STRUCTURAL ANALYSIS
OF HUMIC MATERIALS

A THESIS PRESENTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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SUMMARY

STRUCTURAL ANALYSIS OF HUMIC MATERIALS

A thesis presented by Abdullah Ali Al-Ahmadi for the degree of Doctor of Philosophy in Aston University, Birmingham.

A classical method for structure determination in complex molecules is to degrade the molecule into smaller recognisable fragments. This approach has been applied in the past to humic/coal type analysis but frequently using such powerful reagents that there has been some doubt about the relationship of the very simple degradation products to the original materials. The evidence so far available indicates that in humic acids the units are held together not only by covalent bonding but also by a substantial network of hydrogen bonds, it is thought that it is these secondary interactions which are mainly responsible for the intractability and insolubility of these materials. The purpose of this investigation was to develop milder and more specific methods for the structural degradation of humic acids. The main thrust of the work was towards minimising hydrogen bonding interactions. The particular approaches used were:

(i) Protection of -OH groups. This is a common method for removal of hydrogen bonding interactions. However in these heterogeneous systems it has not been possible so far to obtain quantitative protection. We tried to achieve this by using phase transfer procedures.

(ii) Removal of -OH and >C=O functions. The removal of -OH and >C=O functions not only reduces hydrogen bonding but also the number of active sites. The C-H groups adjacent to >C=O are highly susceptible to oxidative attack and subsequently C-C cleavage occurs at these sites. If -OH and >C=O groups are removed the number of sites for oxidative attack is reduced and the selectivity of the oxidation process increased. Substantial progress was made in converting humic acids to their hydrocarbon-like backbone structure lacking -OH and >C=O groups.

Using a combination of the above methods produces materials sufficiently soluble in organic solvents for analysis by nmr and sufficiently volatile for study in the gc/ms system.

KEY WORDS: Humic acid, phase transfer, reduction, tosylation, methylation.

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INTRODUCTION

1. INTRODUCTION

Humic compounds are among the most widely occurring products on the earth's surface and in the marine environment. They occur in soils, lakes, rivers and in the oceans. In the marine environment, organic carbon averages about 1 mg C/1. Up to date, only 10% of the total dissolved organic carbon has been identified in surface and subsurface waters, leaving 90% termed as "humic compounds"(1). Although much has been learned about the nature and origin of humic compounds and their alteration products, little is known about their detailed molecular structures, properties or the exact biochemical and chemical reactions whereby they were formed. The complexity of structure, non uniformity and general intractability makes the study of these compounds exceedingly difficult.

1.1 General Properties of Humic Compounds

The natural environment contains a large variety of organic compounds which can be grouped into two general categories: i) non-humic substances which include carbohydrates, proteins, peptides, amino acids, fats, waxes, cellulose and many low-molecular weight organic acids; ii) most of the organic matter in natural waters and marine sediments, as in soils, consisting of complex heterogeneous, brown or yellow acidic polymers collectively known as humic matter. They have no specific chemical and physical properties, such as melting point, refractive index, or an exact elementary composition(2).

Humic matter is a relatively stable by-product of the microbial transformations of a great variety of biochemical compounds derived from the dead cells and extra cellular products of various organisms - chiefly land plants and aquatic algae.

Humification occurs under a wide range of environmental conditions - anaerobic as well as aerobic - though the process may be especially intense under aerobic conditions. Being resistant to microbial and chemical decomposition, humic matter tends to accumulate even under aerobic conditions(3). Also in the absence of severe thermal metamorphism, humic matter tends to persist through geological times. It is thought that the more highly aliphatic oil-shale kerogens were formed mainly from algal humus, or "sapropel", whereas the more highly aromatic coals and "coaly" kerogens were of higher plant origin(4,5).

During post depositional alteration of humic matter, the aromatic components become more highly condensed, and oxygen and nitrogen bearing functional groups are split off; the aromaticity of the humus increases, and unsaturated aliphatic chains may condense with each other(6).

The different components of humic matter vary in their resistance to change, for instance, COOH groups are more labile than OH groups, and molecules that are more highly aromatic or more highly condensed are more stable than those that are less so(5). When polymerization has progressed to the point where the humic matter can no longer be dissolved in alkali, the product is called Kerogen. Deep burial leads to further changes, which may be clay mineral catalyzed or thermally promoted(7) in which aliphatic chains are split off and the kerogen or coal becomes more aromatic.

Humic acids play an important role in the formation of the organic portion of peats and humic coals. The characteristic dark-brown colour of soils, peats, and lignite coals is linked to the presence of humic acids in these substances. Humic acids are capable of combining with many elements, i.e. U, V, Ca, Mo, Ni, Cu, and concentrating them in peats and bogs(8).

The division of humic compounds is arbitrary. In early times, they were usually accepted as ethyl alcohol soluble materials which were termed as "Hymatomelanic" acids. At present, this group of compounds is looked upon as a form of humic acid which is soluble in ethyl alcohol. The classification of humic substances into individual groups is based on solubility in water, alkali, or acids. The latter classification is now used commonly. In this classification humic substances are divided into three groups(8): i) humic acids, ii) fulvic acids, iii) humins.

Humic acids are dark-coloured organic compounds that are extracted from soil, peat or sediment by a weak aqueous alkaline solution. They precipitated from the alkaline extract by mineral acids, usually HC1 or H_2SO_4. The remaining tea-coloured solution contain the organic compounds is termed as fulvic acid. Humic and fulvic acids probably have a common type of structure, but differ in the degree of condensation and the character of the side chain groups(5).

Humins are the dark-brown organic residues that remain after the alkaline extract of humic acids is separated from soil. The humins are distinguished by a high ash content and by insolubility in aqueous acids and alkalis. Humins in soils are not specific compound forms, but may be visualized as humic acids that are stably linked with the mineral parts of the soils, and had thus lost their capacity to be extracted from soils.

1.2 PHYSICAL AND CHEMICAL PROPERTIES OF HUMIC COMPOUNDS

Humic acids are high molecular weight compounds, highly polar materials usually defined as heteropolycondensates(9) or complex polyanions(10).

All humic acids possess colloidal properties. Because the humic particles are negatively charged, they absorb positive ions from the electrolytes present in the solution and form compounds called "humates". Depending on the complexity of the structure of the molecule, different humic acids differ among themselves according to the degree of dispersibility and stability to coagulation. The threshold of coagulation of humic acid is determined as the lowest concentration of ions, usually Ca^{+2} and Ba^{+2}, that is required to initiate precipitation of humic acid from an alkaline solution(8). Although alkali humates are readily soluble, those of Ca and Mg are less soluble, or dispersible, and alumino-humates and ferro-humates are insoluble or non-dispersible at the isoelectric point(11). The humic acids because of their high polarity have usually low solubility in organic solvents. This low solubility together with low vapour pressures are limiting factors in the structural analysis of these materials.

Humic acids have a distinctly high carbon content. The elemental composition of a typical humic acids and fulvic acids from peat and various soils, are shown in Table 1(8).

Table 1: A Typical Elementary Composition of Humic Compounds

Acids	%C	%H	%N	%0
Humic acids	52-62	3.0-4.5	3.5-4.5	32-39
Fulvic acids	44-48	4.0-5.5	1.5-2.5	44-48

As can be seen from Table 1, humic acids contain higher percentages of carbon and nitrogen, whereas fulvic acids differ from humic acids by their high oxygen content, i.e., they containing more OH and CO_2H and ether functional groups as compared to humic acids.

Fulvic and humic acids are probably bonded to each other by complex ester linkages which may be broken when extracted with alkali.

1.3 FORMATION OF HUMIC ACIDS

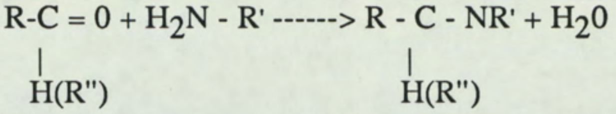
In the 1850s, much attention was given to the hypothesis concerning the origin of humic acids which suggested that humic acids are formed in soil as a result of transformation of cellulose by oxidation and dehydration. Waksman(12) showed that plant carbohydrates decompose quickly under the action of micro-organisms and hence must play an insignificant role in the formation of humus. It was concluded that the chief source of humic material, in soils, is the lignin of plant tissues and the protoplasm of soil micro-organisms, and that the nuclei of humic acids are composed of "ligno-protein complexes" formed by condensation of lignin molecules with the decomposition products of proteins. On the basis of current information, it appears that, Waksman's(12) "ligno-protein" complex may have actually been a form of humic acid or melanin formed from complex transformation products(8) derived from lignin, protein and other natural products.

The structural and functional group relationships between humic acids and lignins have been demonstrated using i.r spectroscopy(13,14).

It was suggested by Erdtman(15) that humic acids are formed by the oxidation of phenols. The phenols are first oxidised to quinones which then polymerise, oxidation of a humic acid in a weakly alkaline medium yields quinones, providing some support for this hypothesis.

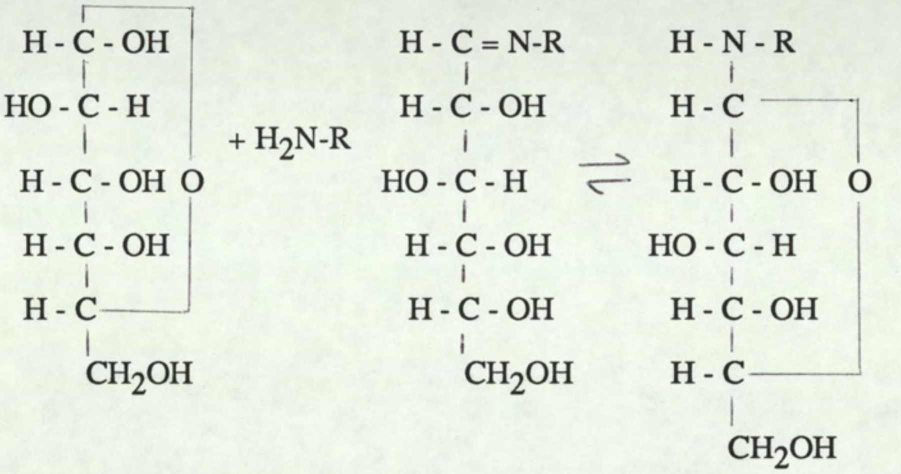
Besides C, H, O, and S compounds, nitrogen compounds are always found in considerable quantities in humic acids. Nitrogen entering into the humic acid structure is of plant and bacterial origin. Hydrolysis of humic compounds in 6N HC1 at 105^{o}C, yielded compounds that contained 40 - 80% of the total nitrogen in the humic acids. The amino acids that are found to be present in the acid hydrolyzates are: phenylalanine, leucine, isoleucine, valine, alanine, glycine, serine, glutamic acid, α - amino-n-butyric acid, γ - aminobutyric acid(16). It has also been shown that nitrogen - containing compounds enter the structure of humic acid as amino-acids and peptides(17). A significant portion of the nitrogen of the humic acid was not released on hydrolysis which would suggest that this part of the nitrogen is present in the form of complex heterocyclic structures(8). It is thought such compounds may be formed via the 'melanoidin' reduction.

It has been shown that interaction of amino-acids with reducing sugars at elevated temperatures led to the formation of dark-coloured amorphous, nitrogen - containing condensation products which were named as "melanoidins"(18). The formation of melanoidins is based on the interaction of the amine group of the amino acid with aldehyde or ketone groups of the sugar as shown in reaction (I):



$$\underset{\displaystyle H(R'')}{\overset{}{R\text{-}C}} = 0 + H_2N - R' \text{------>} \underset{\displaystyle H(R'')}{R - C} - NR' + H_20 \qquad (1)$$

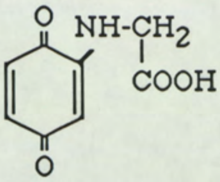
The condensation products between sugar and an amine were first reported by Schiff in 1871. The products of the reaction described by Schiff were named as "Schiff-bases". In solution, Schiff-bases are in equilibrium with their "labile" forms, N-glucosides (II).



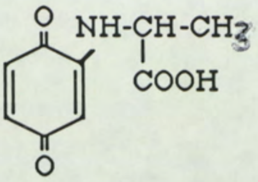
D-Glucose Schiff base N-Glucoside (II)

Schiff-bases form complexes with a series of metals. The ability to form these complexes is explained on the basis of hydroxyl (OH) and azomethine (-CH = N-) groups, and their relative positions in the molecule.

Joffe and Chavin(19) studied the reaction of amino-acids with quinone and demonstrated the formation of the corresponding amino acid aldehydes from which one carbon atom had been removed. At the same time, a condensation reaction takes place between the quinone and the amino acids. The authors isolated the condensation products III and IV.



III



IV

These studies demonstrated that aromatic compounds could participate in the melanoidin reaction and thus established that this reaction could be involved in humic acid formation.

1.4 HUMIC ACIDS AND MELANOIDINS

The melanoidins and humic acids are similar in colour, viscosity, solubility and contain similar functional groups. Characteristic properties of melanoidins and humic acids are given in Table 2.

Table 2: Comparison of Melanoidins and Humic Acids$^{(8)}$

				Functional Groups (meq/g)		
Preparation	Mol.wt.	Eq.wt.	C:N	C=O	Phenolic OH	Total OH
"Standard" melanoidin preparation	1478	245-236	10:1	2.6	6.53	9.6
Humic acid	1400	150-340	10:1	3.2-1.3	6.90	8.6

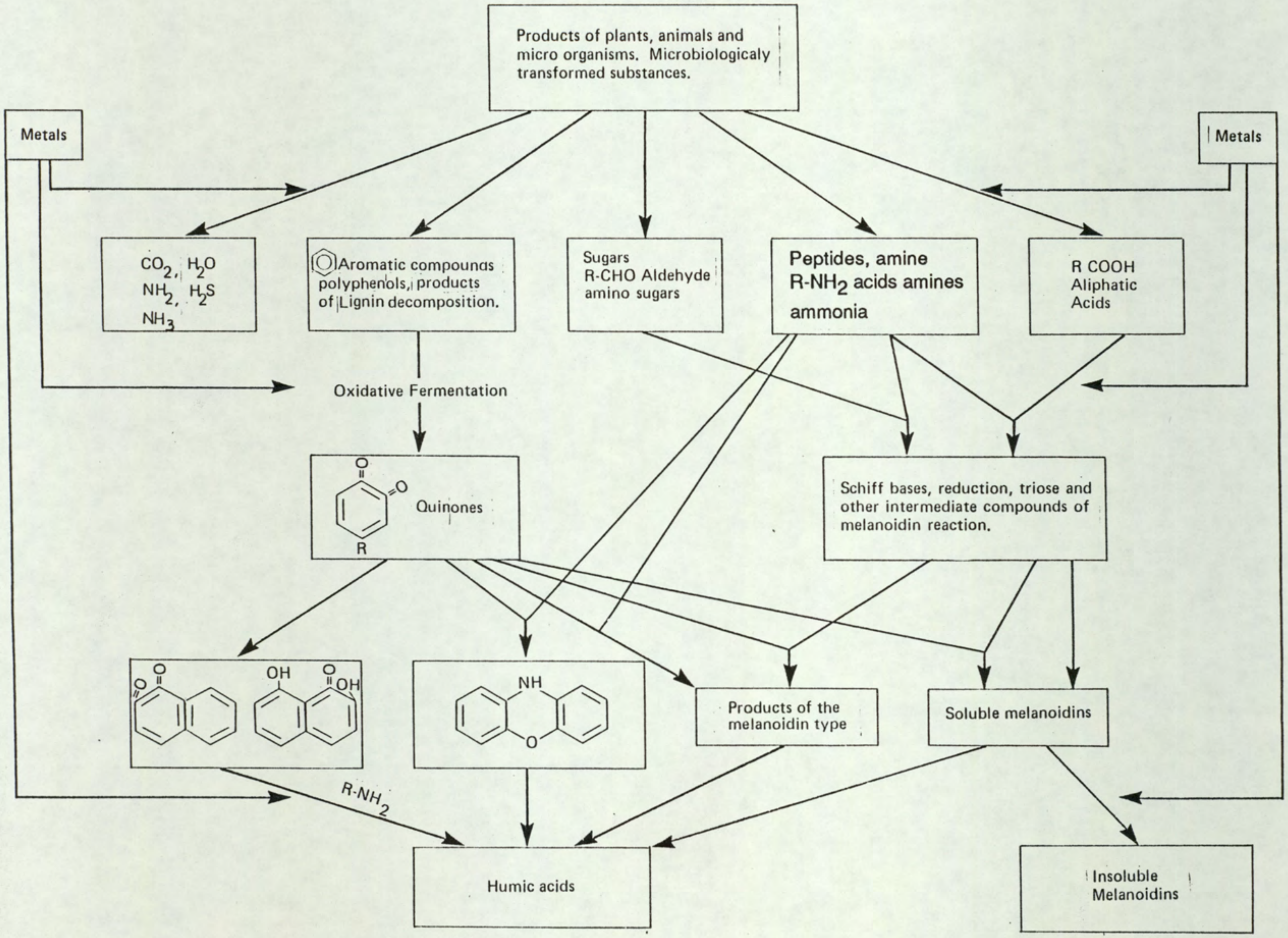
The excretion and decomposition products of bacterial cells and fungi may take part in melanoidin reactions to form dark-coloured substances which, at a later stage, participate in the formation of humic acids under natural conditions. The melanoidin reaction is probably an early and essential stage in the formation of humic acids. Reaction of methyl glyoxal with glycine led to the formation of melanoidins from which 3% fulvic acid, 70-85% hymatomelanic acid and 5-7% humic acid could be extracted(20).

On the basis of current knowledge, the route for the origin of humic acid can be presented as shown in Figure 1(8). The basic starting material of humic acid is the decomposition products of plants, animals and microorganisms. Aromatic compounds (primarily decomposition products of lignin, polyphenols etc.), nitrogen compounds (decomposition products of protein and carbohydrates), and aliphatic acids, may all participate in the formation of humic acid molecules.

It seems likely that one of the first reactions in the formation of humic acid, is the formation of quinones from the oxidation of polyphenols to quinones either biologically(21), or by inorganic species(22). Quinones react with amines and in a majority of instances these reactions can be enzymatically promoted. Reactions of quinones with amino-compounds may result in the formation of diaminobenzoquinones, of nitrogen containing heterocyclic compounds, or of melanin. The amines that react with quinones may be decomposition products of proteins or intermediates in the melanoidins reaction. Quinones may simultaneously be oxidized to form dimers and dienes which, on reaction with amines, may also provide the basic structure for the formation of humic acid molecules.

1.5 STRUCTURE OF HUMIC ACIDS

The determination of the chemical structure of humic acids has been the subject of numerous investigations. However, because of the complexity of the humic material and their lack of uniformity, they have not been completely structurally elucidated. The numerous investigations have produced only limited structural information and regardless of the approach used, the same structural information. Since it has been impossible to obtain the complete structure, many hypothetical structures have been suggested for humic materials. In 1966, Christman and Ghassemi(13) proposed a structure for the humus molecules, Figure 2. Based on the



(8)

Figure 1 Pathways of formation of Humic acids

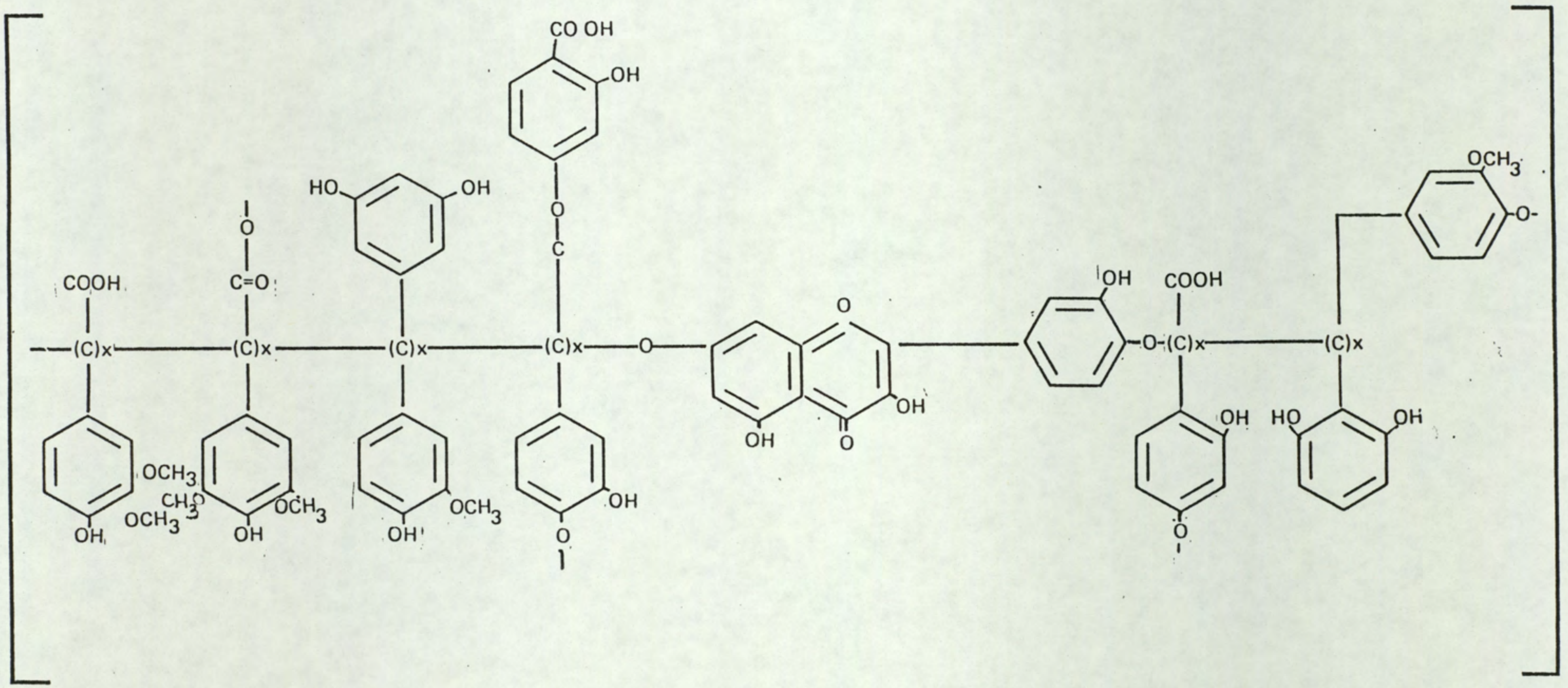


Figure 2: A Structure of the Humus Molecule Proposed by Christman and Ghassemi(13)

assumption that lignin plays an essential part in the humification processes, this suggested formula seems to be useful for theoretical purposes. More recently Haworth (24) has concluded that humic acids are made up of an aromatic 'core' to which are attached, chemically or physically, polysaccharides, proteins, phenols and metals, as indicated in Figure 3.

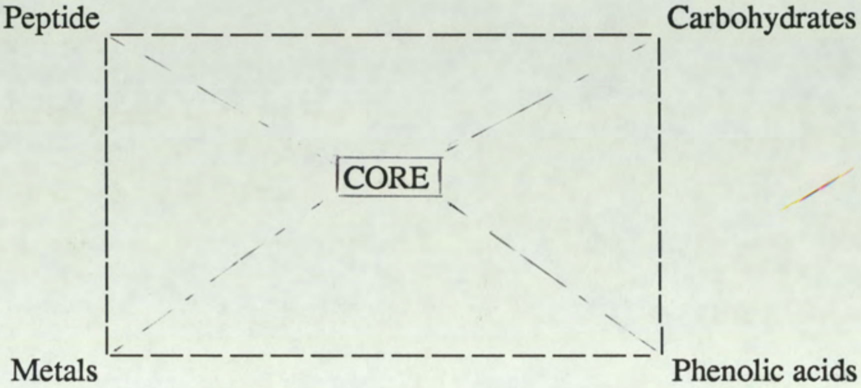


Figure 3: Diagrammatic representation of HA (Haworth, 1971)(24)

Schnitzer and his co-workers(25,26) on the basis of nondegradative studies have suggested that FA's and HA's are polymeric structures made up of hydrogen bonded phenols and benzenecarboxylic acids which are joined by hydrogen bonds as shown in Figure 4.

Very recently, nmr has provided a new dimension in the structural analysis of humic like materials, in particular solid nmr since the insolubility of humic substances has always been a major obstacle in analytical studies. Nmr has made possible detailed and non-destructive analysis of humic substances. On the basis of these nmr studies it has been concluded that humic acid units are held together not only by hydrogen bonding but also by other types of electrostatic bonding and by Van der Waals forces.

