroxide. During the conversion of AlO_4^- to $Al_2O_3.3H_2O$, the AlO_4^- ion is converted to octahedral coordination by polymerisation and addition of water groups. Humic materials interfere in this process by chelating across building sites, thereby inhibiting polymerisation and precipitation. They also inhibit the removal of oxalate, the main degradation product after extensive oxidation (Wilson *et al.*, 1999).

The oxidative transformation of carbohydrates results in carbon dioxide, but during this process carbohydrates are rapidly consumed by microorganisms, which on death leave much of the carbon as polymethylene, presumably derived from the microorganism cell walls. A nice way to demonstrate this (Baldock *et al.*, 1990) is by placing ^{13}C labeled glucose on a soil, and showing that the labeled carbon ends up as $(CH_2)_n$. These are also the thermal transformation products in forest fires. Carbohydrates disappear rapidly on reduction and are not present in Type I, II or III kerogens (Wilson *et al.*, 1987) (Figure 3).

Since lignin is a major component of most but not all decaying vegetation, its transformation is also important. Depending on origin, lignin (Figure 1) may contain syringyl as well as guaiacyl and parahydroxyphenyl units. During oxidative decomposition the phenolic ether linkages are believed to be broken. The resulting monohydroxy- and dihydroxyphenols are believed to be relatively stable but the trihydroxyphenols can rearrange and the aryl ring can be cleaved. The authors were most surprised to find that aliphatic components are formed in this process including aliphatic carboxylic acids (Pang *et al.*, 1990).

The eventual fate of another major plant component, proteins, in unknown. However substantial amounts of nitrogen heterocycles are found in soils and many of these do not derive from DNA or pigments (Stevenson, 1982). Of course most of the protein nitrogen can be assimilated by microorganisms as amino acids and in oxidized form as nitrates and nitrites. However in due course this nitrogen also must eventually become incorporated in complex molecules or lost to the atmosphere as NH_3, N_2O, or other gases.

Pigments are of significant interest. They yield phytol which can be transformed under reducing conditions to phytane or lose one carbon atom to form pristane in highly oxidising environments (Figure 2). Hence the pristane to phytane ratio is very useful for determining the degree of oxidation of the sediments (Didyk *et al.* 1978).

Vegetation Type

Not surprisingly humic structure depends on vegetation type. When lignin is present the product will be quite different from that formed when it is absent. Thus Antarctic humic materials derived from moss are different from those found in dense forests (Wilson *et al.*, 1986). Normally material formed under oak (angiosperm vegetation) differ from that formed under pine (gymnosperm vegetation) because the lignin differs. Thus the origin of the humic material can often be recognised from the macrovegetation on the soil. However if the turnover of microvegetation is more important than that of macrovegetation, microvegetation can be the major contribution to humic material and the lignin origin is unclear from examining the types of trees on the soil (Amalfitano *et al.*, 1995).

In the absence of other factors it is possible to draw illustrative contour plots which demonstrate the effect of climate and hence microbiological activity and input vegetation on the organic matter composition produced (Wilson, 1987) (Figure 5). In principle with increased biological activity, *O*-alkyl carbon (as in carbohydrates) is less stable than aromatic matter which in turn is less stable than aliphatic chains. As more oxidation occurs more carboxylic carbon is also formed.

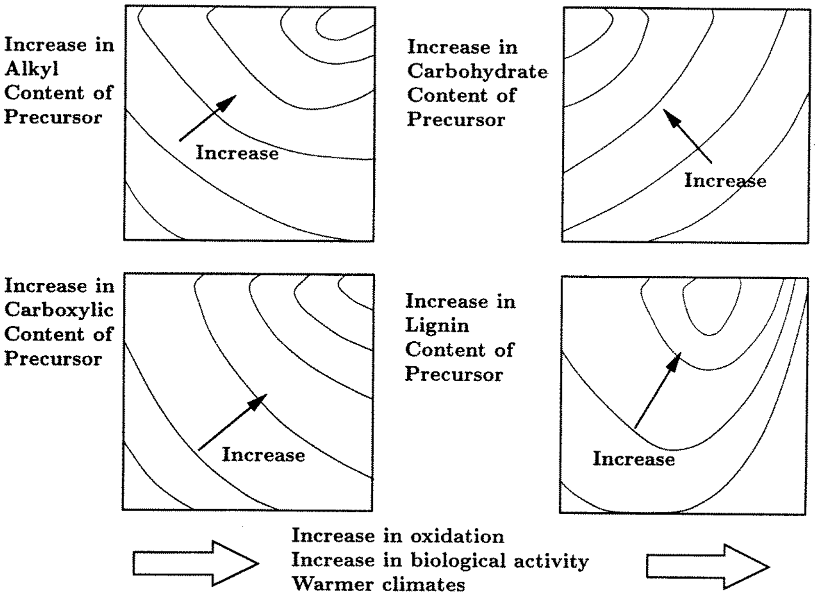

Figure 5. Diagram to illustrate the change in functional group content of humic material with functional group content of precursor vegetation and microbiological activity.