The humic acids from marine sediments and other sedimentary materials

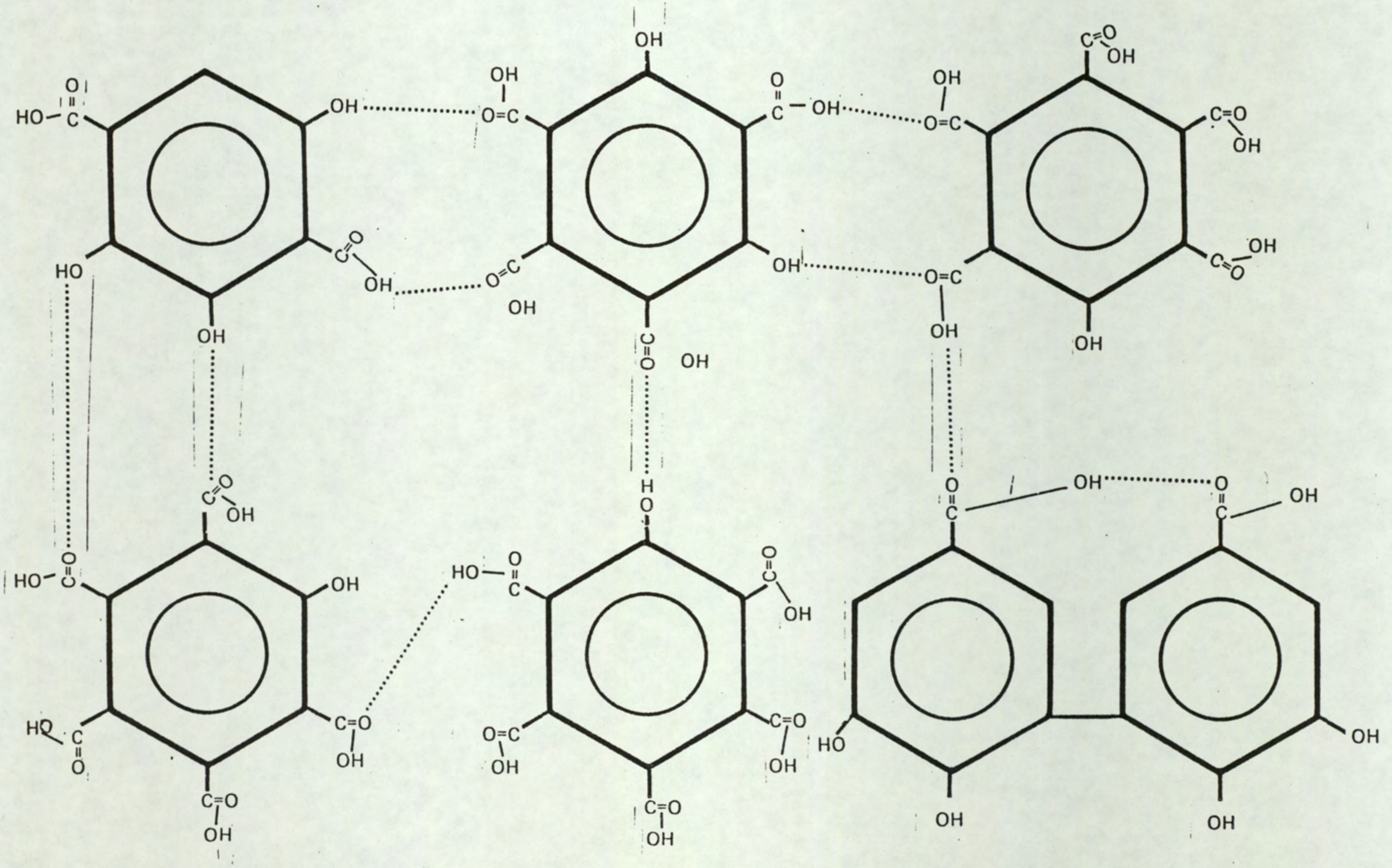
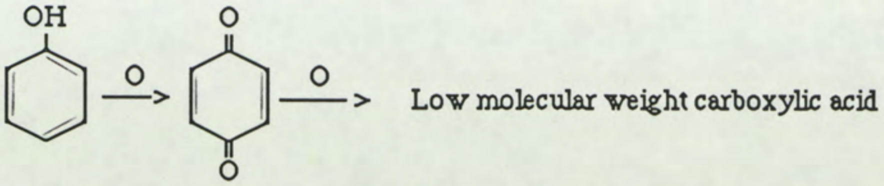
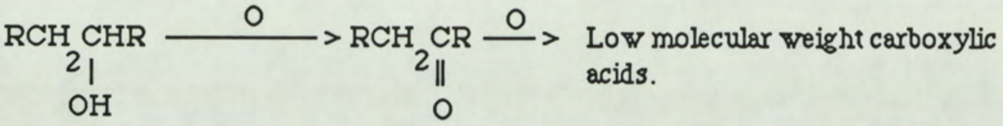


Figure 4: Structure of FA as Proposed by Schnitzer(25,26)

such as soil, peats, and sapropels seem to be made up of several mechanically mixed structural components. One component which is presumed to be derived from aquatic plants is a highly branched, highly cross-linked, mainly aliphatic polymer, which has associated with it carbonyl, carbohydrate, ether and nitrogen functional systems. Another component is a predominantly aromatic polymer, this is the major component in terrestrial systems and is presumably derived from lignin, Nmr spectra indicate that the aromatic polymers derived from soils contain greater numbers of carbonyl functions than are measureable by wet chemical methods and fewer phenolic functions than expected from degradation studies. It would appear that many phenolic functions are introduced in oxidative degradation and are not present in the original structures. The aromatic polymers derived from peats are more highly substituted with phenolic groups and their structures resemble more closely the lignin structure. Aquatic sedimentary humic acids tend to be highly aliphatic structures. A carboxyl group on every third carbon atom has been suggested for these materials (27). The terrestrial humic acids, although they contain paraffinic components, tend to be made up mainly of aromatic units. Whatever their origin and whether predominantly aliphatic or aromatic, all humic acids are highly branched, cross-linked and highly substituted with hydroxyl, carbonyl, ether and nitrogen functional groups. In consequence, these systems are strongly hydrogen bonded and it is thought that it is these secondary interactions which are for a large part responsible for the insolubility in organic solvents and general intractability of humic acids. It is found that when hydrogen bonding is reduced by methylation (28), solubility increases and better resolved, less diffuse spectra can be obtained from the soluble material. It is also advantageous to methylate and remove active hydrogens prior to oxidative degradation. Phenols and alcohols are readily oxidised to >C = O functions and subsequent cleavage of C-C bonds occurs adjacent to the >C = O groups.





$$RCH_2CH(OH)R \xrightarrow{O} RCH_2C(=O)R \xrightarrow{O} \text{Low molecular weight carboxylic acids.}$$

The corresponding methyl ethers do not undergo the initial oxidation to >C = O and, in consequence, oxidation of the methylated derivatives should be more specific and give products more representative of the original structure. Considering the beneficial effects methylation confers on the properties of humic acids, we thought even better results might be achieved by complete removal of hydroxyl and carbonyl functional groups. Their removal would result in a reduction of not only hydrogen bonding interactions, but also dipolar interactions arising from carbonyl functions. In consequence, one might expect the defunctionalised materials to be even more soluble than their methylated counterparts, to give well resolved i.r. and nmr spectra due to their increased carbon content and reduced polarity and to undergo relatively specific oxidative breakdown. In the absence of carbonyl groups and carbonyl producing functions, oxidation should occur mainly at benzylic carbon sites. The main purpose of this investigation was to find relatively mild and specific routes for the removal of hydroxyl and carbonyl functions from humic acids and to obtain if possible defunctionalised hydrocarbon humic backbone structure. We studied in some detail the application of phase transfer catalysts to permit or accelerate heterogeneous reactions and the technique is particularly applicable to the reactions of humic materials which are so frequently heterogeneous in character. It is probably the carboxyl groups which are largely responsible for the strong hydrogen bonding in humic acid.

However, although routes for the removal of carboxyl functions were investigated, we concluded that direct removal of carboxyl functions would not be the method of choice if one wished to preserve a relatively unaltered humic backbone structure. In general, the conditions required for decarboxylation are so vigours that they are likely to produce substantial structural alterations. We have found that the hydroxyl and carbonyl contents of humic acids can be considerably reduced under relatively mild conditions by following the route shown in Figure 5 and discussed below:

(i) $LiAlH_4$ Reduction

Spectral evidence indicates that humic acids are not reduced by $LiAlH_4$ using the usual conditions for reduction of carboxyl functions(29). We have, however, found particular conditions under which carbonyl groups can be completely removed from humic acids.

(ii) Tosylation

Humic acids do not tosylate to any great extent using standard tosylation conditions viz pyridine or aqueous base plus tosyl chloride. If the reaction is carried out under phase transfer conditions, almost quantitative tosylation occurs and the i.r. spectra of the tosylated products show only very weak -OH bands.

(iii) $LiAlH_4$ Reduction of the Tosylated Products

The tosyl groups can be completely removed under the same conditions used for the carbonyl reduction. Reduction of alkyl and benzyl tosylates gives hydrocarbon products:-

$$RCH_2OTS + LiAlH_4 \longrightarrow R-CH_3$$

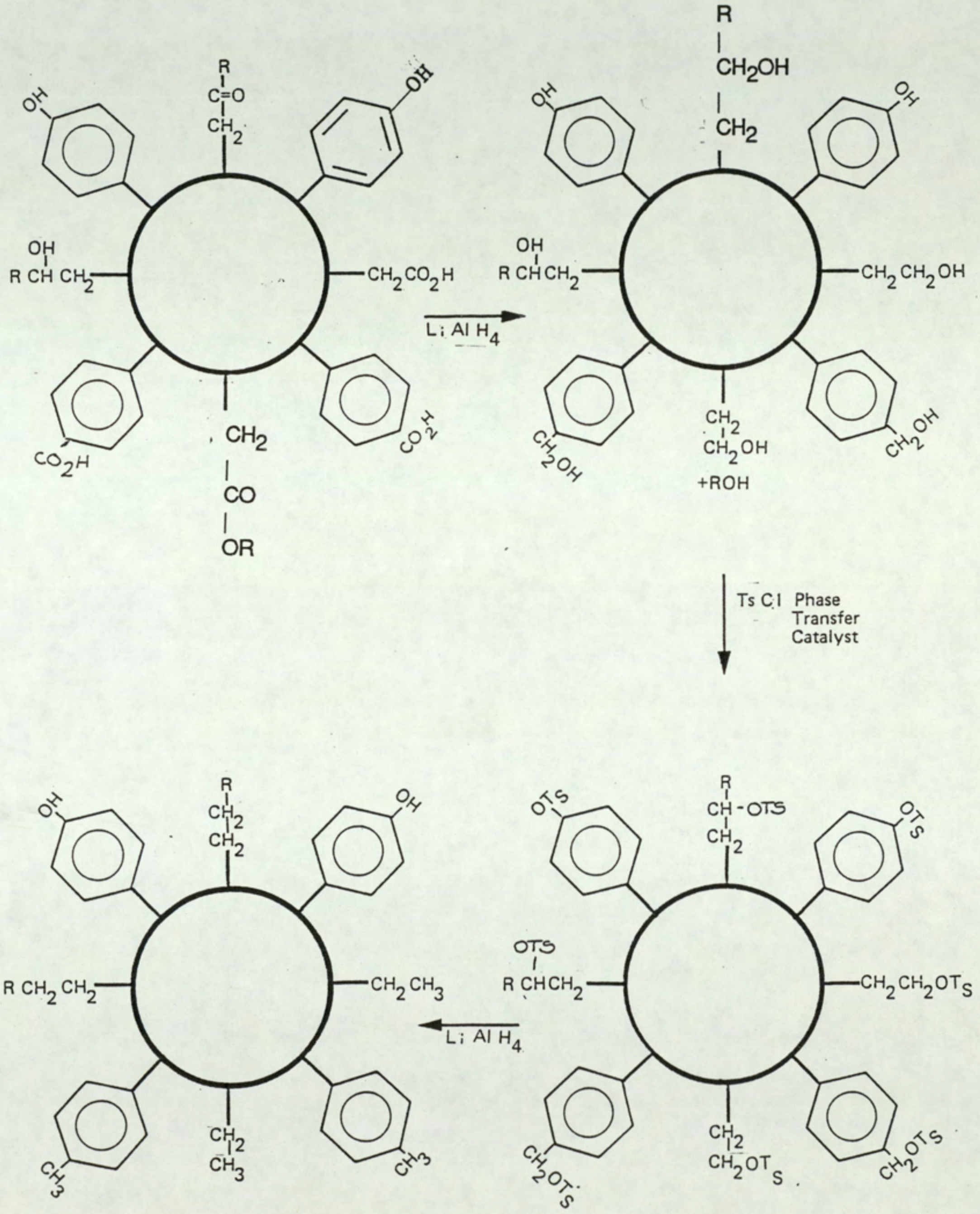


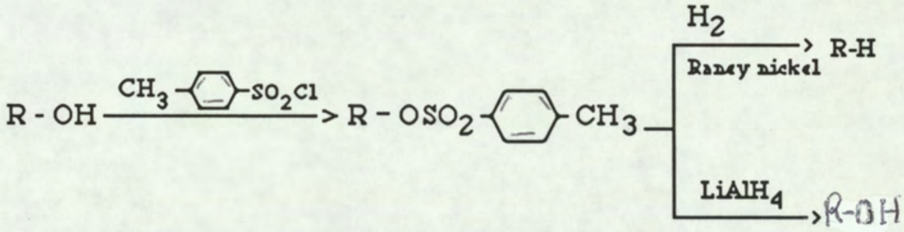
Figure 5 Defunctionalisation Scheme

whereas reduction of aromatic tosylates gives phenols:-

$$Ar - OTS + LiAlH_4 \longrightarrow Ar - OH$$

The reactions discussed above were applied to five humic acids. The first two, Aldrich and Fluka, are available commercially from Aldrich and Fluka and are obtained from peat. Two of the humic acids Norfolk and Reading were obtained from soil and the Lignite humic acid was obtained from brown coal. We have found that the final reduced products show a much narrower and weaker -OH band in the i.r. compared with the original humic acids, which is to be expected since only one type of -OH i.e., phenolic -OH, should now be contributing to the band. Acetylation confirms that the -OH content has been reduced by on average $\approx$70%.

Investigations were also carried out to try and find methods for the removal of phenolic -OH groups from humic acids. There are a number of well authenticated methods$^{(30 - 33)}$ for the removal of phenolic -OH from relatively simple molecules, but of the methods we investigated, tosylation followed by hydrogenation using Raney nickel catalyst.



The formation of the ester salt followed by hydrogenation again with Raney nickel cataylst

$$Ar - OH \xrightarrow{PhNR_2SO_3} ArO.SO_3^- \; HN^+R_2Ph \xrightarrow{KOH\ aq}$$

$$ArO.SO_3^- \; K^+ \xrightarrow[KOH\ aq]{Raney\ nickel} ArH + K_2SO4$$

and the formation of the phosphate ester followed by reduction:

$$\begin{tikzcd}
\mathrm{Ar - OH + Cl - P\,(OEt)_3} \arrow[r, dashed] & \mathrm{Ar - OP\,(OEt)_3} \arrow[d, "H_2"] \\
 & \mathrm{Ar - H}
\end{tikzcd}$$

were unsuccessful when applied to humic systems. Methylation of the five humic acids, using a phase transfer technique was also studied essentially to evaluate the use of phase transfer methods in the alkylation of humic materials.

The products and intermediates from the above reactions were examined using a variety of chromatographic and spectroscopic techniques including gc/ms, i.r. and nmr methods.

The results of these investigations are discussed in detail in the following chapters.

CHAPTER 2

ANALYTICAL METHODS

2.1 INTRODUCTION

Over the past thirty years there has been an ever increasing use of physical methods for characterization of humic substances. Spectroscopic methods, electrometric titration and molecular weight measurements have provided valuable information on the nature and properties of humic substances of widely different origin. Viscosity and electron microscopic measurements have thrown light on the shapes of HA and FA molecules, while radio carbon dating has been used as an index of the ability of humic material to resist decomposition. Thermal methods have been used in structural investigations on humic substances(34). Thermogravimetry (TG), Differential Thermogravimetry (DTG), Differential Thermal Analysis (DTA), and Isothermal heating have been employed to uncover the kinetics and mechanism of the thermal decomposition of humic substances. Schnitzer and Hoffman(35) have shown that the main reactions governing the pyrolysis of humic and fulvic acids are:

(a) dehydrogenation (up to 200°C);

(b) a combination of decarboxylation and dehydration (between 200 and 250°C); and

(c) dehydration at temperatures >250°C.

These methods are useful for the characterization of different humic fractions, for studying reactions of humic substances with clay minerals, metal ions, and hydrous oxides, for indicating generic relationships between humic substances, lignin, coal, etc., and for measuring the aromaticity of humic substances.

In this particular subject we have made much use of two spectroscopic techniques:- i.r. and nmr. In the analysis of the starting materials and products we have also made some use of gc/ms, but to a lesser extent than the other two. Spectroscopic methods have a number of attractive features for the analysis of humic substances:

(a) They are non-destructive

(b) only small sample weights are needed;

(c) they are experimentally simple and do not require special manipulative skills, and

(d) they often provide valuable information on molecular structure and on chemical interaction.

2.2 INFRA RED (i.r.) SPECTROPHOTOMETRY

At first sight this appears to be the method of choice for the structural analysis of humic substances, since i.r measurements are usually carried out on solid samples, but as will be seen from figure 6, using conventional i.r. spectrometers, humic materials generally give very poorly resolved spectra from which only limited structural and functional group information can be obtained. It is a general observation that materials containing large numbers of oxygen functions and which are strongly hydrogen bonded give poorly resolved spectra. However, using modern i.r. spectrometers (Perkin Elmer 559B spectrometer fitted with a 3600 Perkin Elmer data station) with a computer system it is now possible to obtain much improved spectra of humic material (see figure 7). Noise and sloping base line are among the many factors contributing to the poor quality of humic spectra. Programmes are available for the flattening of the base line and reduction of noise. It is also possible using the computer to run repeatable spectra and obtain an average spectrum. Using these various devices much improved spectra are obtained as shown in figure 7.

The i.r. region of the electromagnetic spectrum can yield valuable information on the structure of organic molecules. The masses of the atoms, and the forces holding them together, are of such magnitude that the usual vibrations of organic molecules interact with electromagnetic energy to absorb and radiate in the region(36). For example, all compounds containing the carbonyl group absorb

Figure 6: I.r. Spectrum of Humic Acid using Conventional i.r. Spectrophotometer (compare with Figure 7)

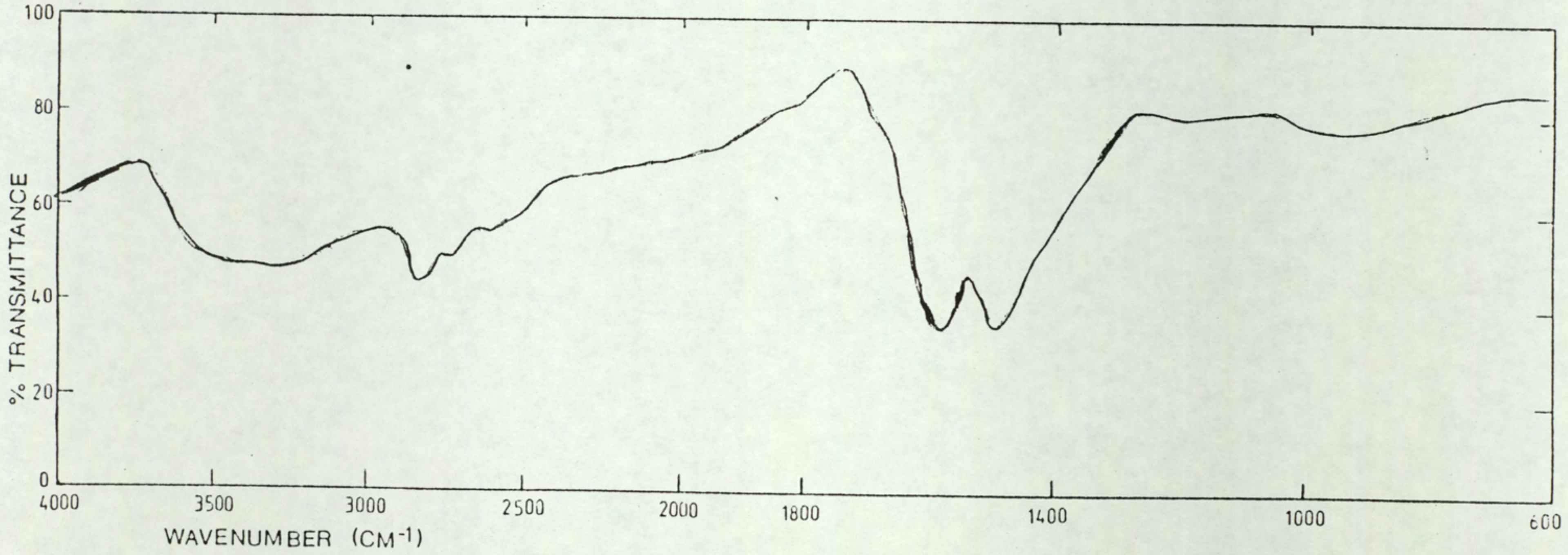
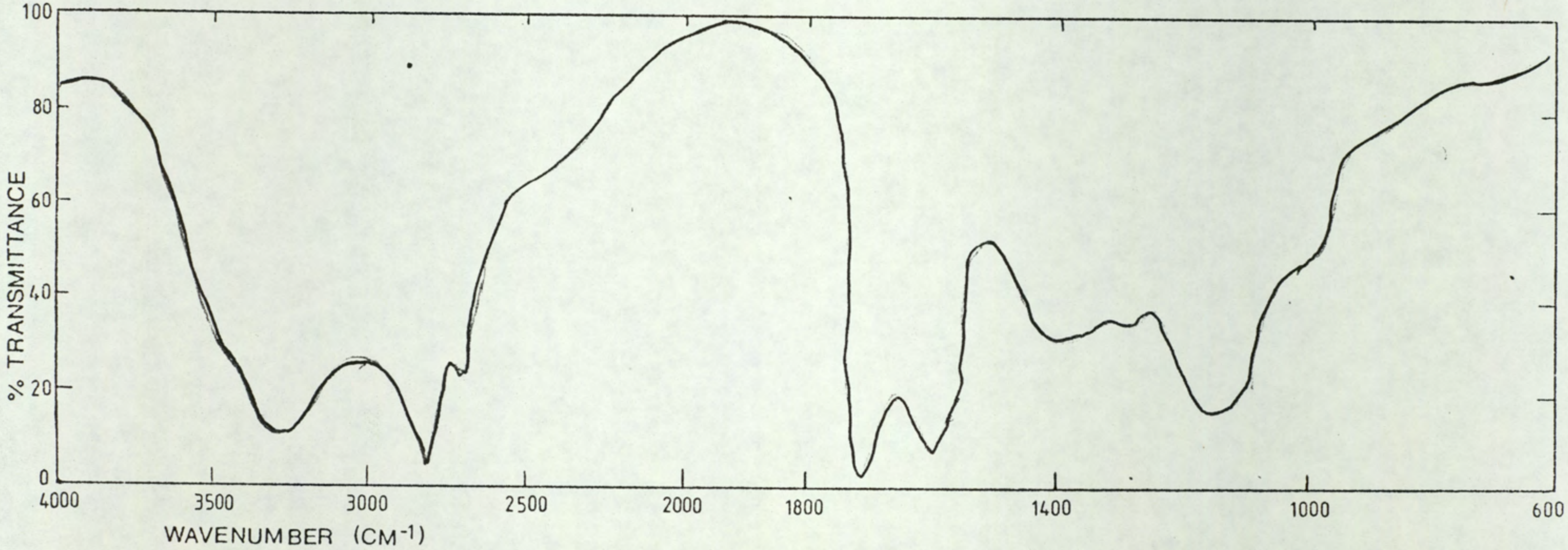


Figure 7: I.r. Spectrum of Humic Acid using Perkin Elmer Spectrophotometer



strongly between 1800 and 1650cm^{-1}, absorption near 1600 and 1400cm^{-1} may indicate the presence of the carboxylate anion. Thus, the position and intensity of an absorption band can be used to confirm the presence of a particular group and to obtain information on its molecular environment. Conversely, the absence of strong group absorption is often indicative of the absence of that group in the molecule, provided that no other effects operate which could shift the band to other regions of the spectrum. The i.r. spectrum provides a physical constant for a particular compound, which is as characteristic as its melting point, refractive index, etc. Infrared spectrophotometry has been found useful for the gross characterization of humic material of diverse origins, for the evaluation of the effects of different chemical extractants (37-39), of chemical modifications such as methylation(40,41), acetylation(37), esterification, and saponification, and for the formation of derivatives(42). It has also been used to detect changes in the chemical structure of soil humic compounds following oxidation, pyrolyses and similar treatments on metal-humate complexes(43-45) and to indicate possible interactions of pesticides and herbicides(46) with humic substances. In the case of humic compounds the assignment of absorption bands to certain groups with the aid of correlation charts is still fraught with considerable uncertainty. It is therefore sound practice to corroborate spectral data with information obtained by other methods.

2.2.1 Preparation of Samples

Most workers in the humic acid chemistry field use the disc technique, which involves mixing a finely ground sample with a suitable matrix material such as KBr, NaCl, KCl or CsBr, and pressing the mixture into a transparent disc. KBr has been found to be the best matrix material down to 400cm^{-1}(47). The matrix material should be pure and dry, but it may pick up traces of moisture even in an air-conditioned laboratory, and therefore special care should be taken in interpreting disc spectra in the OH stretching region. It is advisable to run a blank spectrum of a disc consisting of the matrix material only, each time i.r. spectra are recorded. Theng et al.(48), have drawn attention to the effect of moisture on bands in the 3,300 - 3,000 and 1,750 - 1,500cm^{-1} regions, but these effects should not be exaggerated.