Geochemical Phenomena

Soils and sediments act via aluminosilicates and free oxides as chromatographic agents. Hence rainfall and soil composition can greatly affect the types of humic material present. In many soils organic matter is washed through the profile and behaves like eluent from a column used in chromatography. Just as in conventional chromatography, it collects on the column a distance from the initial elution point. This is normally in the B horizon of a soil, and is, in effect, a chromatographic fraction. Under rainfall some humic materials are washed into streams and rivers. That is, they are fully eluted. The process actually fractionates the organic matter.

The pH and E_h (a measure of the reduction potential) of the soil or sediment can be critical in affecting both the microbiological content as well as the potential for organic substances to be degraded or polymerised. Temperature is important here since it affects transformation rates, but temperature is most significant in fire formation. Fires are common in forests. Fire can affect the composition of humic materials by producing chars of high molecular weight polycyclic hydrocarbons (Skjemstad *et al.*, 1996).

Resynthesis

Not much is known about events immediately after decomposition. Early theories proposed that even under reducing conditions macromolecules were broken down into some sort of organic soup which was then transformed, by addition reactions, to new substances unrecognisable as original plant material. Under reducing conditions this is almost certainly now known not to be true, and it is believed many of the original macromolecules are polymerised, with and without smaller molecular weight co-reactant material. Under oxidation, the situation is much more complex; probably here initial decomposition is more important, but the degree of repolymerisation is unknown and the amount of direct polymerisation has not been established (Figure 6).

Host-Guest Structure

It is clear that a myriad of chemical compounds of different molecular weights compose the organic mixture that makes up the organic matter. Many have extremely high molecular weight. Values for some components as high as 300,000 Daltons have been recorded. It is also clear that this material is intimately bound with structures of relatively low molecular weight.

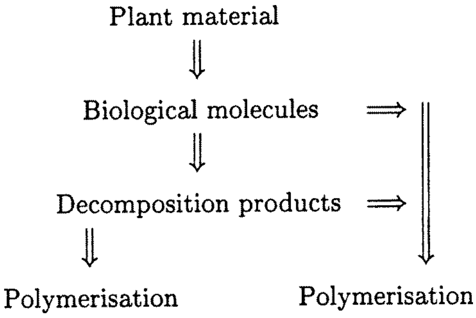

Figure 6. Polymerisation mechanisms.

Material of molecular weights of 50,000 Daltons or greater will have voids in its packing quite capable of occluding smaller molecular weight material, so that the smaller materials are in fact guests. For organic matter produced in reducing environments ^{1}H NMR evidence was proposed (Given and Marzec, 1988) to show the existence of two groups of molecules in bituminous coals with different molecular rigidities, i.e. rigid large hosts and smaller mobile guests. Evidence for a similar structure for brown coals or lignites is based on solubility and reactivity grounds as proposed by Professor Jackson and his group at Monash University (Redlich *et al.* 1985). Hence it is not surprising that we have also found similar evidence for structures produced under oxidising environments.

A good example of this evidence comes from organic matter associated with bauxite which becomes soluble in sodium hydroxide used in the process for producing alumina from bauxite. Despite attempts to remove low molecular weight organic matter by dialysis, specific molecular weight fractions are obtained which still contain low molecular weight organic matter. In differential thermal analysis studies on various dialysed high molecular weight (>25,000 Da) fractions, the loss of mass up to 200°C was attributed to loss of volatile organics as well as surface and bound water (Smeulders *et al.*, 2000). These molecules are trapped in the macromolecular matrix. This mass loss was found to be between 9.3% and 17.6%, depending on which fraction was being examined. If the amount of water and volatile organics adsorbed on a humic extract solid is calculated and compared with that calculated by summing the amount for each molecular weight fraction adjusted for mass, the numbers differ. This shows that the water and volatile organic holding capacity for the different fractions changes when the different molecular weight materials are separated. It suggests that the various humic molecular weight fractions agglomerate together in structures where some water or volatile organic binding sites on each humic substance are held by other humic species.