Humic substances contain a relatively high concentration of OH groups and these absorb strongly in the 3,400 - 3,200cm^{-1} region.

2.2.2 Infrared Spectra of Humic Acids

The main absorption bands are; 3,400cm^{-1} hydrogen bonded OH, 2,900cm^{-1} aliphatic C-H stretch, 1,725cm^{-1} C=O of CO_2H, C = O stretch of ketonic carbonyl, 1,630 cm^{-1} aromatic C = C hydrogen bonded C = O of carbonyl , double bond conjugated with carbonyl, COO^-, 1,450cm^{-1} aliphatic C - H, 1,400cm^{-1} COO, aliphatic C-H, 1,200 cm^{-1} C-O stretch or OH deformation of CO_2H, 1,050cm^{-1} Si-O silicate impurity.

In general, spectra of humic acids of diverse origins are very similar, which may indicate the presence of essentially similar chemical structures. Even the much improved spectra did not reveal sufficient detail for us to make useful distinctions between humic materials.

2.3 NUCLEAR MAGNETIC RESONANCE

Nuclear magnetic resonance (nmr) spectroscopy is one of the most valuable spectroscopic techniques available to organic chemists. It is the method of structure analysis determination to which chemists first turn for information. Mass spectroscopy provides information about the molecular weight and formula of a molecule of unknown structure, and infrared spectroscopy provides information about the kinds of functional groups in the unknown. Nuclear magnetic resonance spectroscopy does not replace or duplicate either of these techniques; rather, it complements them. Nmr spectroscopy provides a 'map' of the carbon-hydrogen framework of an organic molecule. Taken together, the three techniques often allow us to obtain complete solutions for the structures of even rather complex unknowns.

When certain nuclei such as ^{1}H and ^{13}C are placed in strong magnetic fields their spins orient either with or against the applied field. On irradiation with radiofrequency (rf) waves, energy is absorbed and the nuclear spins "flip" from the lower energy state to the higher energy state. This absorption of rf energy is detected, amplified, and displayed as a nuclear magnetic resonance (NMR) spectrum.

The nmr spectrum is obtained by irradiating a sample with a constant frequency rf energy and slowly changing the value of the applied magnetic field. Different kinds of ^{1}H and ^{13}C nuclei come into resonance at slightly different applied fields, and we therefore see a different absorption line for each different kind of ^{1}H and ^{13}C. The nmr chart is calibrated in delta (δ) units, where $1\delta = 1$ part per million (ppm) of spectrometer frequency. For example, a ^{1}H spectrometer operating at 60 MHz frequency would have $1\delta = 60$ Hz. Tetramethylsilane (TMS) shows both ^{1}H and ^{13}C absorptions at unusually high values of the applied magnetic field and is therefore used as a reference sample to which all other peaks are compared. The TMS absorption occurs at the right hand (upfield) edge of the chart and is arbitrarily assigned a value of 0 δ. All other nmr absorption normally occurs downfield (left) of the TMS reference line.

Both ^{1}H and ^{13}C nmr spectra display four general features:

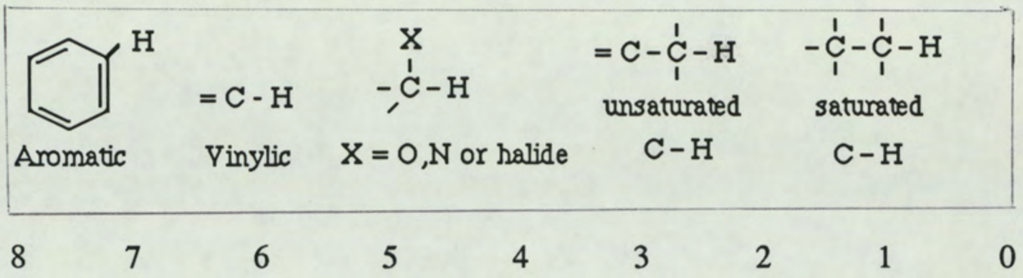
1. Number of resonance lines. Each nonequivalent kind of ^{1}H or ^{13}C nucleus in a molecule can give rise to a different resonance line.
2. Chemical shift. The exact position of each peak is the chemical shift of the nucleus. Chemical shifts are due to the effects of electrons in the molecule.

3. Integration. The area under each nmr absorption peak can be electronically integrated so that we can determine the relative number of nuclei (^1H or ^{13}C) responsible for each peak.
4. Spin-spin splitting. Neighbouring nuclear spins can couple, splitting nmr peaks into multiplets. The nmr signal of a ^{13}C nucleus bonded to n-protons splits into n + 1 peaks. Similarly, the nmr signal of a ^1H nucleus neighboured by equivalent adjacent protons splits into n + 1 peaks.

Because of electronic constraints inherent in ^{13}C nmr spectrometers, most spectra are run in the proton noise - decoupled mode. Operating in this manner provides maximum sensitivity and gives a spectrum in which each non equivalent carbon shows a single unsplit resonance line. Carbons that are sp^3 hybridized absorb in the upfield region from 0 to 100 δ; sp^2 hybridized carbons absorb from 100 to 200 d. Operating in the gated decoupled mode causes a loss of sensitivity, but provides a spectrum that can be electronically integrated to measure the number of carbon nuclei responsible for each peak. Operating in the off resonance mode also causes a loss of sensitivity, but provides a spectrum in which spin-spin splitting is observed. Each carbon resonance is split into a multiplet depending on the number of protons to which it is bonded: quaternary carbon resonances remain as singlets, tertiary carbons (R_3CH) appear as doublets, secondary carbons (R_2CH_2) appear as triplets, and primary carbons (RCH_3) appear as quartets.

Proton nmr spectra are even more useful than ^{13}C spectra. The sensitivity of ^1H instruments is high, and normal spectrometer operating conditions provide spectra that show spin-spin splitting and can be integrated. Proton resonance usually falls into the range 0 - 10 δ downfield from the TMS reference point, and the exact chemical shift of an absorption indicates the chemical environment of the nucleus responsible for that signal.

The great majority of ^{1}H nmr absorptions occur from 0 to 8 δ, and this range can be conveniently divided into five regions as shown in Table 3.



<u>Chemical Shift (δ)</u>

A specific resonance peak is often split into a multiplet due to spin-spin coupling with the spins of protons on adjacent carbons. The spins of equivalent protons do not couple with each other, but a proton with n-equivalent neighbouring protons gives a signal that is split into n + 1 peaks with coupling constant J.

Nmr studies were until recently carried out on solutions. Over the past ten years or so there has been a development of instrumentation and techniques for the examination of solid samples by nmr.

2.3.1 Nmr of Humic Acids

Nmr has been used intensively as an analytical tool for the structural elucidation of humic acids. However, poor resolution, low sensitivity and, most important, the need for complete solubility have limited the usefulness of this technique and early attempts to obtain usable spectra were disappointing. The development of nmr spectrometers with higher resolution has now made it possible to obtain useful ^{1}H and ^{13}C spectra for humic acids in both the solid and solution states.

The ^{13}C nmr spectra of Fluka, Aldrich and Lignite humic acids dissolved in D_20/OD^- are shown in figures 8,9,10.

The ^{1}H nmr spectra of Aldrich and Fluka humic acids dissolved in

Figure 8: ^{13}C nmr Spectrum of Humic Acid (Fluka)

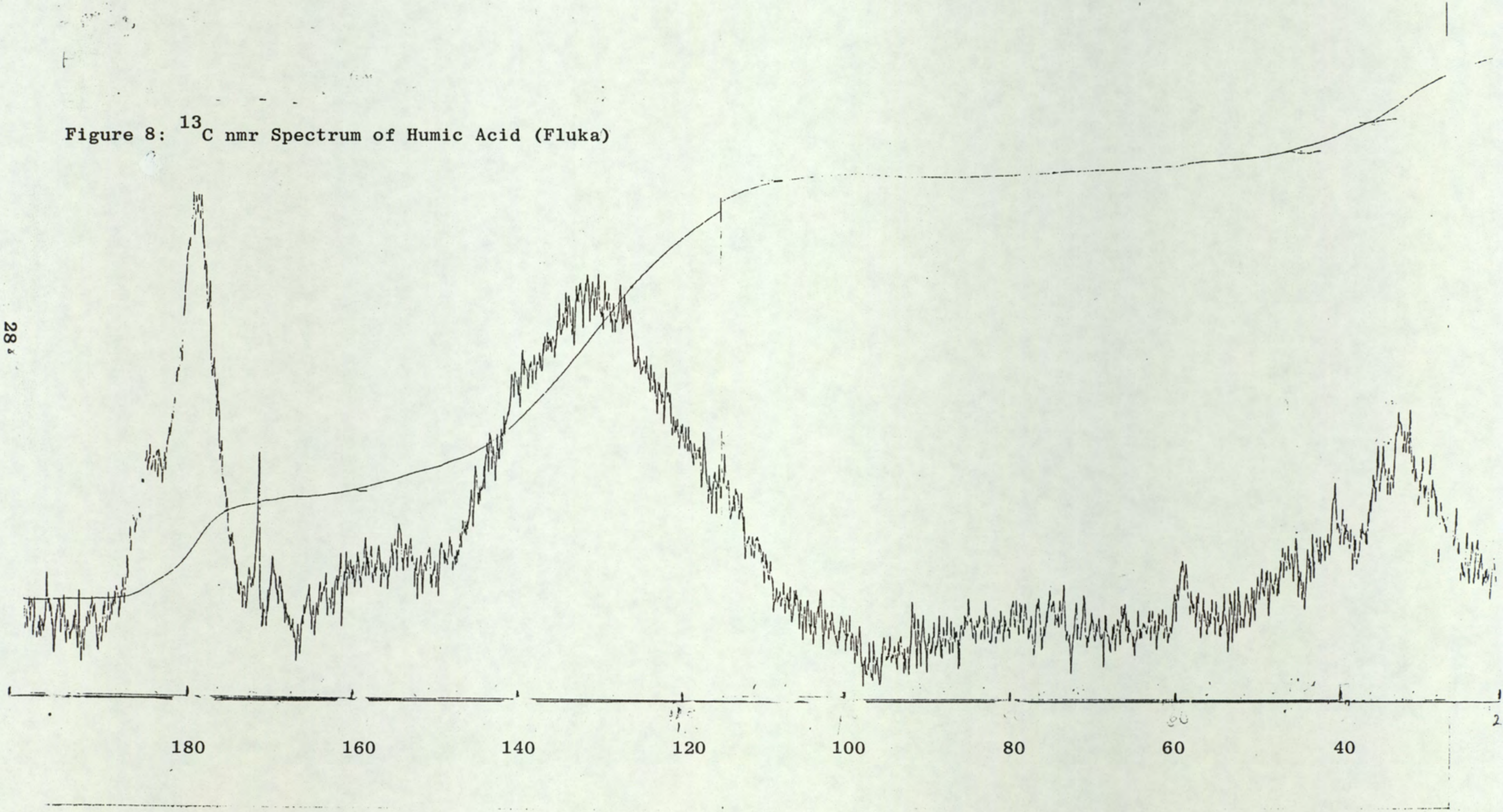
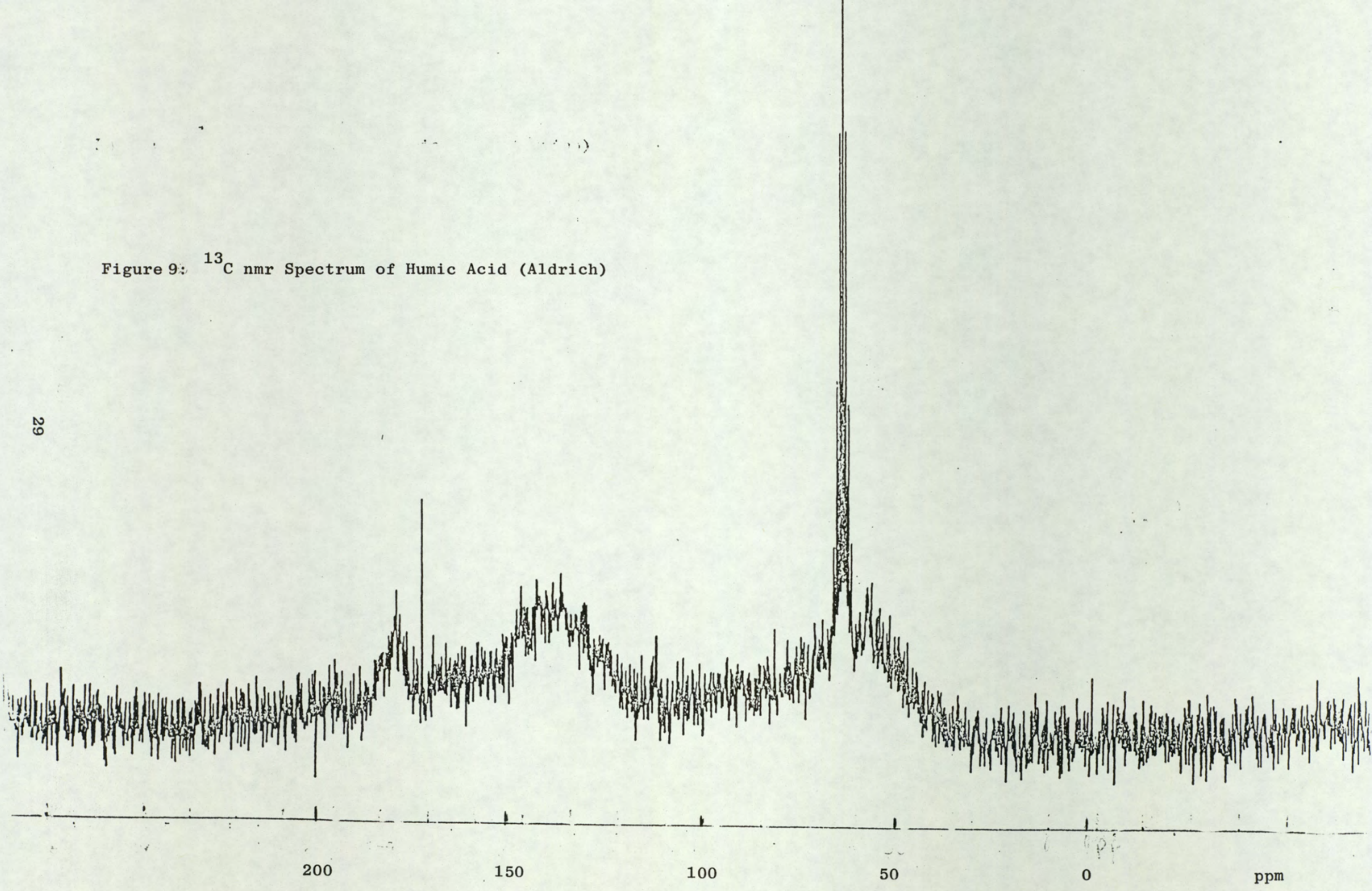


Figure 9: ^{13}C nmr Spectrum of Humic Acid (Aldrich)



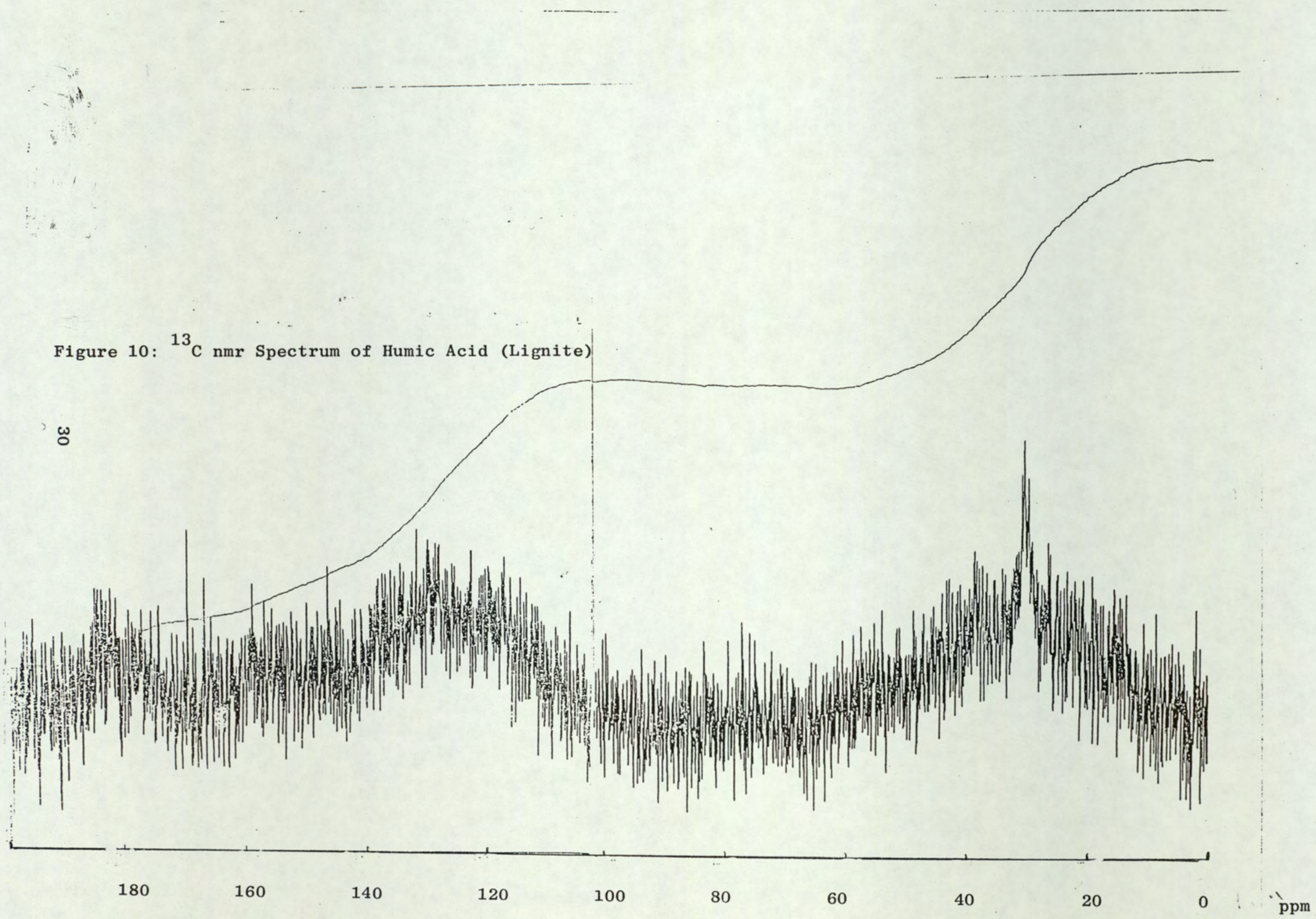


Figure 10: ^{13}C nmr Spectrum of Humic Acid (Lignite)

DMS_O-d_6 are shown in figures 11,12.

The solid ^{13}C nmr spectra of Aldrich and Fluka humic acids are shown in figures 13,14. The ^{13}C solution spectra of the Fluka and Lignite samples were obtained using the high field 400 MHz. Bruker instrument of the University of Warwick.

2.3.2 ^{13}C Nmr Spectrum Fluka

There are many overlapping absorptions in the region 0 - 55 ppm which can be assigned to paraffinic carbons. The major peaks in this region are at 32 - 34 ppm which is the chemical shift of methylene carbons in either large chain paraffinic structures or cycloparaffins. It has been suggested that cyclic structures are more likely than large chain structures. There are also relatively strong absorptions between 35 and 45 ppm probably arising from branched hydrocarbons. The region 50 - 100 ppm shows absorptions arising from oxygen and nitrogen substituted carbons as in carbohydrates, ethers, alcohols and amines. In this sample there is a strong peak at 59.6 ppm indicative of aliphatic ether carbon and peaks at 73 - 77 ppm generally attributed to carbohydrate structures. The absorptions in the region 110 - 160 ppm can be assigned to aromatic and olefinic carbon resonances. Because alkenes are not usually found in high concentration in humic acids, aromatic carbons are expected to be the major contributors to peaks in this region. The major band at 130 ppm can be assigned to aromatic carbon attached to a proton and the minor band at 150 ppm to aromatic carbon attached to oxygen.

There are strong peaks arising from carbonyl carbons in the region 170 - 190 ppm. The advantages of the high field instrument are its excellent resolution and sensitivity, and it is possible to distinguish various types of carbonyl carbon. The peaks at 170 - 172 ppm probably represent contributions from aromatic carboxylic acids, the very strong peaks at 179 - 180 ppm probably are due to straight chain aliphatic carboxylic acids and the absorption in the 183 - 6 ppm region can be

Figure 11: ^{1}H nmr Spectrum of Humic Acid (Aldrich)

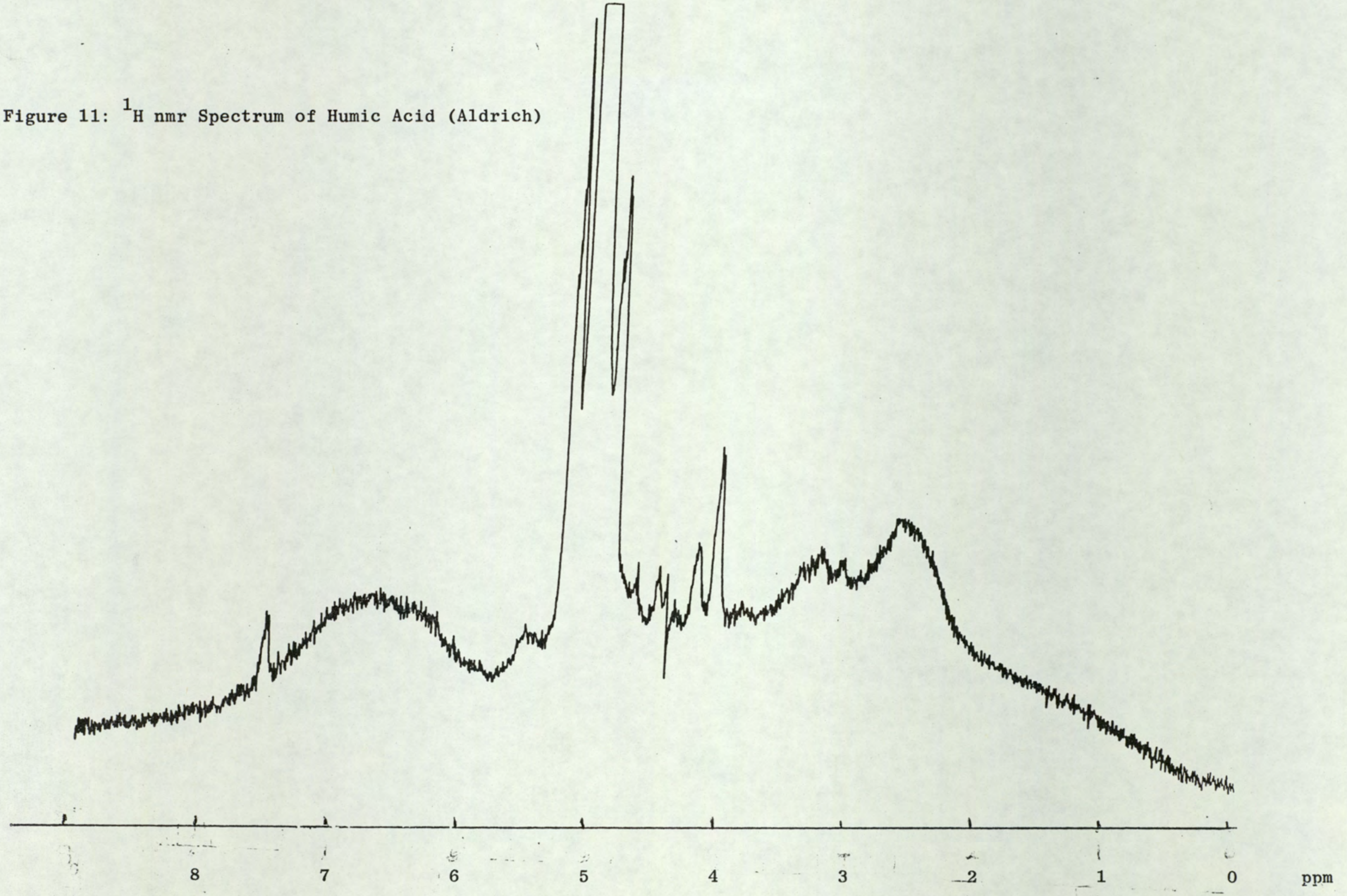
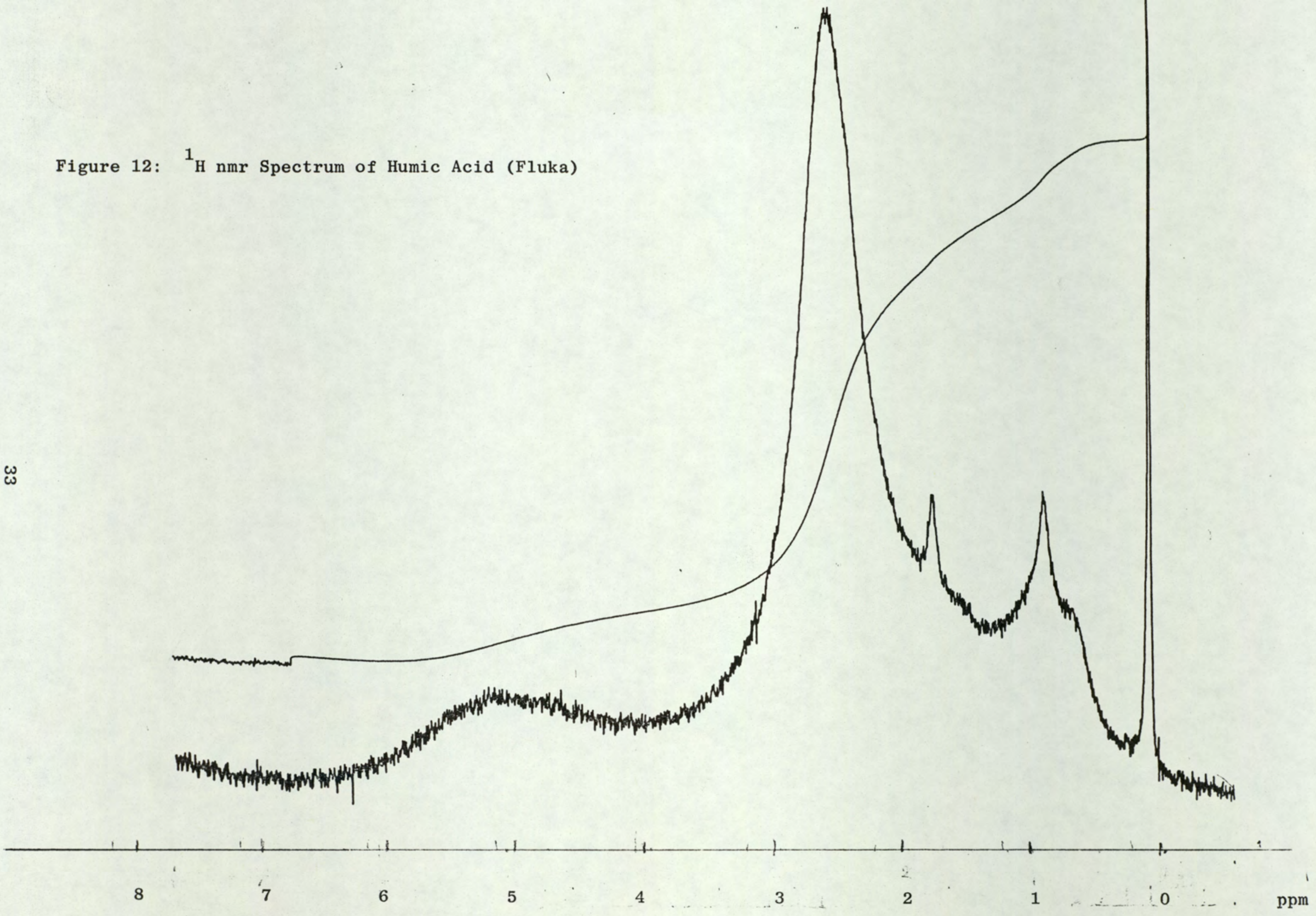
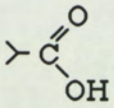


Figure 12: ^{1}H nmr Spectrum of Humic Acid (Fluka)



assigned to branched chain carboxylic acid.