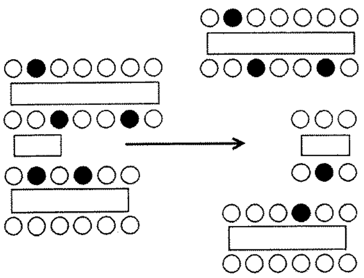

Figure 7. Model to explain the water and volatile holding capacity of humic substances before and after fractionation. Rectangles = humic substances, length equivalent to molecular weight, O = water, ● = organic volatile, The arrow represents the process of dialysis.

This is depicted in Figure 7 (left side), where both water and small organic volatiles (circles) occupy binding sites and the bound molecules can bridge macromolecules (rectangles). After separation by dialysis (Figure 7, right side) the large molecules are separated and this process generates more sites for adsorption of small molecules. Thus the water and volatile content of the unseparated material is not the same as the mass weighted sum of the water content of the separated materials. Mass loss data expressed as first derivative plots (differential thermal gravimetric analysis, (DTG, Figure 8), indicates temperatures at which rapid mass loss occurs which allows further information to be gathered. Such plots also show that secondary volatile material is present which gives superimposed inflections in DTG plots.

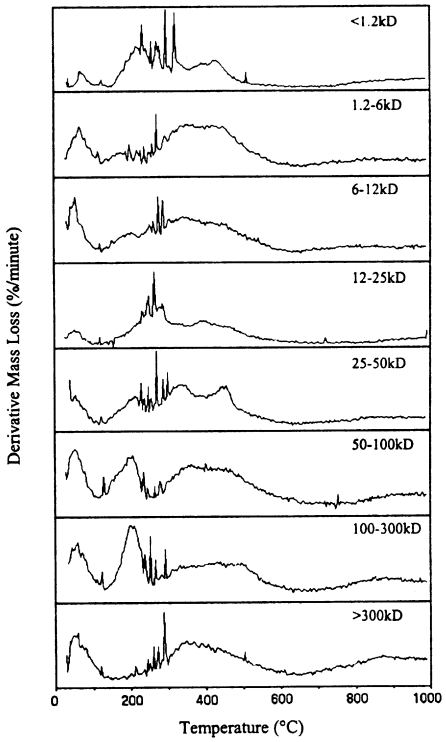

Figure 8. Differential thermal gravimetric analysis (DTG) plots of humic molecular weight fractions.

With pure compounds enthalpies can assist in identification. However, thermal events are not discrete for georganic matter since a variety of materials may be volatilising at the same time or changing phase in some other way. Since the ΔH of vaporisation of water is known (44 kJ mol^{-1}) (Aylward and Findlay, 1991) it should be possible to determine whether the thermal events at lower temperatures are due to loss of water or not.

It is difficult to obtain accurate values for endotherms for individual events but those below 250°C can be summed. Fractionated material produces two separate endotherms at 140°C and 210°C ($\Delta H = 21.69$ kJ mol^{-1} if calculated as water). This is a lot lower than that expected for water ($\Delta H = 44$ kJ mol^{-1}). Moreover a further endotherm was observed at about 250°C – 272°C (see arrow Figure 9), a temperature unlikely to be due to volatilisation of bound water, and an event, which in any case, has an inappropriate ΔH. Thus there is considerable evidence for volatile material other than water being present from enthalpy data. All events are endothermic up to 250°C as expected for evaporation and are not decarboxylation reactions as they proceed well above 250°C (Vassallo and Attalla, 1992; Leinweber and Schulten, 1992). This is additional evidence that the events are evaporations which arise from trapped species and not decompositions. However, it is shown below that most of the trapped species can only be removed by methylation.

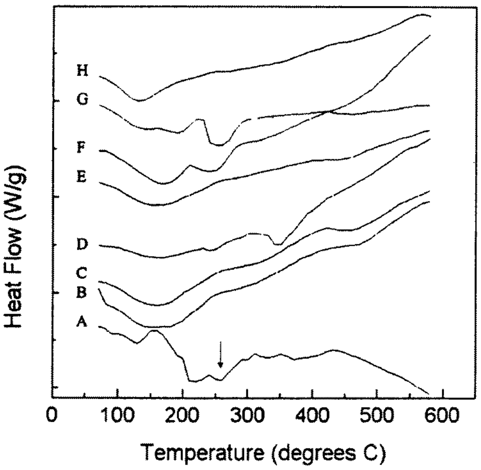

Figure 9. Modulated Differential Scanning Calorimetry plots for humic molecular weight fractions. A = <1.2 kD; B = 1.2-6 kD; C = 6-12 kD; D = 12-25 kD; E = 25-50 kD; F = 50-100 kD; G = 100-300 KD; H = >300 kD.