There are no strong absorptions above 185 ppm indicating the absence of ketonic and aldehydic carbon.

The carbonyl : aromatic : aliphatic ratio is approximately 2.5 : 9 : 3 indicating the high aromatic character of this humic acid.

2.3.3 ^{13}C nmr Spectrum Aldrich

This sample was run on a 100 MHz Jeol instrument and the resolution is very poor compared with that of the previous sample run on the high field instrument. The main absorption bands are in the same regions but without good resolution it is not possible to make the same detailed structural assignments. It can be said that this sample appears to be more highly aliphatic in character.

2.3.4 ^{13}C D_2O/OD^- Solution Nmr Lignite

This sample was run on the high field Bruker instrument, but gave a poorer spectrum than the Fluka sample. There is a great deal of background noise. This may be due to the presence of free radicals in the sample which destroys the resolution. Here again one can only look at the main features such as carbonyl, aromatic, aliphatic ratios which are about 1 : 7 : 7. The strongest peaks are at 30 ppm, indicative of a high contribution from cyclic structures.

2.3.5 ^{13}C Solid Nmr

These are not so well resolved and do not have the detail of the high field Fluka solution spectrum.

(a) Aldrich spectrum (figure 13)

Figure 13: ^{13}C Solid nmr Spectrum of Humic Acid (Aldrich)

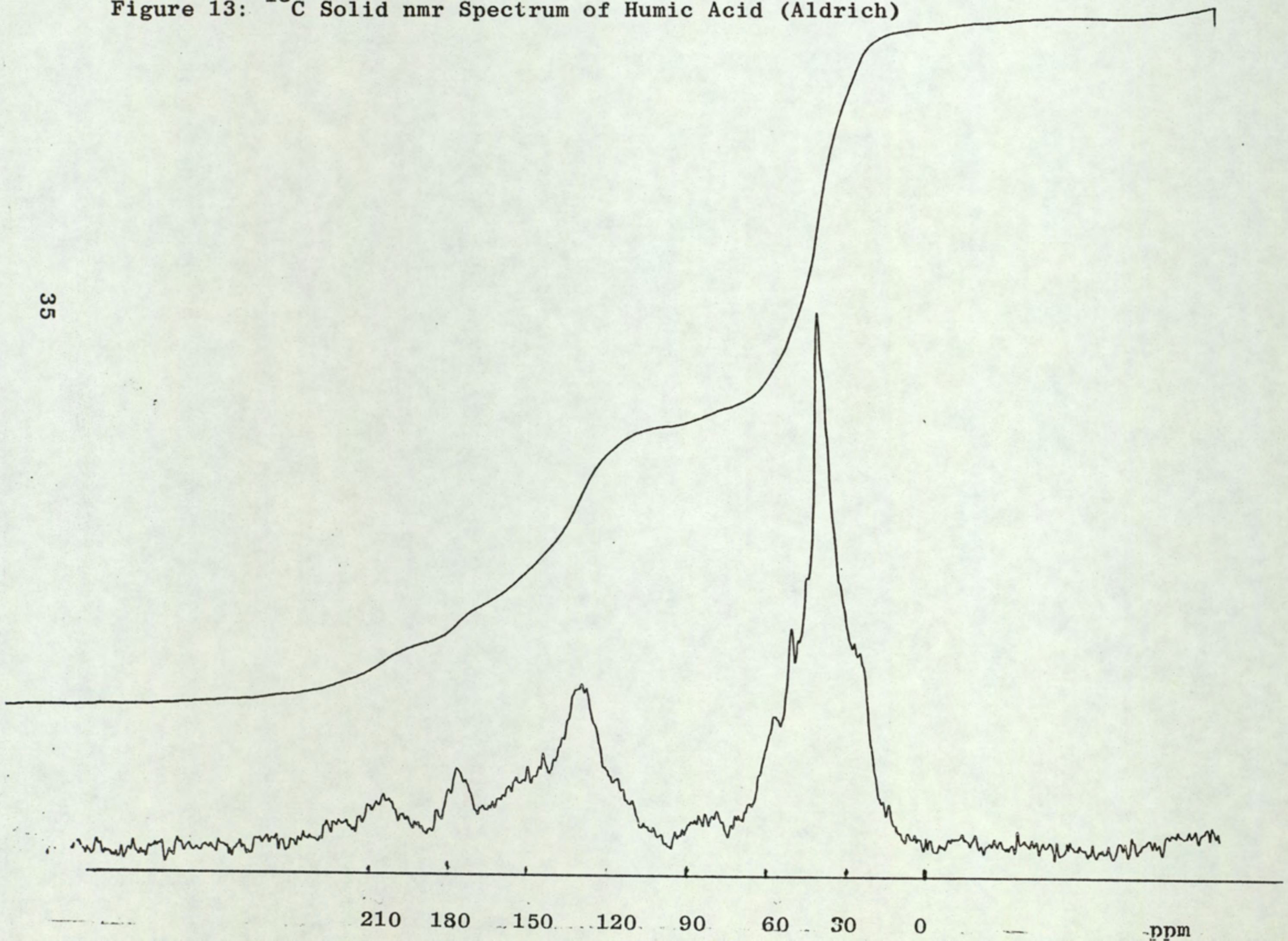
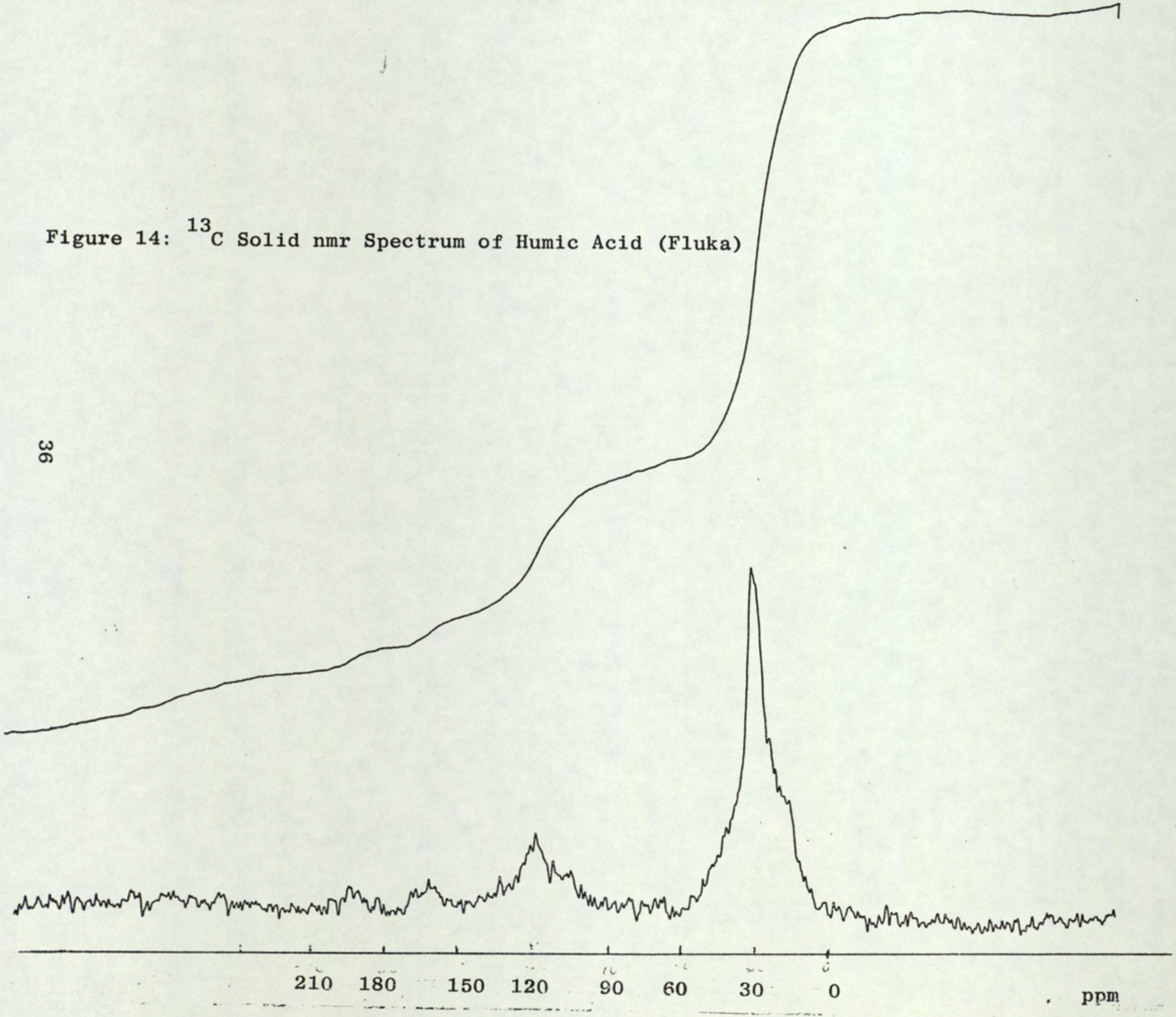


Figure 14: ^{13}C Solid nmr Spectrum of Humic Acid (Fluka)



This is very similar to the solution spectrum (figure 8).

(b) Fluka spectrum (figure 14)

Here the aromatic/aliphatic ratio is reversed compared with the solution sample. There are obviously problems still to be worked out in the ^{13}C solid/solution analysis of humic materials.

2.3.6 ^1H nmr Fluka

High molecular weight and chemical heterogenity of the humic acids characterize ^1H nmr spectra by very broad absorption signals. In spite of this, the spectra give very useful information mostly on the alkanes and aromatic proton absorption ranges. Indeed, the ^1H nmr Fluka spectrum shows two signals at 0.87 and 1.27 ppm arising from protons of $-CH_3$ and $-CH_2-$ groups, respectively, belonging to branched aliphatic chains. Noteworthy is the broad signal between 7.0 and 8.8 ppm arising from aromatic protons.

2.3.7 ^1H nmr Aldrich

^1H Nmr spectra were recorded in deuterated dimethyl sulphoxide (DMSO-d_6) solutions. DMSO-d_6 was preferred to other nmr organic solvents since it is a good solvent for humic substances (49-52) and the higher concentrations obtained increase the signal to noise ratio. The spectrum shows absorption between 7.4 and 8.8 ppm due to aromatic protons, and shows two signals of 0.87 and 1.27 ppm arising from protons of $-CH_3$ and CH_2 groups, respectively, belonging to branched aliphatic chains.

2.3.8 ^1H Nmr Lignite, Reading and Norfolk

These were not so well resolved and showed some absorption between 7.4 and 8.8 ppm, due to the presence of aromatic protons.

2.4 GAS CHROMATOGRAPHY/MASS SPECTROSCOPY

The term 'chromatography' is now used to refer to a variety of related separation techniques, but all work on a common principle. The mixture to be separated is dissolved in a solvent and added to an adsorbent stationary phase (Al_2O, SiO_2). Different compounds adsorb to the stationary phase to different extents and as a mobile phase is passed over the stationary phase they migrate at different rates and hence separate. In liquid chromatography a solvent is used as the mobile phase. In gas chromatography a carrier gas such as nitrogen or helium rather than a liquid solvent is used as the mobile phase.

In practice, a small amount of sample mixture is dissolved in a small amount of solvent and injected by syringe into a heated inlet of a gas chromatograph. The sample is immediately vaporised and swept through a column of stationary phase by a stream of carrier gas (mobile phase). As pure separated components come off the end of the column each is detected by a detector and registered on a recorder. In a combined gas chromatograph/mass spectrometer, the pure separated components are passed into the mass spectrometer which acts as a detector. If capillary columns are used the components can pass directly from the gas chromatograph to the mass spectrometer. With non-capillary columns a device is required to deal with the difference in pressure between the gc and the ms.

At its simplest, mass spectroscopy gives a measure of the molecular weight of a molecule. In addition, it is often possible to obtain useful structural information by determining the masses of fragments produced when high-energy molecules fly apart.

A small amount of the sample is introduced into the mass spectrometer and bombarded by a stream of high energy electrons. The exact energy of the electron beam varies but it is commonly about 70 electron volts (eV). When a high energy electron strikes an organic molecule it knocks out a valence electron from the molecule producing a cation radical.

$$\mathrm{RH} \longrightarrow \underset{\text{cation radical}}{\mathrm{RH}^{+} + e^{-}}$$

In addition to causing ionisation electron bombardment transfers such a large amount of energy that cation radicals fragment and are then sorted magnetically, according to their mass-to-charge ratio (m/z). It is possible to obtain structural information from interpretation of fragmentation patterns.

The two main problems that arise in gc/ms are unresolved gas chromatographic peaks and the large amount of data that is produced. These problems can be overcome by the use of a computer, which is used not only as a high speed data acquisition system but also to control the operation of the mass-spectrometer. The first step is to establish under computer control a repetitive scan over the desired mass range. During the recording of part of the cycle the computer collects data and simultaneously begins determining peak intensities and masses. While the magnet is returning to the starting point calculations are completed and the spectrum is written into some external memory device for future use. Upon command the computer can produce a recording of total ion current throughout the run. The total ion current recording will be very similar to the recording from the normal gas chromatograph (see figure 15). The computer can then search out and plot the spectrum revealed at the top (or side if desired) of any specific gc peak or, it can search all the spectra for an ion mass characteristic of a particular functional group or type of compound (e.g .m/z 74 for methyl esters of fatty acids). It can also detect slight variations in spectra recorded at different points on a gc peak and extract the mass spectra of individual components of an unresolved mixture. The ability to deal with complex materials would appear to make computer

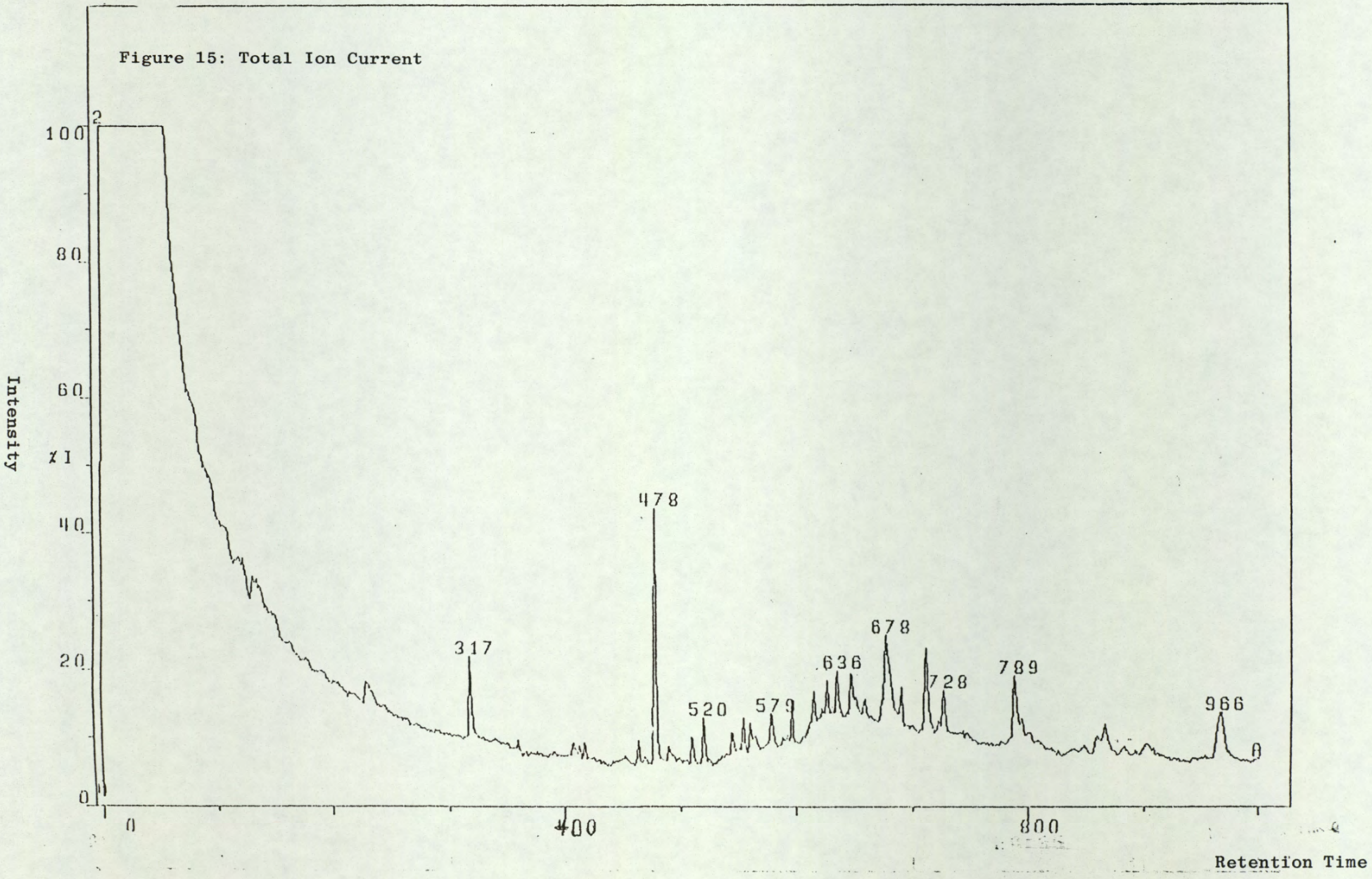


Figure 15: Total Ion Current

gc/ms a very useful technique for the analysis of humic materials. However because of their strong hydrogen bonding and involatility these materials do not give useful gc/ms spectra. Even after methylation to remove hydrogen bonding the products are not sufficiently volatile to be detectable by gc/ms. Mild tosylation of humic acid gave some good, resolvable peaks in gas chromatography. Total ion current (TIC) from the sample was used to generate a chromatogram by specifying the compounds in terms of their scan numbers in the mass spectrometer. TIC's of tosyl derivatives of Aldrich, Fluka, Norfolk, Reading and Lignite humic acids are discussed in chapter 4.

CHAPTER 3

REDUCTION

3 REDUCTION OF HUMIC ACIDS

Humic substances contain a high percentage of hydroxy and carboxylic acid functions and the resultant strong hydrogen bonding is one of the main factors which complicates the structural analysis of these materials. The usual method of removing hydrogen bonding is to protect the active hydrogen functional groups. This is commonly achieved by methylation or acetylation.

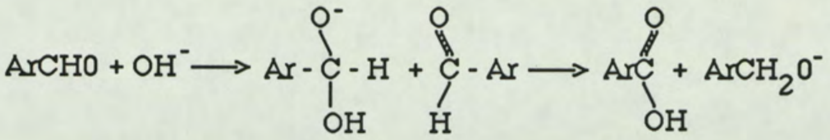
$$R\text{-}OH + CH_3I \dashrightarrow R\text{-}OCH_3$$

$$R\text{-}OH + (CH_3CO)_2O \dashrightarrow R\text{-}OCO\,CH_3$$

The methylated and acetylated products are more soluble in organic solvents and more amenable to structural analysis using conventional separation and spectroscopic techniques.

It occurred to us as discussed in the introduction, that even better results might be achieved by complete removal of hydroxyl and carbonyl functional groups. The route which appeared most promising was first to reduce carbonyl functions to hydroxyl and then to remove the hydroxyl groups. Reduction methods have not been very widely used in the study of humic acid, the few previous investigations which have been carried out are discussed below.

Reactions which add hydrogen to a compound to form covalent linkages are generally called reductions. The most useful reducing agents in organic chemistry are hydrogen, (generally used with a solid catalyst), metals such as zinc and sodium which transfer electrons to substrate, and metal hydrides which transfer hydride (H^-) anions. In the electronic transfer process the metals are oxidized to their cationic species. Hydride transfer reactions can also take place between certain molecules in basic media to yield oxidation and reduction products. For example the Cannizzaro reaction, for aldehydes lacking an α - hydrogen atoms, benzaldehyde, for instance, when treated with base, initially forms an addition product which then transfers a hydride ion to the aldehyde to give the reduction product (the alcoholate anion), and the residual oxidation product forms the acid. The reaction is completed by transfer of a proton from the acid to alcoholate to form the alcohol and the acid anion.

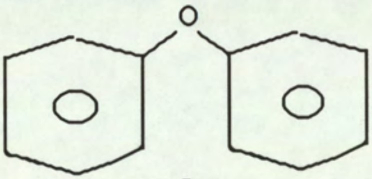


$$\mathrm{ArCHO} + \mathrm{OH}^- \longrightarrow \mathrm{Ar}-\underset{\mathrm{OH}}{\overset{\mathrm{O}^-}{\mathrm{C}}}-\mathrm{H} + \underset{\mathrm{H}}{\overset{\mathrm{O}}{\mathrm{C}}}-\mathrm{Ar} \longrightarrow \mathrm{Ar}\underset{\mathrm{OH}}{\overset{\mathrm{O}}{\mathrm{C}}} + \mathrm{ArCH_2O^-}$$

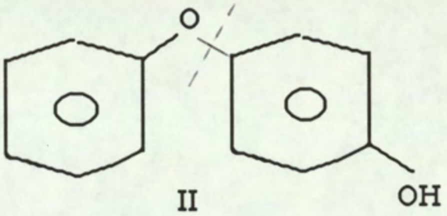
In the past, reduction of humic acids has been carried out mainly by methods involving active metals.

3.1 REDUCTION WITH SODIUM AMALGAM

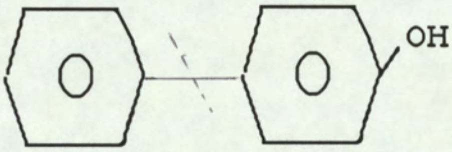
Burges et al(53) used the sodium amalgam (Na/Hg) technique for the degradation of humic acids and isolated yield of 30-35% ether soluble product from the digests. These products, composed for the most part of mixtures of phenols and phenolic carboxylic acids, were readily oxidized to a dark residue on standing. Since that time the technique has been used by a number of workers(54-59) who obtained lower yields of ether or solvent soluble materials. Amongst the many compounds identified, the most readily released were 3-methoxy-4-hydroxybenzenecarboxylic acid (vanillic acid) and 3-5 dimethoxy-4-hydroxy benzenecarbxylic acid (syringic acid), and these were common to all digests despite differences in the ratios of reagents and reactants used. Piper and Posner(60) studied the mechanisms of reaction, investigated the optimum conditions for Na/Hg degradations and established the importance of the ratio of reactant to reagent and the time of reaction. Their work showed that the same products were released for hydrolysed and unhydrolysed substrate, and illustrated the importance of using finely ground amalgam(61). It also showed that the amounts of monomers released increased with time during 30 hours of reaction. The results suggested that under the experimental conditions employed, the best results were obtained by using 25mg of the more highly oxidized humic acid (such as those extracted with pyrophosphate) or 50mg of less oxidized material (e.g. alkali extracted humic acid) in an alkaline medium (15 cm^3 of 0.5M NaOH) containing 30g of 5% sodium amalgam. Whilst the reaction conditions used by Burges et al(53) met these requirements, those used by other workers did not, and this might account for their lower yields. Degradation on model compounds by Piper and Posner(60) give indications of some relevant linkages which could be cleaved in Na/Hg digests. Although the mechanisms involved are not known, degradation is thought to result from attack by atomic hydrogen (H·). These atoms combine readily to form hydrogen gas (H_2), and this might account for the necessity to use excess amalgam. The hydrogen acts as an electrophile, as indicated by the products released during degradation of compounds (1-IV).



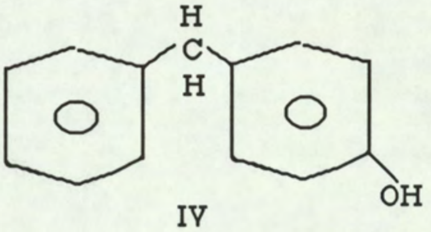
I



II



III



IV

Compound I gave benzene and phenol, II gave equal amounts of phenol because the electron density of para-carbon is increased by the activating effect of the 4-hydroxy group, and this would favour cleavage as indicated by the broken line. Again a similar activating effects caused (III) to cleave to equal amounts of benzene

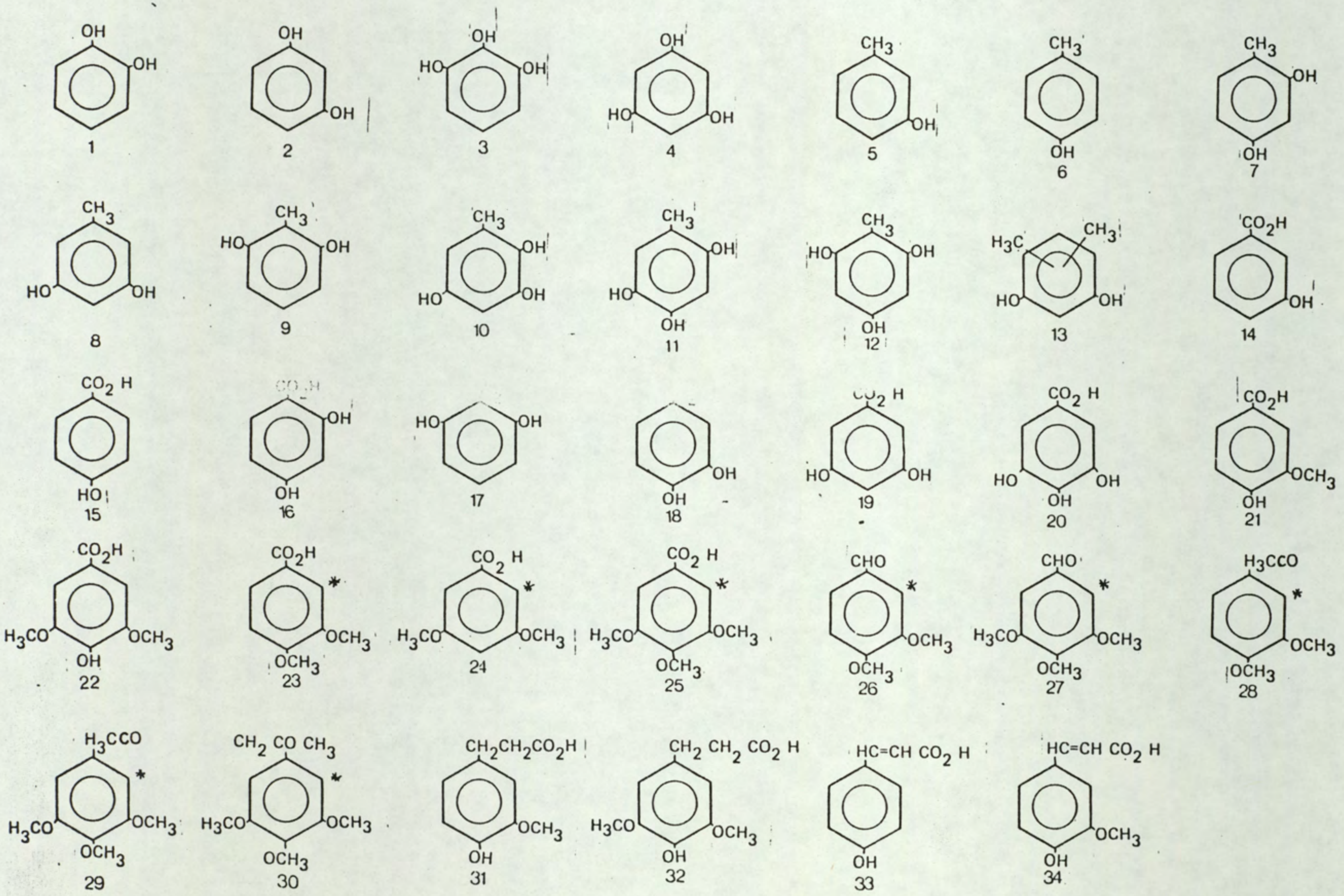
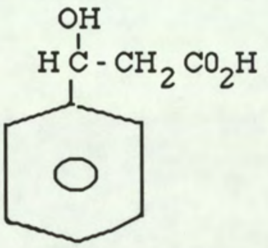


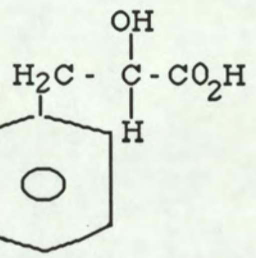
Figure 16: Products identified in the digest from sodium amalgam degradations of Humic Acids$^{(58)}$

and phenol, but (IV) did not cleave because excess electron density cannot reside on the bridging methylene carbon. Figure16 summarizes the products (Burges et al(53), Piper and Posner(60), Dormaar(56), Schnitzer and Ortiz de Serra(57), and Martin et al(59) which have been identified in humic acid-Na/Hg digests. Model studies by Piper and Posner(60) indicated that compounds 1,2,15,20,21 and 4-hydroxybenzenol (hydroquinone) did not degrade when subjected to their reaction conditions, and this suggested that the identified phenolic structures in humic acid digests could reasonably be assigned to polymer structures. Later, however, Martin et al(59) showed that varying extents of degradations occurred in their digests in the cases of compounds 4,5,16,17,19, benzoquinone, 2-hydroxy-5-methylbenzene carboxylic acid, and several hydroxynaphthenic acids. Decarboxylation was the principal reaction observed in the cases of the dihydroxybenzenecarboxylic acids, but the partial destruction of the trihydroxybenzene structures indicates that the yields reported for such compounds in humic acid digests were not representative of the amounts released as carboxylic acid derivatives. Variation in reaction conditions (e.g. the amounts of the reductant used and the maintenance of anaerobic conditions in the alkaline media might account for some of the differences in the apparent stabilities of the compounds examined.

On the basis of the studies of degradation of model substances by Martin et al(59) we cannot be certain that all of the non carboxylic phenolic structures in Figure 16 represent primary structures in the humic acid polymer. Also, the fact that none of the phenolic carboxylic acids listed have a methyl group attached to an aromatic ring suggests that such structural types did not exist in the original polymer, or (if they did), were decarboxylated when released into the digest. It would appear that the various cleaved structures were linked by aromatic ether linkages, or were present as biphenyl structures where activating (hydroxyl or methyl) substituents were ortho or/and para to the linking bond. The model studies, and the compounds identified would suggest also that methylene and longer aliphatic bridging structures were not cleaved. For detection of the phenolic compounds and their quantitative estimations generally used two-dimensional chromatography and co-chromatography with standards. Compounds marked with an asterisk in Figure 21 (taken from the data of Schnitzer and Oritz de Serra)(58). These compounds were methylated prior to g.c.m.s. and micro-infrared analysis, and it is impossible to state which of the structures originally had free hydroxyls. However, it is highly likely that the distributions of OH and OCH_3 substituents were the same as for the other similar compounds in Figure 16 It is interesting to note that these workers isolated the aldehyde and keto structures 26,27,28,29 and 30, and this is good evidence for the presence of carbonyl groups in humic substances. The side chain double bonds of substituted propenoic acids 33 and 34 would be expected to rapidly hydrogenate, and Piper and Posner(60) suggested that these might arise by dehydration of appropriate hydroxyl derivatives (V and VI) during the drying of the ether extracts.



V



VI

Despite the reducing conditions of the reaction many of the compounds in Figure 16 contain the carboxyl group. This provides strong evidence that this functional group, as well as hydroxyl, methoxyl and carbonyl are present as substituents on aromatic nuclei of humic substances. This inference could not be made so strongly for carbonyl and carboxyl groups solely on the basis of oxidative degradation reactions.

3.2 ZINC DUST REDUCTION

The zinc-dust distillation of humic substances has been described by

Cheshire et al(62,63) and by Hansen and Schnitzer(64,65). The later authors have also used zinc-dust fusion, and the products which have been obtained by both procedures are summarized in Figure 17. For the distillation process Cheshire et al used 0.5g quantities of humic acid previously treated with (6 MHCl) and mixed with 30g purified zinc dust, and they placed an additional 10g of the dust at the exit port of the pyrex tube used for the reaction. Distillation was carried out in a stream of the H_2 gas at 500 - 550°C. For fusion Hansen and Schnitzer combined 0.5g of substrate with 3g of Zn dust, 5g $ZnCl_2$ and 1g NaCl and heated them to a temperature of 300°C. As Figure 17 shows, the products isolated were composed of fused aromatic structures. The listed compounds are naphthalene(35), anthracene(36), 1, 2-benzanthracene(37), fluorene(38), 2, 3-benzofluorene(39), fluoranthene(40), phenanthrene(41), chrysene(42), pyrene(43), triphenylene(44), perylene(45), coronene(46), carbazole(47), and acridine(48). In addition, a number of derivatives of these were identified which included methylated naphthalenes (1-methylnaphthalene, 2- methylnaphthalene, and 1, 2, 7 -trimethylnaphthalene), methylated anthracene (1-methyl- anthracene and 9-methylanthracene), methylated phenanthracenes (2-methylphen- anthrene and 3-methylphenanthrene), methylated pyrenes (1-methylpyrene and 4-methyl pyrene), benzpyrenes (1, 2 benzopyrene and 3, 4-benzopyrene), and naphtho-(2', 3': 1,2)-pyrene, 1, 1,2-benzoperylene, and homologues of carbazole and acridine. The yields of products from zinc-dust distillation reactions are invariably very low, often less than 1% and the recovery of 3% of starting material as a pale yellow oil, by Cheshire et al (63) was considered to be good. Hanson and Schnitzer(64) obtained yields of the order of 0.6 - 0.7% for humic acid, whilst those for fulvic acid were somewhat lower.

Assuming a 10% recovery of products and calculating on a functional group-free basis, they postulated that fused aromatic structures could account for 25% and 12% of the components in fulvic and humic acids respectively. Based on their

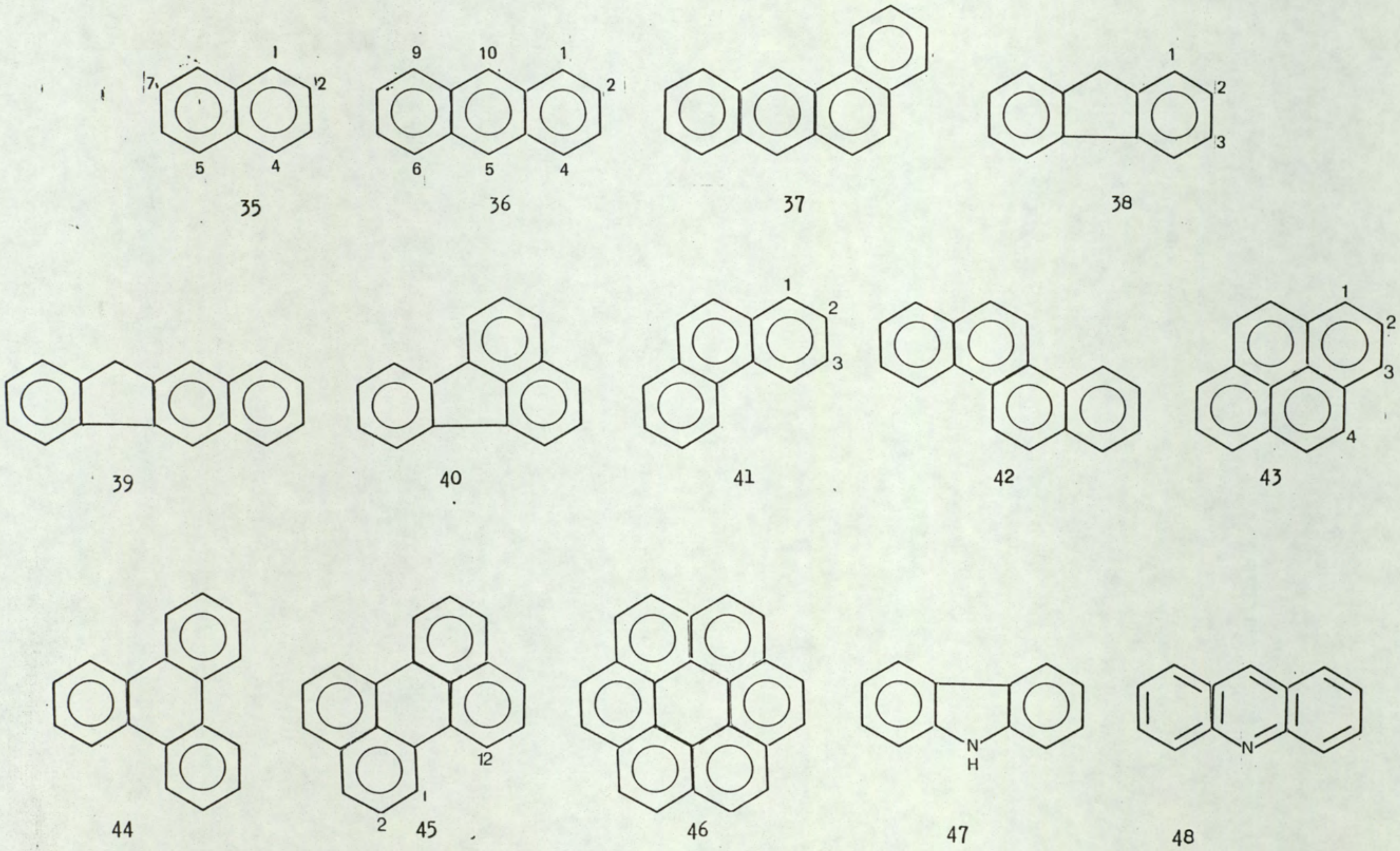


Figure 17 Products identified in the digests from degradation of humic materials in Zinc-dust distillation of fusion reactions (64)

data from zinc-dust distillation experiments, Cheshire et al (62) and Haworth(24), proposed that humic substances were built around a polynuclear aromatic 'core'; combining this with other data from hydrolysis, oxidation, reduction and electron spin resonance (e.s.r.), they suggested that polysaccharides, simple phenols, proteins or peptides, and metal were attached to this 'core'.

The results from such drastic procedures must be interpreted with caution. In their later paper Cheshire et al showed that 3, 4 - and 3, 5-dihydroxybenzoic acids, furfural (from dehydration of pentose sugars), and quinone polymers gave polycyclic aromatic structures for zinc-dust distillation at 500-550°C.

At 400°C only small yields of anthracene were obtained from the hydroxybenzenes, and no aromatic structures were detected in distillates from furfural and polymers of ortho and para-benzoquinone. In addition, the same products were isolated in the same proportions from the distillation at the higher and lower temperature and it was concluded that the products identified were largely released from the humic materials.

We conclude that judgement cannot yet be passed on the usefulness of zinc-dust distillation and fusion techniques for work with humic substances. An obvious experiment which could help decide whether or not the fused aromatic structures detected were 'core' products or artefacts, would be to subject compounds released by other reductive techniques (such as the sodium amalgam method), and the undegraded residue to zinc-dust distillation at 400°C, and to compare the yields of fused aromatic structures from both substrates.

3.3 REDUCTION BY $NaBH_4$

Maximove and Gelbko(66) have estimated the contents of quinone and ketone groups present in the humic acid from various sources, namely soil, peat and brown oxidized coal. This was done by reducing the humic acid by using 0.1M solution of sodium borohydride in dimethylformamide (DMF), which quantitatively reduces quinones to hydroquinones, and ketones to secondary alcohols. Choudhry(67) has used a rather active reducing agent, namely a solution of bivalent iron in alkaline triethanolamine (Fe-TEA method), for determination of quinoid groups. This method can be performed within a short time period and under mild conditions. The reaction proceeds stoichiometrically and is accompanied by the consumption of one reducing agent equivalent per quinone carbonyl, i.e. is completed at the stage of hydroquinone formation(66). The overall content of $C = O$ groups (B, in Table 4) in milliequivalent (meq)/g of HA was determined with respect to the amount of 0.1M solution of sodium borohydride in dimethylformamide (DMF), which was consumed. In this reaction the carbonyl groups of ketones and quinones are converted into secondary alcohol and hydroquinones, respectively. The ketone-carbonyl content has been calculated from the difference between the overall consumption of the reducing agent (B) and the amount of the quinones determined by reductometric titration according to the Fe-TEA procedure (A), to standardize the data, last figures are divided by two. The result of those calculations (see Table 4) shows that the ketone carbonyl content in HA of different origins differs within a narrow range. It has been reliably shown by Maximov and Glebko(66) that functional groups, being chiefly and undoubtedly of quinonoid origin and readily subject to reversible redox transformations, are contained in the molecules of natural humic acids.

None of the methods used previously filled our particular needs. The sodium amalgam reaction required relatively fierce conditions, was non specific, caused uncontrolled cleavage of the backbone structure and gave products containing carboxylic acid functions. The zinc-dust and high pressure hydrogenation reactions removed the appropriate functions but, like the sodium amalgam reductions, did not proceed in a controlled stepwise fashion. If such vigorous conditions are required for

Table 4

Contents of quinoid and Ketonic groups in humic acid from various sources. From Maximov and Glebko(66)

Source	Quinone groups[a] (meq/g) A	$NaBH_4$ used to reduce C = 0 group meq/g B	Ketone groups C = 0 meq/g $\frac{B - A}{2}$
Woodland soil	1.05	2.92	0.93
Peat	1.26	2.75	0.78
Brown coal	1.33	2.90	0.78
Brown coal	2.04	3.43	0.69
Weathered brown coal	2.51	3.95	0.72
Brown Coal	1.91	3.72	0.91
Weathered brown coal	1.80	3.85	1.09
Weathered brown coal	2.98	4.05	0.53
Weathered brown coal	3.27	5.01	0.87
Weathered bituminous coal	3.27	5.08	0.90
Weathered hard coal	3.26	-	-

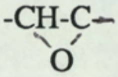
reduction it is unlikely that the products are highly representative of the starting materials. In contrast the reduction with $NaBH_4$ and Fe-TEA are so mild and so highly specific that only active carbonyl functions are reduced, the carboxyl function one of the groups we wished to remove, is not affected by these reagents. The reagent which appeared most likely to fulfil our requirements was lithium aluminium hydride.

3.4 LITHIUM ALUMINIUM HYDRIDE

Lithium aluminium hydride is a very powerful reducing reagent which reacts with a variety of functional groups and reacts under relatively mild conditions compared with reagents such as sodium amalgam and zinc-dust. Table 5 lists the common functional groups which may be reduced with ethereal solutions of lithium aluminium hydride. The groups are listed approximately in order of the ease with which they are reduced. However, its selectivity is limited making it necessary to develop more selective reducing hydrides for specific purposes. Most polar unsaturated groups are reduced by the reagent under normal conditions, but it is possible to carry out selective reduction by using protective groups(68) and special conditions. Amidoxime and guanidino groups are not attacked and ethers and ketals react only in special instances(69). Isolated double and triple bonds in olefins and acetylenes are not saturated by $LiAlH_4$ under normal conditions(70). When working with this very useful reagent, one should never forget that it is a pyrophoric dust and safety precautions should be observed.

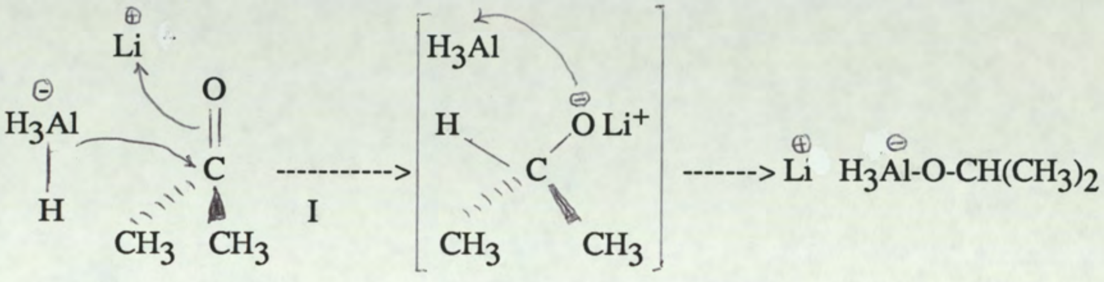
Table 5

Common Functional groups reduced with lithium aluminium hydride (The groups are listed approximately in order of the ease of reduction with lithium aluminium hydride).

Functional group	Product
$-CHO$	$-CH_2OH$
$>C=O$	$>CH\text{-}OH$
$-COCl$	$-CH_2OH$
 $-CH\text{-}C-$ (epoxide, O)	$CH_2\text{-}\underset{OH}{\overset{\mid}{C}}\text{-}$
$-CO_2R$	$-CH_2OH+ROH$
$-CO_2H$ or $CO_2^{\ominus}\,Li^{\oplus}$	$-CH_2OH$
$-CO\text{-}NR_2$	$CH_2 - NR_2$ or $\left[\begin{array}{l}-CH\text{-}NR_2\\ \mid\\ OH\end{array}\right] \text{--->} -CHO + R_2NH$
$-CO\text{-}NHR$	$-CH_2\text{-}NH\text{-}R$
$-C\equiv N$	$-CH_2\text{-}NH_2$ or $[-CH = NH]\xrightarrow{H_2O} -CHO$
$>C = NOH$	$>CH\text{-}NH_2$
$-\underset{\mid}{\overset{\mid}{C}}\text{-}NO_2$ (aliphatic)	$-\underset{\mid}{\overset{\mid}{C}}\text{-}NH_2$ and other products
$-CH_2\text{-}O\text{-}SO_2\text{-}C_6H_5$ or $-CH_2Br$	$-CH_3$
$>CH\text{-}O\text{-}SO_2\text{-}C_6H_5$ or $>CH\text{-}Br$	$>CH_2$

3.4.1 Reduction of Aldehyde and Ketones

The reaction of an aldehyde or ketone with lithium aluminium hydride involves the transfer of a hydride anion from the metal atom to the carbonyl carbon atom; this transfer is presumably assisted by the prior or concurrent association of the carbonyl oxygen atom with a lithium cation, as illustrated below. The resulting aluminium hydride lithium alkoxide pair, which exist transiently, if at all, after transfer of the hydride anion, is converted to a lithium alkoxyaluminium hydride.



$$\xrightarrow[\text{II}]{(CH_3)_2\,CO} \overset{\oplus}{Li}\quad H_2\overset{\ominus}{Al}\,[0\text{-}CH(CH_3)_2]_2 \xrightarrow[\text{III}]{(CH_3)_2\,CO} \overset{\oplus}{Li}\,H\overset{\ominus}{Al}\,[0\text{-}CH(CH_3)_2]_3$$

$$\xrightarrow[\text{IV}]{(CH_3)_2\,CO} \quad \overset{\oplus}{Li}\overset{\ominus}{Al}[O\text{-}CH(CH_3)_2]_4$$

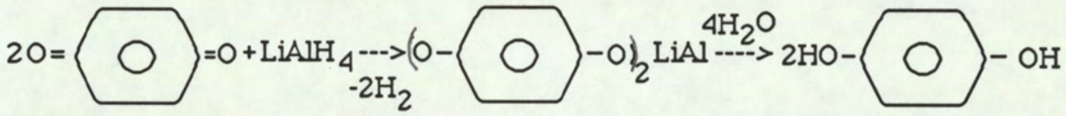
This hydride transfer step (I) may be followed by analogous steps (II, III and IV) so that as many as four molecules of ketone are reduced by one molecule of the metal hydride to form a tetraalkoxyaluminium anion. There is considerable evidence indicating each successive hydride ion transfer to be slower than the preceding step (rate I>II>III>IV). Removal of a hydride anion from the metal complex would be expected to become more difficult as the number of electronegative alkoxyl substituents in the complex is increased. Because of this reactivity difference it is possible to prepare reducing reagents such as lithium aluminium trimethoxyaluminium hydride and lithium tri-t-butoxyaluminium hydride, which are less reactive and more selective than lithium aluminium hydride.