^{1}H and ^{13}C NMR analysis of molecular weight fractionated material is very useful in supporting this proposition. Discrete resonances should not be observed for macromolecules, yet they are seen in high molecular weight fractions. In larger molecular weight fractions these are almost all confined to the aliphatic region. The ^{1}H NMR spectrum of all fractions of molecular weight greater than 25 kD fraction showed few discrete resonances in the aromatic region. These fractions appear to form molecular aggregates with alkanes and aliphatic fatty acids. Discrete aromatic resonances were not seen for >300 kD molecular weight fractions indicating the absence of small aromatic compounds in this fraction.

Two dimensional heteronuclear multiple quantum correlation and homonuclear chemical shift correlation spectroscopy (COSY) NMR analysis (Ruiz-Cabello *et al.* 1992; von Kienlin *et al.*, 1991; Wilker *et al.*, 1993) allowed some further information to be deduced about the fractions and identified some guests. The results from heteronuclear correlation analysis show several strong couplings in the aromatic region of the spectrum. Coupling in the aromatic region was observed between the carbon at 129.50 ppm with the proton at 8.08 ppm. The ^1H and ^{13}C chemical shifts suggest the compound to be benzene-1,4-dicarboxylic acid. The ^{13}C resonance at 120.49 ppm was strongly coupled to the aromatic proton at 7.55 ppm, and the carbon at 121.39 ppm was coupled to the proton at 7.96 ppm. The ^1H and ^{13}C chemical shifts suggest the compound to be 5-methylbenzene-1,3-dicarboxylic acid.

In the COSY aliphatic spectrum coupling was observed in the aliphatic region, with the protons in the triplet at 3.20 ppm (J = 7.6 Hz) being coupled to the protons in a quintet at 2.10 ppm (J = 7.6 Hz). Integration, coupling and location of the resonances indicate that the compound contains a three-carbon alkyl chain attached to highly electronegative functional groups at each end with the two CH_2 groups adjacent to the electron withdrawing groups being equivalent. This ^1H NMR data is thus consistent with the compound 1,5-diphenylpentan-1,5-dione.

Several couplings were observed between protons in the aromatic region. The proton at 7.96 ppm is coupled to the strong resonance at 7.55 ppm (unresolved doublet, J = 1.4 Hz). A J value of 1.4 Hz suggests *meta* coupling. Hence the results indicate that the protons are located *meta* to one-another in different chemical environments on the same aromatic ring. The coupled protons located at 7.55 ppm and 7.96 ppm were the same protons observed in the ^1H–^{13}C 2-D NMR spectrum. The integration results show that there are twice as many protons at 7.55 ppm as there are at 7.96 ppm. The small coupling constant, integration results, and the chemical shifts, like the heteronuclear correlation experiment, also suggest that 5-methylbenzene-1,3-dicarboxylic acid is present in the 12-25 kD fraction.

If the fractions are methylated with tetramethylammonium hydroxide (TMAH) (Hatcher *et al.*, 1995) and analysed by GC/MS a wide variety of compounds were identified including benzenecarboxylic acids, *n*-alkanes, and fatty acids. These three families of compounds represented the majority of the compounds released by the fractions. Many of the compounds released from the Bayer organic fractions by methylation were analysed as methyl esters, particularly the fatty acids and benzenecarboxylic acids.

A typical GC/MS chromatograph of the methylated Bayer organic fractions is shown in Figure 10. The chromatogram shows the large number of compounds released from the molecular weight fractions when they are methylated. Methylation with TMAH acts to release small molecules trapped in the macromolecules by forming esters and ethers with carboxylic and phenolic groups respectively, thereby breaking the hydrogen bonding that holds the molecules in place. Without