3.4.2 Reduction of carbonyl compounds

Aldehyde and ketones are reduced rapidly with 1 equivalent of hydride to the primary and secondary alcohols, while quinones react with 2 equivalents of hydride to give mostly hydroquinones.

$$\mathrm{4R\text{-}CHO+LiAlH_4 \text{----}\!\!> (R\text{-}CH_2\text{-}O)_4\,LiAl \xrightarrow{4H_2O} 4R\text{-}CH_2\text{-}OH+LiOH+Al(OH)_3}$$

$$\mathrm{4R_2CO+LiAlH_4 \text{------}\!\!> (R_2CH\text{-}O)_4\,LiAl \xrightarrow{4H_2O} 4R_2CH\text{-}OH+LiOH+Al(OH)_3}$$

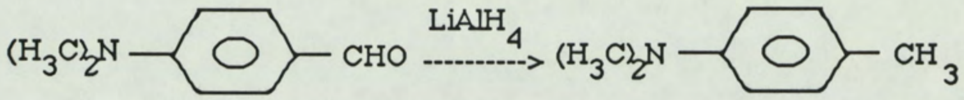


$$\mathrm{+\ LiOH + Al(OH)_3}$$

Lithium aluminium hydride normally has no effect on isolated C-C multiple bonds but double and triple bonds conjugated with a polar multiple bond are reduced. The normal reduction procedure (addition of the compound to a lithium aluminium hydride solution) when applied to cinnamaldehyde furnishes 3-phenyl-l-propanol, while the reverse mode of additions carried out by adding the calculated amount of hydride solution to a solution of cinnamaldehyde at temperatures below 10^{o}C, results in an excellent yield of cinnamyl alcohol$^{(74)}$:-

$$\mathrm{H_5C_6\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}OH \xleftarrow{LiAlH_4} H_5C_6\text{-}CH = CH\text{-}CH_O \xrightarrow{LiAlH_4} H_5C_6\text{-}CH = CH\text{-}CH_2\text{-}OH}$$

Aromatic carbonyl compounds substituted in ortho or para positions by electron releasing groups, and the corresponding rich heteroaromatic compounds with nitrogen as the heteroatom, are hydrogenolysed by a large excess of lithium aluminium hydride under forcing conditions, for example p-dimethylaminobenzaldehyde gives a 78% yield of N,N - dimethyl-p-toludine(75):



3.4.3. Reduction of Carboxylic acids and their Derivatives

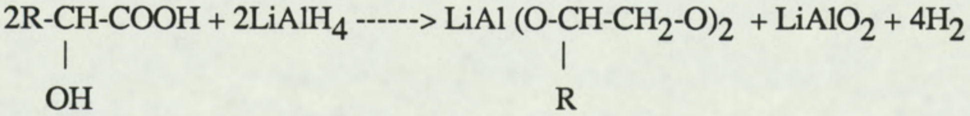
On contact with $LiAlH_4$, carboxylic acids instantly evolve 1 equivalent of hydrogen and then react at a moderate rate in THF at 0^oC, taking up 2 equivalents of hydride for reduction to the alcohol stage in about 6h(73).

$$4R\text{-}COOH + 3LiAlH_4 \text{------>} LiAl\,(O\text{-}CH_2\text{-}R)_4 + 2\,LiAlO_2 + 4H_2$$

$$LiAl\,(O\text{-}CH_2\text{-}R)_4 + 4H_2O \text{------>} 4R\text{-}CH_2\text{-}OH + LiOH + Al\,(OH)_3$$

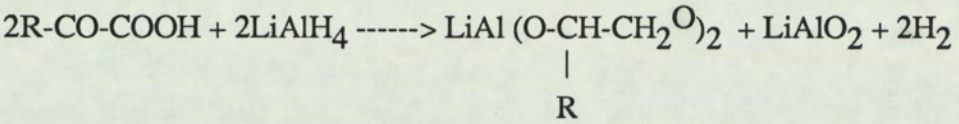
The reaction is a two-step procedure, the first step involving formation of a salt with evolution of hydrogen and the second being the actual reduction. In diethyl ether, some difficulties have been noted in the reduction of carboxylic acids, e.g. triphenylacetic acid is not reduced in this solvent at 25^oC(76).

Hydroxyl and amino groups do not interfere in the reduction of hydroxy and amino acids, other than by the decomposition of an amount of hydride equivalent to the active hydrogen atoms(76).



$$2R\text{-}\underset{\substack{|\\ OH}}{CH}\text{-}COOH + 2LiAlH_4 \text{------>} LiAl\,(O\text{-}\underset{\substack{|\\ R}}{CH}\text{-}CH_2\text{-}O)_2 + LiAlO_2 + 4H_2$$

Keto acids are reduced by $LiAlH_4$ to the corresponding diols:



$$2R\text{-}CO\text{-}COOH + 2LiAlH_4 \text{------>} LiAl\,(O\text{-}\underset{\displaystyle R}{\underset{|}{CH}}\text{-}CH_2O)_2 + LiAlO_2 + 2H_2$$

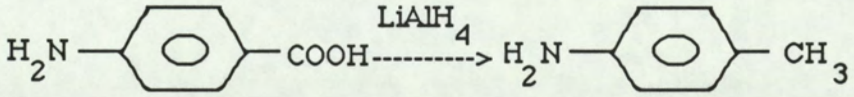
Double bonds in aliphatic unsaturated acids are generally not affected by the reagent. For example, 2,4-hexadienoic acid (sorbic acid) affords sorbyl alcohol in a yield of 92%(76).

$$H_3C\text{-}CH{=}CH\text{-}CH{=}CH\text{-}COOH \xrightarrow{LiAlH_4} H_3C\text{-}CH{=}CH\text{-}CH{=}CH\text{-}CH_2\text{-}OH.$$

However, if conjugated with the carboxyl group as in cinnamic acid, the double bond undergoes concurrent reduction yielding 3-phenyl-l-propanol in a yield of 85%.

$$H_5C_6\text{-}CH{=}CH\text{-}COOH \xrightarrow{LiAlH_4} H_5C_6\text{-}CH_2\text{-}CH_2\text{-}CH_2OH.$$

Aromatic acids with electron releasing groups, like the corresponding aldehydes and ketones, are reduced under forcing condition to the corresponding methyl derivatives. For example, p-aminobenzoic acid gives p-toluidine in yield of 47%(75).



3.5 REDUCTION OF HUMIC ACIDS WITH LITHIUM ALUMINIUM HYDRIDE

The reaction of humic acids with $LiAlH_4$ should result in the reduction of aromatic carboxyl functions to benzylic alcohols

$$Ar\,CO_2H \text{------->} ArCH_2OH$$

aliphatic carboxyl functions to primary alcohols

RCO_2H -------> RCH_2OH

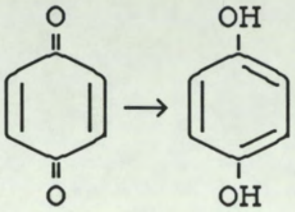
aldehydic functions to primary alcohols

RCHO -------> RCH_2OH

ketone functions to secondary alcohols

C = O ------> CHOH

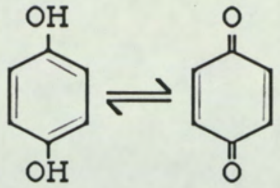
and quinones to phenols.



It was hoped then to remove the -OH functions by conversion to tosyl or other derivatives which will be discussed in detail in a later chapter. Although $LiAlH_4$ is a powerful reagent for the reduction of carbonyl functions it appeared from i.r that the carbonyl functions were not affected by $LiAlH_4$ under normal conditions. The i.r spectra of the $LiAlH_4$ treated materials showed no reduction of the C = O peak at 1720 cm^{-1} and no indication of any change in the -OH band at 3300 cm^{-1}. It would be expected, if $-CO_2H$ were converted to CH_2OH, that the -OH stretching band would decrease in width due to the reduction in the strength of the H-bonding in alcohols compared with carboxylic acids. It seems likely that the main reason for the lack of reaction of humic acid with $LiAlH_4$ is that it is essentially the heterogenous reaction of two solids. Although diethyl ether is low boiling and the conditions of the reaction are exceedingly mild, the reason for the choice of diethyl ether is that it is the best solvent for $LiAlH_4$. At least 30% of $LiAlH_4$ had approximately dissolves in diethyl ether.

$LiAlH_4$ is less soluble in other commercially used stronger higher boiling ethers such as THF and DME. However, since it was thought that the reaction might be promoted by higher temperatures attempts were made to reduce the HA's with $LiAlH_4$ in DME but the i.r spectra of the products still gave no indication that reduction had occurred even under the more severe temperature conditions. The reaction in THF was rather more promising when a humic acid was refluxed with an excess of $LiAlH_4$ in THF for 48h. The i.r spectra of the product showed a decrease in the $C = O$ absorption at 1720 cm^{-1} relative to the aromatic $C = C$ at 1600 cm^{-1}, there was an increase in the aliphatic -CH stretch and changes in the shape, position and intensity of the -OH absorption at 3400 cm^{-1}. A number of reactions were then carried out to ascertain conditions required for complete removal of the $C = O$ absorption. It was found that refluxing of the HA for six days with an excess of $LiAlH_4$ followed by stirring for another six days at room temperature gave a product which showed no $C = O$ absorption in the i.r spectra. Rather surprisingly, the six days reflux followed by the six days stirring at room temperature was more effective in removing $C = O$ than reflux for 12 days. All humic acids were then reduced using the 6 + 6 reduction conditions. The i.r spectra of the reduced products are given in Figures (18-22) and the hydroxyl contents as determined by acetylation of the reactants and reduced products are given in (Table 16 page 83).

It is essential that the reduced products be dried in a vacuum desiccotor or vacuum oven. If dried in air some oxidation occurs and a small $C = O$ peak reappears. It seems that some reduced functional groups are very readily reoxidised by air. It is unlikely that aliphatic alcohols are undergoing oxidation under these conditions. We have observed that even the benzyl alcohols which have more labile C-H bonds than the aliphatic alcohols do not readily undergo air oxidation. The -OH groups most likely to be undergoing oxidation are the hydroquinone functions. This is a very labile oxidation reduction system.



It has been observed that sonic waves promote heterogeneous reactions. For example the Wurtz-type coupling of organic halide RX (R=alkyl, aryl ,benzyl, X = Cl, Br and I)(77) and organometallic chlorides R_3MCl (R = alkyl, aryl, M = Si, Sn)(78) using lithium wire at room temperature proceeds at a convenient rate only in the presence of sonic waves. Ultrasound accelerates the Reformatsky reaction(79), the $LiAlH_4$ reduction of aryl halides(80), the Barbier reaction(81) and the catalytic reduction of alkenes and ketones to hydrocarbons(82). Since the reduction of the HA's with $LiAlH_4$ proceeded so slowly and since ultrasound appeared to promote other heterogenous reactions the effect of sonication on the $LiAlH_4$ reduction of HA's was investigated. Irradiation with ultrasonic waves for 6h of 1g of a HA's in the presence of an excess of $LiAlH_4$ in THF gave a product whose i.r indicated that it had undergone no significant reduction. Since it appeared that our relatively mild sonication technique did not effect any spectacular increase on the reduction rate we did not investigate the sonication reaction any further. Since, however, HA reactions are almost always heterogeneous this is a technique which is worthy of more detailed investigation in the future. It is possible that more powerful sonication apparatus would be required to break up the intransigent humic materials.

Figure 18: I.r. Spectrum of Reduced Humic Acid (Aldrich) (Compared with i.r. spectrum of Humic Acid page 94)

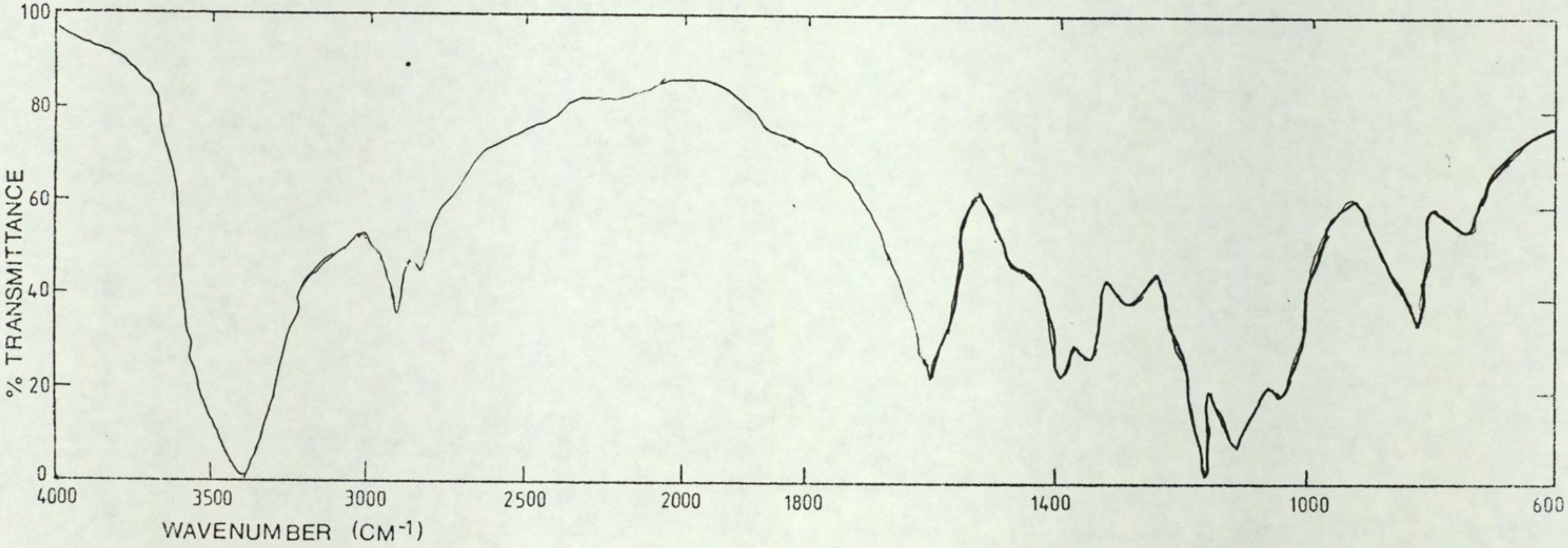


Figure 19: I.r. Spectrum of Reduced Humic Acid (Fluka) (Compared with i.r. spectrum of Humic Acid Page 95)

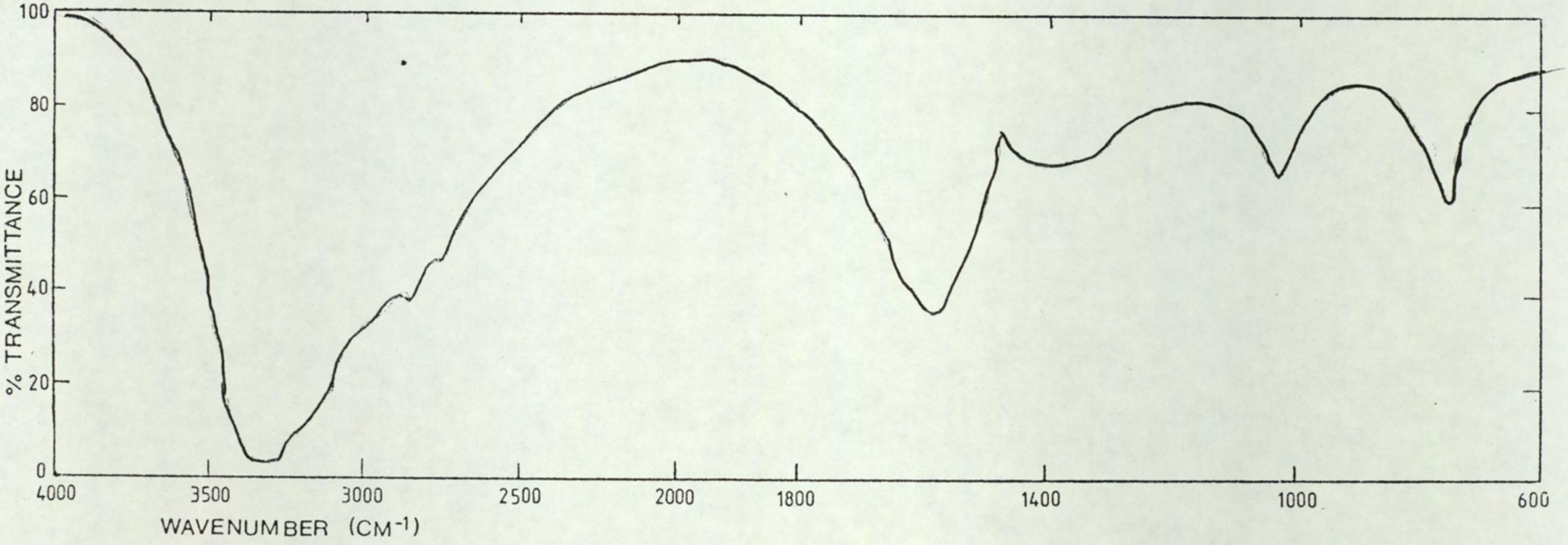


Figure 20: I.r. Spectrum of Reduced Humic Acid (Lignite) (Compared with i.r. spectrum of Humic Acid page 96)

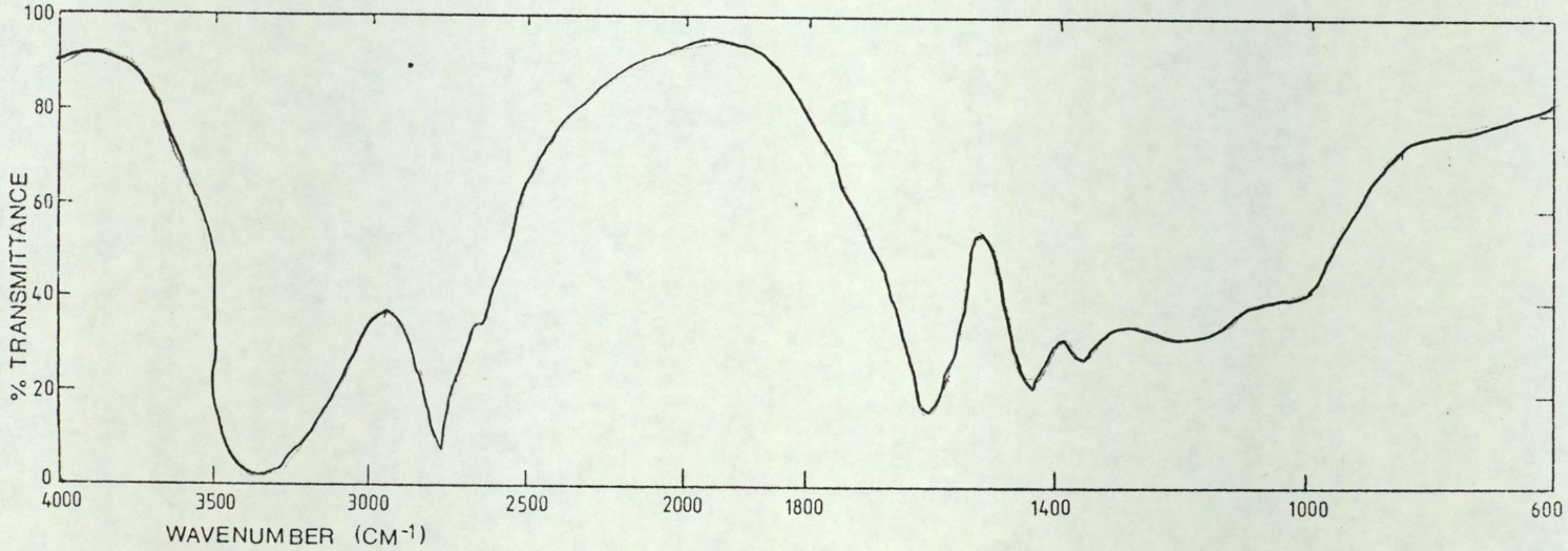
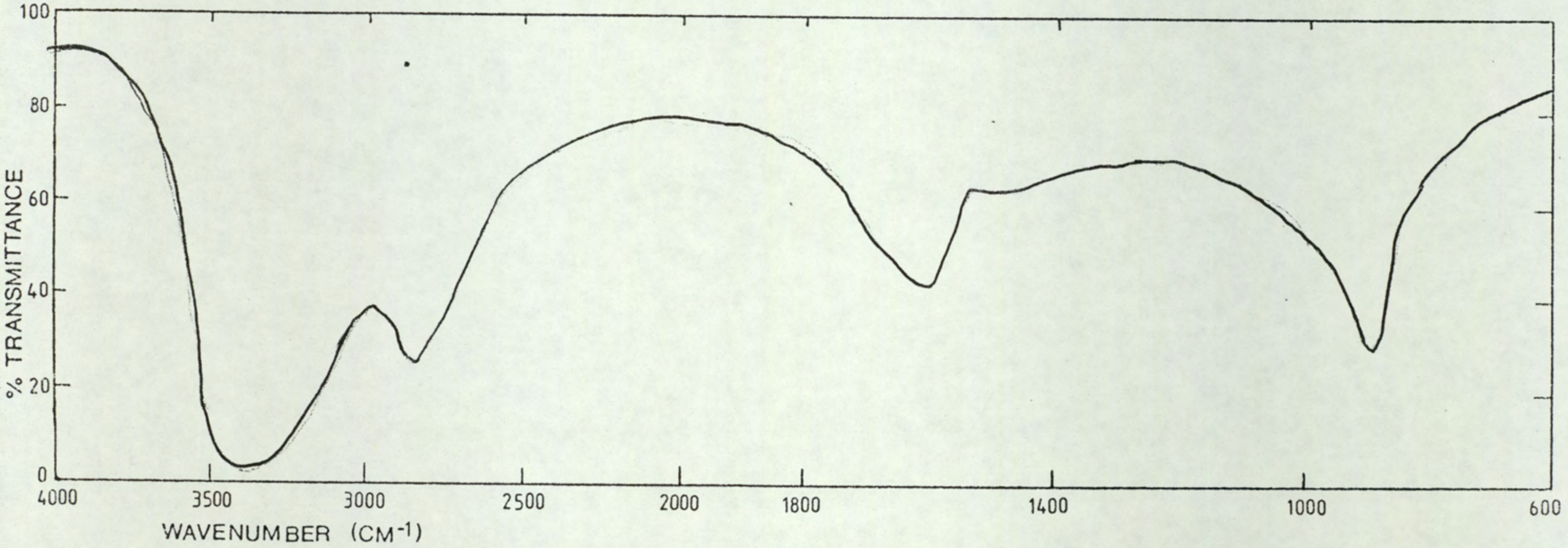


Figure 21: I.r. Spectrum of Reduced Humic Acid (Norfolk) (Compared with i.r. spectrum of Humic Acid page 97)



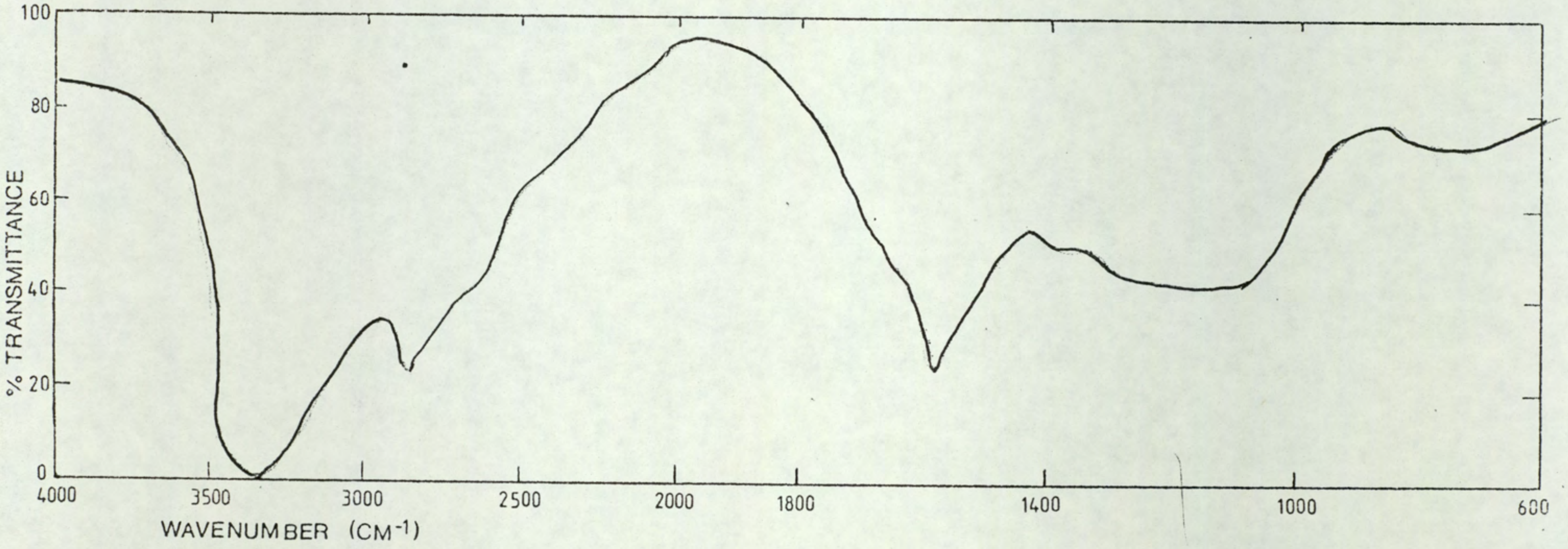
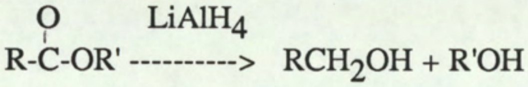


Figure 22: I.r. Spectrum of Reduced Humic Acid (Reading) (Compared with i.r. spectrum of Humic Acid page 98)

As discussed in later chapters on methylation, phase transfer catalysis is another technique widely applied to promote heterogeneous reactions. A brief investigation of the PTC reduction of HA was carried out. The HA was treated with 5g of $LiAlH_4$ and 30 cm^3 of t-butylammonium hydroxide for 48 hours. There was no indication of reduction and since we could not easily find a more rapid reduction technique for the HA's, we continued to use our effective but time consuming reduction with $LiAlH_4$ in THF.

If the HA's contained ester functions as previous investigations have shown then the $LiAlH_4$ would not only reduce C = O functions but would also cleave the ester groups.



$$\mathrm{R\text{-}\overset{\overset{O}{|}}{C}\text{-}OR'} \xrightarrow{LiAlH_4} \mathrm{RCH_2OH + R'OH}$$

It could also cleave certain types of labile ethers such as benzyl or allyl. So, as well as acting as a reduction agent, the $LiAlH_4$ could also act as a relatively mild and specific cleavage reagent giving structurally meaningful fragments.

The $LiAlH_4$ reduced materials were methylated and the methylated products were examined by i.r and highfield nmr and gc/ms.

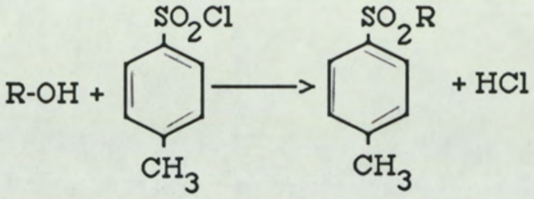
The i.r spectra were well resolved but did not give any new information, in contrast, the high field nmr spectra did in some cases yield new structural information.

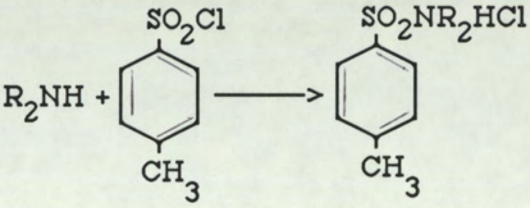
CHAPTER 4

TOSYLATION

4.1 INTRODUCTION

p-Toluene sulphonyl chloride (tosyl chloride) reacts generally with -OH and -NH functions to give derivatives known as tosylates.





These reactions are carried out in basic media. The tosyl chloride is so slowly hydrolysed by water that with relatively reactive -OH and =NH functions tosylation can be achieved in aqueous base. Weakly basic amines and unreactive -OH derivatives react so slowly with tosyl chloride that most of the acid chloride is hydrolysed by aqueous alkali before a reasonable yield of the sulphonamide is produced. With such compounds excellent results are normally obtained by carrying out the reaction in pyridine solution.

The alkyl esters of sulphonic acids are in general hydrolysed by hot aqueous alkali to the corresponding alcohol and sulphonic acid, under the same circumstances dehydration may also occur to give an olefin.

$$C_2H_5OSO_2Ar \text{ --------> } C_2H_4 + Ar\ SO_3H$$

Reduction of alkyl esters of sulphonic acids with $LiAlH_4$ generally results in the formation of a hydrocarbon by replacement of the sulphonic group by hydrogen.

$$R\ OSO_2Ar \xrightarrow{LiAlH_4} RH + Ar\ SO_3H$$

This reduction provides a simple and elegant method of replacing a hydroxyl group by hydrogen. It has been applied successfully to the preparation of branched chain paraffins from the corresponding alcohols and reduction of p-toluene sulphonic esters in the sugar series has been exploited to a considerable extent for the replacement of hydroxyl by hydrogen although there are some exceptions where the sulphonyl group is reduced off to leave the original hydroxyl group. The application of this reduction method to the replacement of hydroxyl in steroids has, however been less uniformly successful(104).

The reduction of sulphonate esters by a complex metal hydride reagent such as $LiAlH_4$ is a nucleophilic substitution reaction with the hydride ion acting as a nucleophile.

$$M^+H^- + R\text{-}CH_2{}^{\delta+} - {}^{\delta-}OSO_2Ar \text{ -----------> } R\text{-}CH_3 + Ar\,SO_3\,M$$

In aromatic systems the common mode of substitution is electrophilic, nucleophilic substitution occurs only very slowly under extraordinary conditions. Thus the sulphonic esters of phenols are reduced usually by $LiAlH_4$ back to phenols.

In the introduction to this thesis it was pointed out that reduction followed by tosylation, then further reduction appeared to present a reasonable route (see Fig. 5) for the mild, controlled removal of a substantial proportion of oxygen functions from humic materials. Evidence so far available suggests that in humic acids oxygen is present usually in the form of ethereal, carbonyl and hydroxyl functions.

The carbonyl groups are present mainly in the form of esters, carboxylic acids and quinones and to a lesser extent as ketonic functions. Reduction of aliphatic carboxylic acid and ester functions with $LiAlH_4$ will give alcohols.

$$R\text{-}CO_2H \xrightarrow{LiAlH_4} RCH_2OH$$

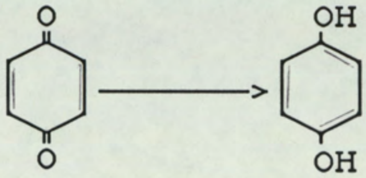
$$R - CO_2R' \xrightarrow{LiAlH_4} RCH_2OH + R'OH$$

Reduction of aromatic carboxylic and ester functions will give benzyl alcohols,

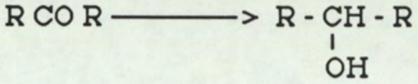
$$ArCO_2H \xrightarrow{LiAlH_4} Ar\,CH_2OH$$

$$ArCO_2R' \xrightarrow{LiAlH_4} Ar\,CH_2OH + R'\,OH$$

Reduction of quinones will give phenols,



Reduction of alkyl or aryl ketones will give secondary alcohols,



$$R\,CO\,R \longrightarrow R-\underset{OH}{\underset{|}{C}H}-R$$

Thus in the $LiAlH_4$ reduced humic acids, oxygen should be present as ethereal and hydroxyl functions. On tosylation this will produce tosylates of the form,

$R\,CH_2\,OTs$ $R_2\,CHOTs$ $R_3\,COTs$

$Ar\,CH_2\,OTs$ and $Ar\text{-}OTs$

On subsequent reduction with $LiAlH_4$.

$RCH_2\,OTs \dashrightarrow RCH_3$

$R_2CH\,OTs \dashrightarrow R_2CH_2$

$R_3C\,OTs \dashrightarrow R_3CH$

$ArCH_2\,OTs \dashrightarrow ArCH_3$

$Ar\,OTs \dashrightarrow ArOH$

Assuming hydroxyl and carbonyl functions predominate over ether and quinone functions, this process should result in the removal of a substantial proportion of oxygenated functions from humic acids.

It has been reported that phenolic tosylates can be reduced to hydrocarbons by hydrogenation with a Raney Nickel Catalyst(83).

$$\mathrm{Ar\,OTS} \xrightarrow[\mathrm{Ra\text{-}Ni}]{H_2} \mathrm{Ar - H}$$

whereas,

$$\mathrm{R\text{-}OTS} \xrightarrow[\mathrm{Ra\text{-}Ni}]{H_2} \mathrm{R\text{-}OH}$$

Thus, if after reduction with $LiAlH_4$ the humic material is retosylated, subsequent hydrogenation should remove the remaining phenolic -OH functions.

Four methods for the tosylation of the $LiAlH_4$ reduced humic materials were investigated.

i) The humic acid was suspended in pyridine and p-toluene sulphonyl chloride was added and the solution was left standing for various lengths of time.

ii) The humic acid was dissolved in 10% aqueous sodium hydroxide and p-toluene sulphonyl chloride was added and the mixture left standing for various lengths of time.

iii) The humic acid was suspended in dry THF and tetrabutylammonium hydroxide was added, then it was left stirring for 24h, and p-toluene sulphonyl chloride was then added and the solution was left stirring for various lengths of time. (The phase transter catylst technique will be discussed on page 89).

iv) The humic acid was suspended in THF and 10% sodium hydroxide the mixture cooled to 0°C and p-toluene sulphonyl chloride was added and the mixture was placed in a refrigerator for various lengths of time.

4.2 SPECTRA OF TOSYLATED HUMIC ACIDS

Infra red spectroscopy can be used to give an indication of whether tosylation has taken place. Tosylation gives rise to two new absorption peaks at (1370 cm^{-1}) and (1175 cm^{-1}). These new peaks are due to the S = O bands, the 1370 cm^{-1} peak arises from S = O asymmetric stretch and the 1175 cm^{-1} peak from the symmetric stretch. In addition the 3400 cm^{-1} band, due to the -OH stretch, decreased and changed shape on tosylation, (see figures 23-24).

4.3 SULPHUR CONTENT

Measuring the sulphur content also gives some measure of tosylation since in these complex systems tosyl groups may be adsorbed rather than reacted and in the phase transfer reaction the catalyst may also be adsorbed into the system. We have not used sulphur content as a quantitative measure of tosylation, but only as a qualitative measure in conjunction with i.r. data. Details of tosylated derivatives are given in tables (6-10).

i) method using pyridine
ii) method using aqueous sodium hydroxide
iii) method using phase transfer catalyst
iv) method using THF and sodium hydroxide

The time = 6 weeks, 24h

i.r peaks l = large
m = medium
s = small

Table (6) %S and Spectra Characteristics of Aldrich HA and Tosylated Derivatives

Sample	%S	3400 cm^{-1}	1370 cm^{-1}	1175 cm^{-1}
Aldrich HA	4.5	Broad		
Tosylated i) 6 weeks	7.36	decrease (m)	increase (l)	increase (s)
Tosylated ii) 1 week	4.44	no change	increase (s)	no change
Tosylated iii) 24h	6.21	decrease (l)	increase (l)	increase (l)
Tosylated iv) 24h	5.67	decrease (m)	increase (s)	increase (s)

Table (7) %S and Spectra Characteristics of Fluka HA and Tosylated Derivatives

Sample	%S	3400 cm^{-1}	1370 cm^{-1}	1175 cm^{-1}
Fluka HA	7.8	Broad		
Tosylated i) 6 weeks	9.2	decrease (m)	increase (l)	increase (s)
Tosylated ii) 1 week	7.7	no change	increase (s)	no change
Tosylated iii) 24h	9.1	decrease (l)	increase (l)	increase (l)
Tosylated iv) 24h	8.9	decrease (m)	increase (s)	increase (s)

Table(8) %S and Spectra Characteristics of Lignite HA and Tosylated Derivatives

Sample	%S	3400 cm^{-1}	1370 cm^{-1}	1175 cm^{-1}
Lignite HA	2			
Tosylated i) 6 weeks	4.5	decrease (m)	increase (l)	increase (s)
Tosylated ii) 1 week	1.9	no change	increase (s)	no change
Tosylated iii) 24h	5.3	decrease (l)	increase (l)	increase (l)
Tosylated iv) 24h	4.6	decrease (m)	increase (s)	increase (s)

Table (9) %S and Spectra Characteristics of Reading HA and Tosylated Derivatives

Sample	%S	3400 cm^{-1}	1370 cm^{-1}	1175 cm^{-1}
Reading HA	2.2			
Tosylated i) 6 weeks	4.3	decrease (m)	increase (l)	increase (s)
Tosylated ii) 1 week	1.9	no change	increase (s)	no change
Tosylated iii) 24h	4.5	decrease (l)	increase (l)	increase (l)
Tosylated iv) 24h	3.8	decrease (m)	increase (s)	increase (s)

Table (10) %S and Spectra Characteristics of Norfolk HA and the Tosylated Derivatives

Sample	%S	3400 cm^{-1}	1370 cm^{-1}	1175 cm^{-1}
Norfolk HA	1.2			
Tosylated i) 6 weeks	3.5	decrease (m)	increase (l)	increase (s)
Tosylated ii) 1 week	1.1	no change	increase (s)	no change
Tosylated iii) 24h	3.3	decrease (l)	increase (l)	increase (l)
Tosylated iv) 24h	2.3	decrease (m)	increase (s)	increase (s)

Since method (III) using the phase transfer catalyst gave consistently the best results this method was employed for all subsequent tosylations.

4.4. Gc/ms ANALYSIS OF TOSYLATION PRODUCTS

Chloroform soluble extracts of the tosylated products were examined by gc/ms. The TIC's of the volatile products are given in figures 25-29 and Tables (11-16), also details of the types of species detected using the SIMs technique. (Secondary ion mass spectrometry)

Figure 23: I.r. Spectrum of Tosylated Humic Acid (Aldrich)

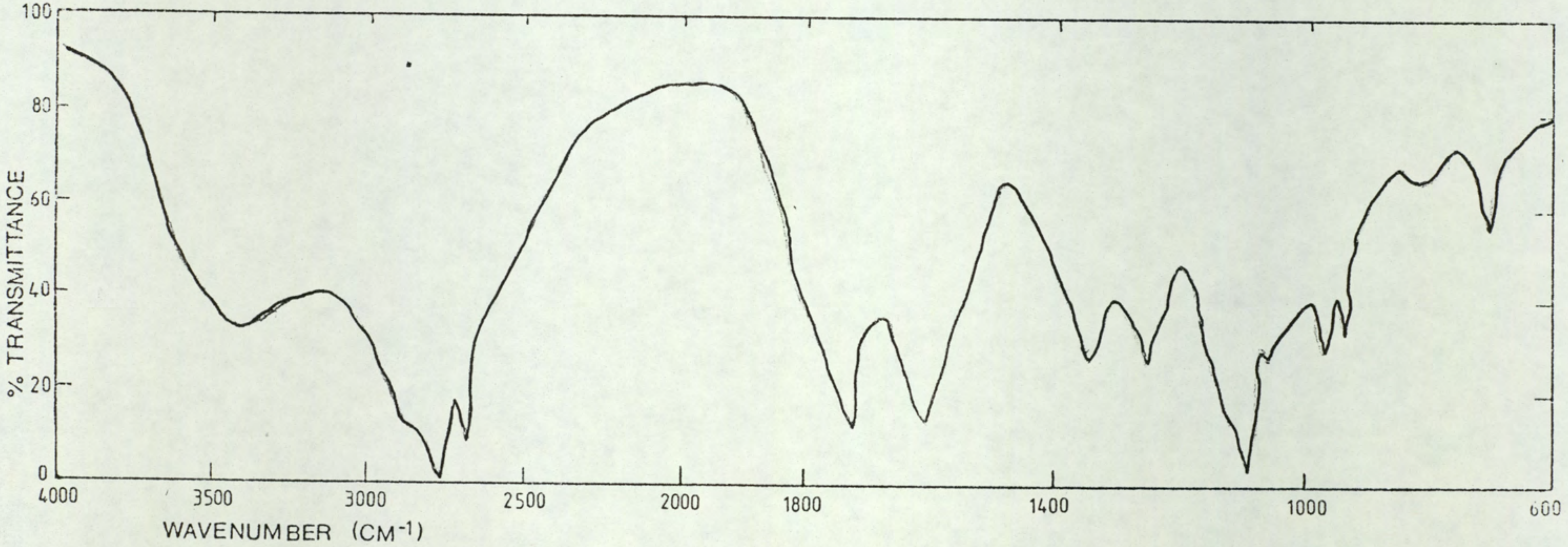


Figure 24: I.r. Spectrum of Tosylated Humic Acid (Fluka)

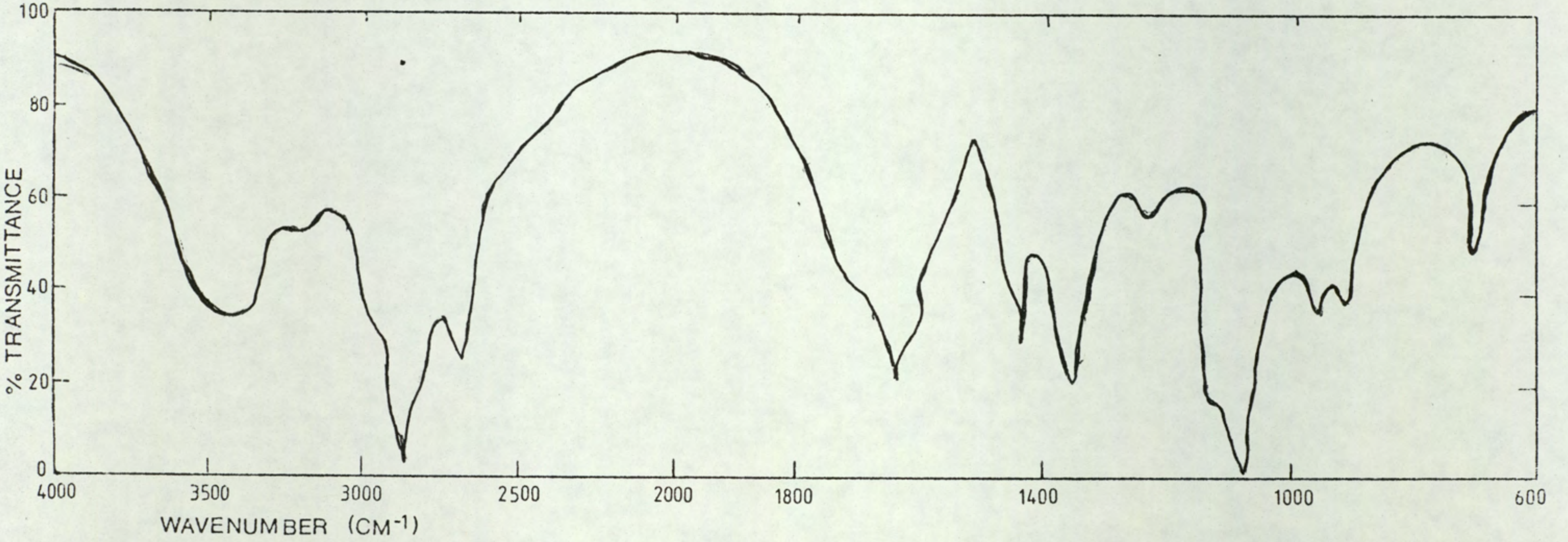


Figure 25: TIC of Chloroform extract of Reduced-Tosylated-Reduced Humic Acid (Reading)

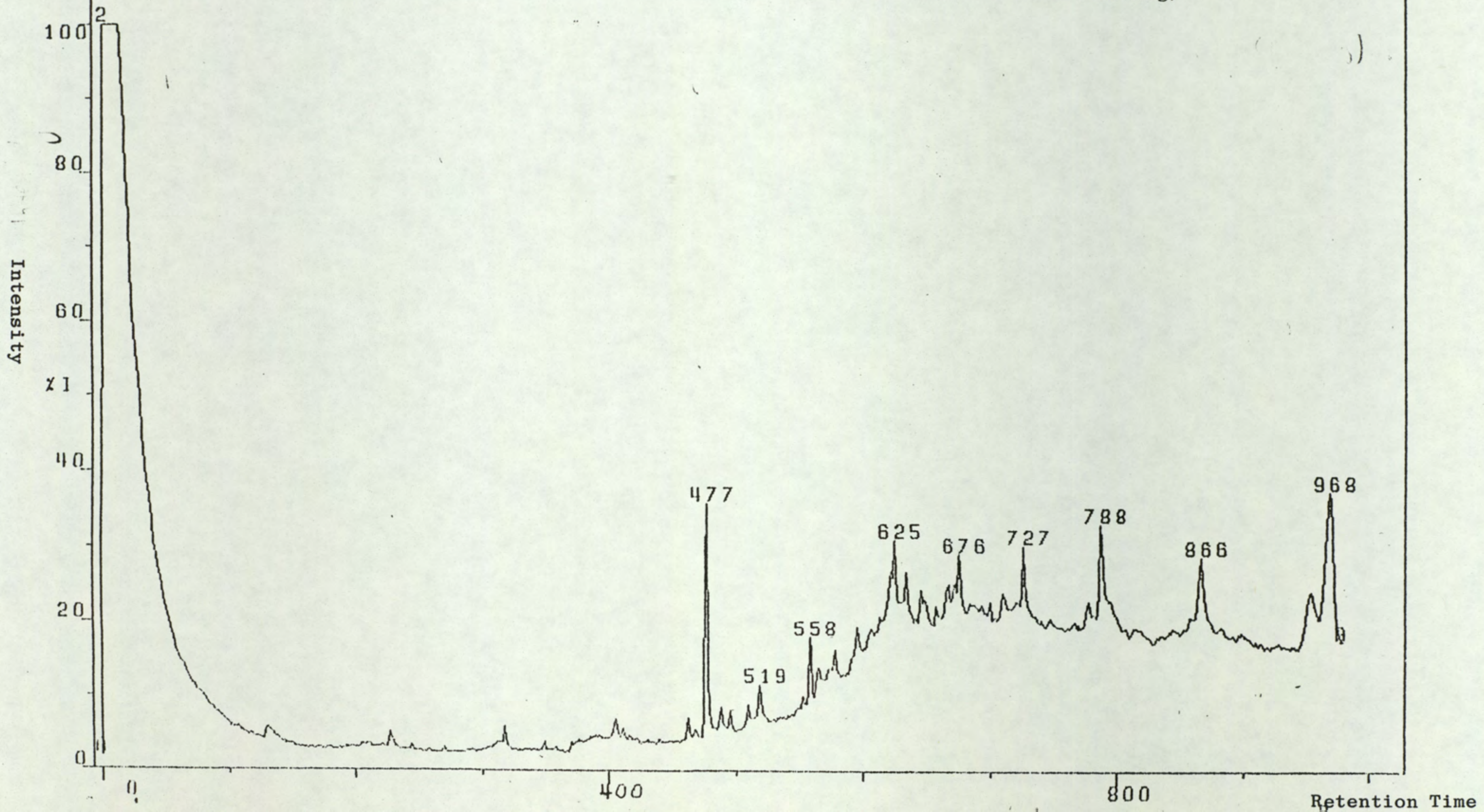
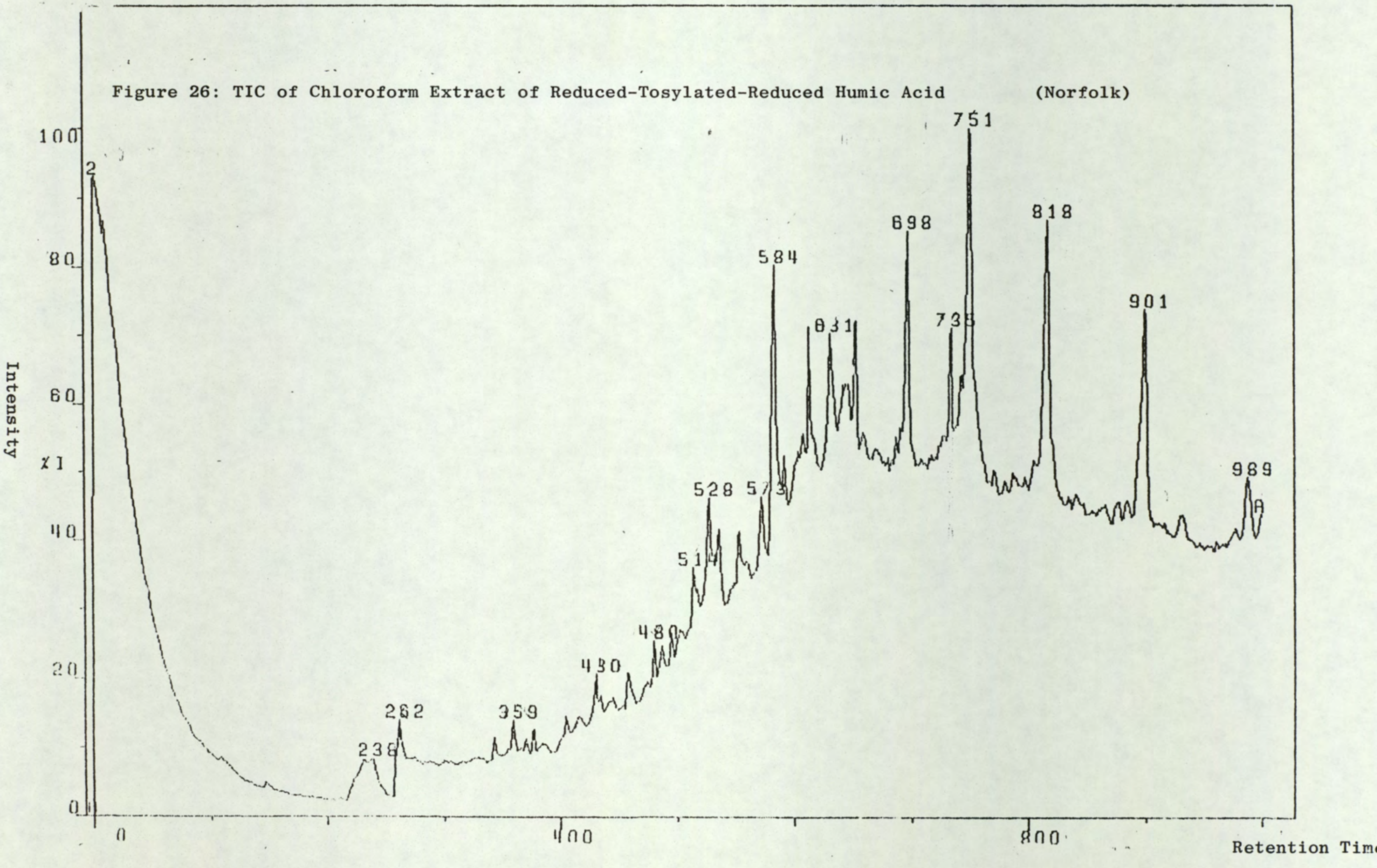