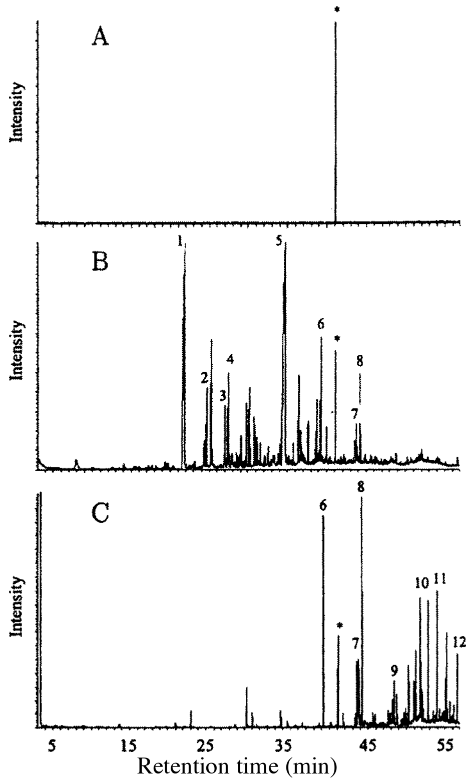

Figure 10. GC/MS spectra of molecular weight fractions (A) before methylation (B) after methylation of low molecular weight humic material (<1.2 kD) and (C) after methylation of the high molecular weight humic material (100-300 kD). The numbering of compounds is as follows: (1) 3-Methoxybenzoic acid methyl ester, (2) 1,4-Benzenedicarboxylic acid methyl ester, (3) 1,4-benzenedicarboxylic acid dimethyl ester, (4) 1,3-benzenedicarboxylic acid dimethyl ester, (5) † 4-hydroxy-2-methoxyphenyldicarboxylic acid monomethyl ester or 2,4-dihydroxyphenyldicarboxylic acid dimethyl ester, (6) hexadecenoic acid methyl ester, (7) 7-octadecenoic acid methyl ester, (8) 1-octadecenoic acid methyl ester, (9) C_{23}-Alkane, (10) C_{25}-Alkane, (11) C_{27}-Alkane, (12) C_{29}-Alkane,

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* C_{20} internal standard.

† Correction made recently by Professor Wilson to assignment (5): this peak is not due to 4-hydroxy-2-methoxy-3,5,6-trimethylbenzoic acid methyl ester as given in the original publication.

methylation the molecules are held tightly in the macromolecules matrix. When the unmethylated fractions are analysed by GC/MS no small molecules are detected (Figure 10A), only the internal standard (C_{20}) is identified. This result indicates that the small molecules are tightly hydrogen bonded in the macromolecule structures by forming molecular aggregates.

With methylation *n*-alkanes were identified in the chromatograms with carbon chain lengths ranging from C_{11} to C_{29}. *n*-Alkanes are derived from algal, microbial and higher plant sources. The distribution of the *n*-alkanes in these fractions suggests that they were derived from the waxes of higher plants.

Fatty acids were found to be one of the main chemical classes released by the methylation of the Bayer organic fractions. Fatty acids with carbon chain lengths ranging from C_7 to C_{22} were identified as products of the fractions. Identified fatty acids were found to have both monocarboxylic and dicarboxylic acids on their structures as well as unsaturations in several of the products. Numerous C_{18} isomers were identified as products from the organic fractions. Numerous substituted benzene mono- and dicarboxylic acid compounds were identified as methylation products from the Bayer organic fractions.

It is not clear whether these host-guest complexes formed by these highly oxidised humic molecules also exist in solution. The deprotonated conjugate bases, phenoxide and carboxylate would not form strong hydrogen bonds under these conditions due to repulsion forces of similarly charged species under strongly basic conditions but under neutral pH they may still hydrogen bond. In either case, during precipitation intramolecular hydrogen and intermolecular hydrogen bonding may occur. In the process large molecular voids may be formed which could occlude other molecules. It may well be true that some of these occluded molecules also hydrogen bond but the presence of alkanes shows that for some molecules this is not always the case.

These host-guest interactions may occur in a variety of humic macromolecule compounds in the environment. It is also possible that the formation of these host-guest interactions through hydrogen bonding or physical entrapment trap other molecules including organic pollutants. While chelation of metal ions is important this may be a mechanism of breaking hydrogen bonding intra- or intermolecular interactions which create voids or indeed a mechanism of forming other voids. Thus chelation may release guests or entrap others. The destruction of host-guest complexes might be expected to be achieved more readily than necessary for the breaking of covalent bonds. Indeed it has been demonstrated that UV radiation can degrade humic substances (Scmitt-Kopplin *et al.*, 1998; Ong and Bisque, 1968; Havel *et al.*,1998; Fetsch *et al.*, 1998; Frimmel 1998) rather than polymerise them. This reaction could be through oxidation of phenols to quinones, breaking hydrogen bonding and then subsequent release of small molecular weight guests.

Suggestions for Future Research

Isotope ratio gas chromatography mass spectroscopy can lead to important information of the source of geo-organic compounds. A single compound can have a different isotope ratio depending on source or formation mechanism. Solid state 2 and 3 dimensional NMR will enlighten us further on coal and humic structure. New separation methodology is urgently needed.

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