Figure 26: TIC of Chloroform Extract of Reduced-Tosylated-Reduced Humic Acid (Norfolk)



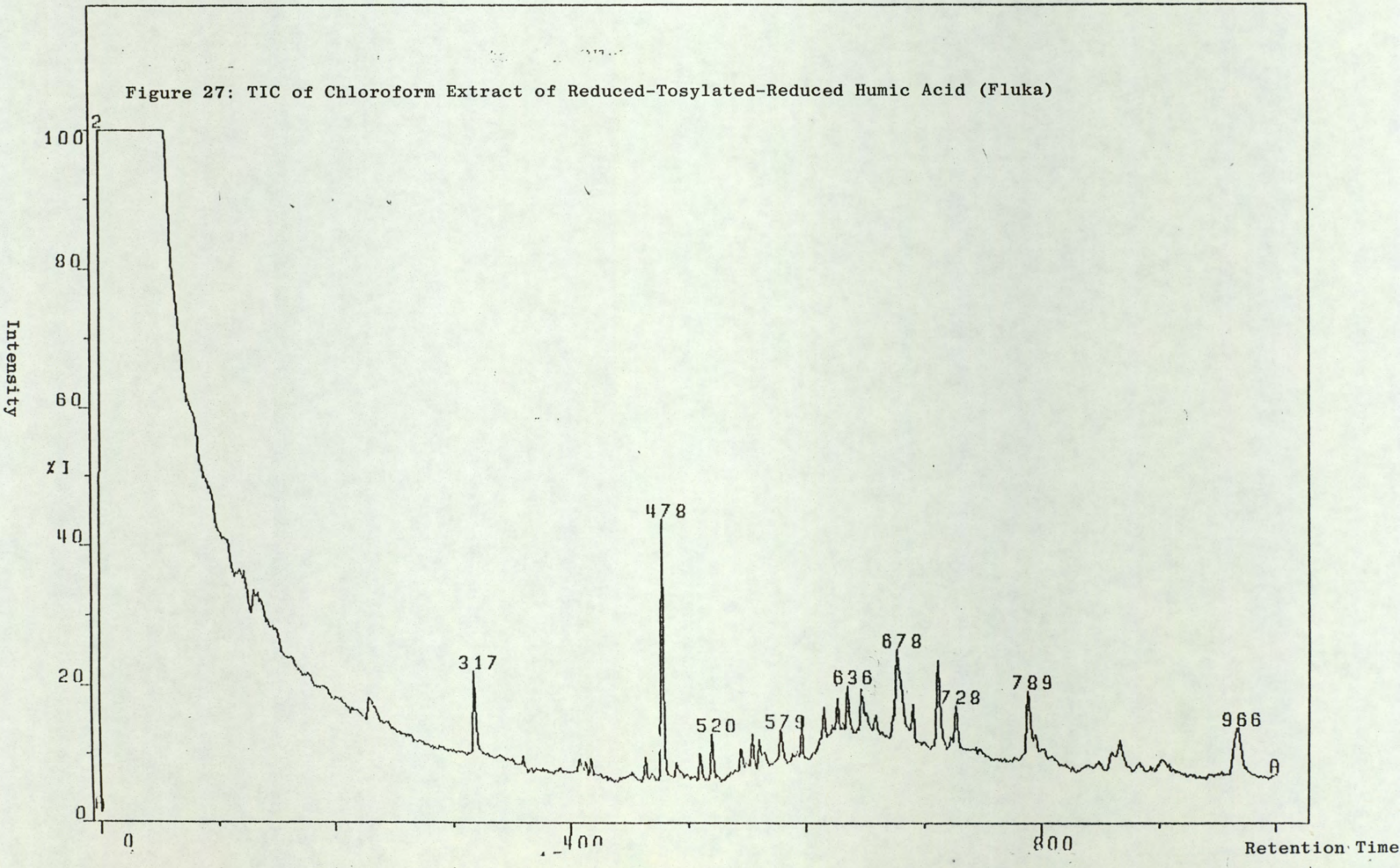
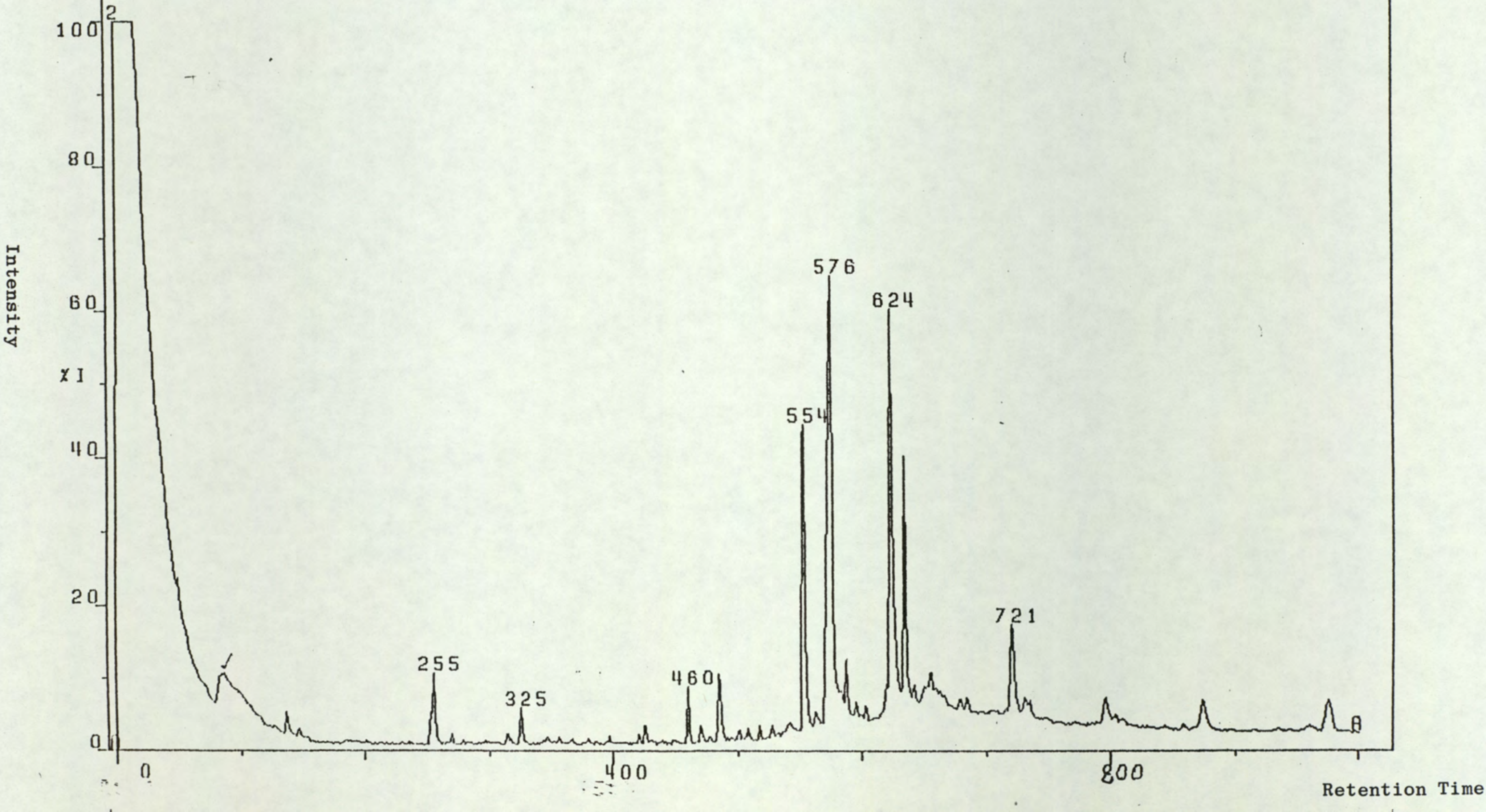


Figure 27: TIC of Chloroform Extract of Reduced-Tosylated-Reduced Humic Acid (Fluka)

Figure 28: TIC of Chloroform Extract of Reduced-Tosylated-Reduced Humic Acid (Lignite)



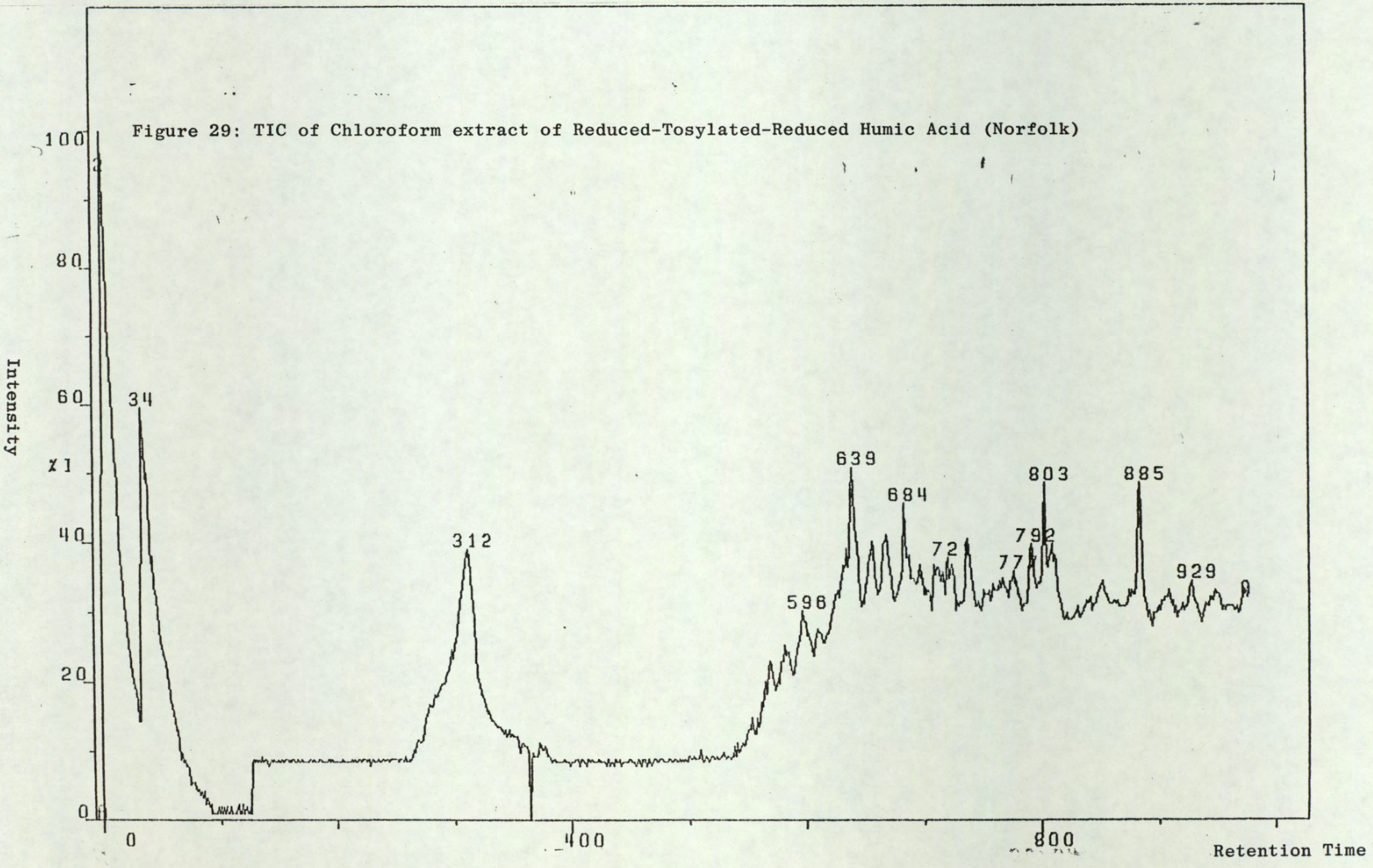


Figure 29: TIC of Chloroform extract of Reduced-Tosylated-Reduced Humic Acid (Norfolk)

Table (11) Reduced Tosylated Product - READING Classes of compounds detected by gc/ms

Alkanes C_{15} - C_{30}	strong
Alkylaromatics	weak
Aromatic -O-	weak

Table (12) Reduced Tosylated product - NORFOLK Classes of compounds detected by gc/ms

Alkanes C_{15} - C_{30}	strong
Alkyl benzenes	strong
Alkyl aromatic diols	medium
Other diols	medium
Furans	weak
Anthracenes	weak

Table (13) Reduced Tosylated Product - FLUKA Classes of compounds detected by gc/ms

Alkanes	C_{15} - C_{30}	strong
Alkyl benzenes		strong
Alcohols		medium
Indenes		weak

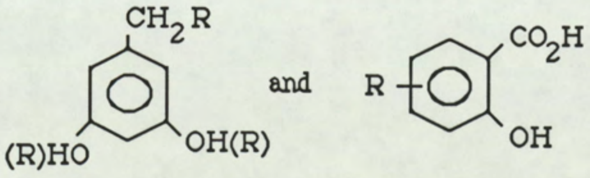
Table (14) Reduced Tosylated product - LIGNITE Classes of compounds detected by gc/ms

Alkanes	C_{15} - C_{30}	strong
Alkyl benzenes		strong
Alcohols		medium
Alkyl aromatic diols		medium

Table (15) Reduced Tosylated Product - ALDRICH Classes of compounds detected by gc/ms

Alkanes	C_{15} - C_{30}	strong
Alkyl benzenes		weak

Much further work will have to be done before the type of information obtained from gc/ms can be translated into real structures. It is apparent that all the humic acids examined incorporated large alkyl chains in their structure. Also in the Norfolk, Fluka and Lignite derived materials there appeared to be a significant contribution to the structure of units with alkyl chains attached to benzene rings. In the Aldrich and the Lignite there was evidence also for contribution from materials of the type shown below, presumably derived from lignin.



4.5 REDUCTION OF TOSYLATED HUMIC ACID

Tosylate groups were removed by treatment with $LiAlH_4$ under the conditions previously used for the reduction of the HA's that was refluxing for six days in THF followed by standing at room temperature for six days. These conditions were found to be most successful for the removal of tosyl groups. The hydroxyl content of the detosylated product was measured using the acetylation procedure of Brooks et al(84).

$$ROH + (CH_3CO)_2O \longrightarrow CH_3COOR + CH_3COOH$$

$$H_2O + (CH_3CO)_2O \longrightarrow 2\,CH_3COOH$$

Table (16) The Hydroxyl Contents

Humic Acid	Reduced Humic Acid	Reduced detosylated
Aldrich 12.4 meq OH/g	9.50 meq OH/g	3.40 meq OH/g
Fluka 11.6 meq OH/g	10.12 meq OH/g	3.83 meq OH/g
Reading 24.19 meq OH/g	15.32 meq OH/g	1.7 meq OH/g
Lignite 11.19 meq OH/g	9.91 meq OH/g	2.30 meq OH/g
Norfolk 15.80 meq OH/g	5.54 meq OH/g	4.24 meq OH/g

Assuming the validity of the acetylation procedure these figures indicate that about 70% of oxygen can be removed using our procedures. The i.r spectra also show that substantial changes have occurred in the character by the oxygenated functions. In the original humic acid spectra, the -OH stretch absorption covers a range from 3400 cm^{-1}, it is very strong, very broad with various submaxima indicating contributions from many types of -OH and oxygen (see Figure 30 page 94), whereas the final reduced products show a much narrower weaker -OH band in the i.r which is to be expected since only one type of -OH, i.e phenolic -OH should now be contributing to the band.

The $LiAlH_4$ reduced products were retosylated using the phase transfer procedure. The i.r spectra indicated that a significant proportion of the phenolic OH groups had been tosylated. Attempts were then made to remove the tosyl groups by hydrogenation using a Raney Nickel Catalyst. The material appeared to take up hydrogen but even after several weeks there was no evidence from the i.r that tosyl groups had been removed. This was surprising since when the reaction was carried out on a model compound, 2-naphthol tosylate, this compound was readily reduced to give a good yield of the reduced products.

It is generally observed that non activated aryl halides are not reduced by $LiAlH_4$, since this reduction is a nucleophilic substitution and aryl systems are usually not susceptible to nucleophilic attack. It has been found that aryl halides are reduced by $LiAlH_4$ if the reaction is carried out in an ultrasonic bath.

$$\mathrm{Ar - X + LiAlH_4 \text{ --------->} Ar - H}$$

Since tosyl groups are better leaving groups than halo groups, it seemed possible that the tosyl groups might be removed ultrasonically.

The tosylated derivatives were reduced with $LiAlH_4$ in an ultrasonic bath for several hours but there was no evidence from the i.r that any reduction had occurred.

Another method which has been used for the removal of phenolic -OH is to convert the OH to its ester salt (II) essentially involving transfer of SO_3 to the phenolic OH,

$$2PhNR_2 \xrightarrow{ClSO_3H} PhN^+R_2HCl^- + PhNR_2SO^-_3 \quad (I)$$

$$\underset{(II)}{ArOH \dashrightarrow ArO.SO^-_3\ H^+NR_2Ph}$$

and then to hydrogenate again with a Raney Nickel Catalyst,

$$ArO.SO_3^-\ HN^+R_2Ph \xrightarrow[-PhNR_2]{KOH\ aq} ArO.SO_3^-\ K^+ \xrightarrow[KOH\ aq]{Raney\ Ni} ArH + K_2So_4$$

This method appeared hopeful since the hydrogenation is carried out in base and with sulphonate functions on the humic acid it was thought the materials might be soluble in base and there was a greater chance of reaction than with the heterogeneous system. However, again there was no evidence from the i.r that reaction had occurred. Attempts were also made to derivatise the phenolic groups of the humic acids by conversion to the phosphate esters:

$$Ar - OH + Cl - P(OEt)_3 \dashrightarrow Ar - O - P(OEt)_3$$

since these esters, like the tosyl derivatives, can be reduced to the hydrocarbon:

$$Ar - O - P(OEt)_3 \xrightarrow{H_2} Ar - H$$

We reacted the humic acid with the chlorophosphate using a number of different conditions including phase transfer catalysis, but i.r. analysis of the products gave no indication of incorporation of the -P $(OEt)_3$ group into the humic acids.

Although we were unsuccessful in completely removing -OH groups from the humic acids, this mode of approach would appear to be the most promising method for defunctionalisation of the humic acids, all that remains is to find a successful method for the removal of phenolic -OH.

Prior to following the route discussed in this chapter much time had been spent trying to decarboxylate the humic acids following the Benkeser(103) procedure.

This method is very succcessful when applied to model compounds. The carboxylic acid is reacted with silicochloroform and a tertiary amine in a polar aprotic solvent.

$$\mathrm{R - CO_2H \;+\; Si\,HCl_3 \;+\; R_3N \;\xrightarrow{CH_3CN}\; R\text{-}CH_3}$$

We applied this procedure to the humic acids, titration evidence indicated that decarboxylation was not occurring to any great extent.

CHAPTER 5

METHYLATION

5.1. INTRODUCTION

Whatever their origin and whether predominantly aliphatic or aromatic all humic acids are highly branched, cross-linked and highly substituted with hydroxyl, carbonyl, ether and nitrogen functional groups. In consequence these systems are strongly hydrogen bonded and it is thought that it is these secondary interactions which are for a large part responsible for the insolubility in organic solvents and general intractability of humic acids. Much research has gone into developing methods for the protection of active hydrogen sites and reducing the hydrogen bonding interactions. Methylation and acetylation have been the most extensively investigated protective techniques.

Methods for the methylation of carboxyl and hydroxyl functions in humic acids were studied in some detail by Briggs and Lawson(85). Several humics were methylated by reaction with:

(i) Methanol and concentrated sulphuric acid.

(ii) Methyl iodide and silver oxide in dimethylformamide.

(iii) Methyl iodide and barium oxide and hydroxide in dimethylformamide.

(Methods (ii) and (iii) were used originally by Kuhn(86) for the methylation of sugars).

(iv) Methyl iodide and solid sodium hydroxide(87).

(v) Dimethyl sulphate and aqueous sodium hydroxide.

(vi) Dimethyl sulphate and solid sodium hydroxide

(vii) Dimethyl sulphate and anhydrous potassium carbonate in acetone (this method has been used widely for the methylation of hydroxyanthraquinones)(88).

They found that methods (ii-vi) were unsuitable because methylation was slow or incomplete, or for other reasons; e.g. silver oxide appeared to cause some oxidation and barium compounds gave products with a high ash content. Diazomethane was not tested because previously it had been found that its reaction with humic acids always led to products with increased nitrogen contents. The first and last methods were found to be the most useful, the former esterifies carboxyl groups only while the latter methylates both hydroxyl and carboxyl groups. After three treatments of the humic acid with dimethyl sulphate and potassium carbonate in acetone a product was obtained with a methoxyl content of 20.8% which was raised very slightly by prolonged fourth treatment to 21.1%. I.r. measurements made on the product with 20.8% methoxy, showed a peak, not present in the spectrum of the original humic acid, in the 1660 cm^{-1} region. Alkaline hydrolysis reduced the methoxyl content to 5.5% and eliminated the peak at 1660 cm^{-1}.

Esterification of humic acid with methanol and sulphuric acid yielded after two prolonged treatments a product with maximum methoxyl content of 11.2% The i.r spectrum of this product when compared with that of the original humic acid, showed the expected increase in absorption at 1740 cm^{-1} due to the formation of methyl esters but no change at 1660 cm^{-1}.

Functional group analysis indicated that methylation was incomplete and quantitative methylation could not be achieved even after repeated reaction. It was thought this may be due to the presence in the humic acid of hydroquinones or other functions containing hydroxyl groups bonded or activated by conjugated carbonyl groups.

It is found that where hydrogen bonding in humic material is reduced by methylation, and organic solubility is increased, better resolved, less diffuse spectra can be obtained from the soluble material.

Barton and Schnitzer(89) introduced a nondestructive method for the spectral analysis of humic acid based on exhaustive methylation, extraction of the methylated material into benzene, followed by separation over Al_20_3. The method has been extended or modified by Ogner and Schnitzer(90) and Khan and Schnitzer(91) to include thin - layer and gas chromatographic separation, and identification of compounds by mass spectrometry and i.r spectrophotometry.

One of the main problems associated with trying to react materials such as coals and humic acid is their lack of solubility, only in basic solution is there any possibility of obtaining a homogeneous type reaction with humic acids and even in base there is some micellar colloidal type character to the solutions. In the reactions commonly carried out on humic material the reactant will be in one phase and the humic in another e.g. in alkylation, the alkylating agent will be in the organic phase and the humic material in the basic aqueous phase. In oxidising acid or neutrilizeing media, the oxidising agent will be in the aqueous phase while the humic acid is insoluble. For most reactions which proceed by a bimolecular mechanism the transformation occurs only in the same phase and close to one another. There are examples of successful heterogeneous reactions but these generally depend on some slight solubility of the one reactant in the other or of both in a mutual cosolvent. Organic chemists have looked for solutions to mutual insolubility problems for many years. A number of approaches have been looked at and have been successful for example the use of dipolar aprotic solvents. These substances commonly have lipophilic alkyl groups and polar functional groups so that they dissolve in water and organic media. Dipolar aprotic solvents have certain disadvantages, they are generally difficult to purify and to remove after reaction is complete. In the late sixties another technique emerged as a method for overcoming the mutual solubility problem, that was phase transfer catalysis. The general technique called phase transfer catalysis can be applied to a number of different types of reactions. The common procedure is to use concentrated solutions with an aqueous and an organic phase. The catalyst, usually a cation with organic groups which are sufficiently large to provide solubility in organic solvents, is added to the reaction medium. The catalyst will transport a nucleophile from the aqueous layer across the phase boundary to attack a substrate in organic phase. The product remains in the organic layer and the catalyst can then transport another nucleophile back across the phase boundary into the aqueous layer and repeat the cycle. A typical example is the catalytic conversion of 1-chloro octane(94) into 1-cyano octane shown schematically below:

$$\begin{tikzcd}
Q^+CN^- + R-Cl \arrow[r] \arrow[d, shift left] & R-CN + Q^+Cl^- \text{ organic phase} \arrow[d, shift left] \\
Q^+CN + Na^+Cl^- \arrow[u, shift left] \arrow[r, shift left] & Na^+CN^- + Q^+Cl^- \text{ aqueous phase} \arrow[l, shift left] \arrow[u, shift left]
\end{tikzcd}$$

interface

Q^+ = quaternary ammonium cation.

It has been shown as in the example given above, that those reactions involving nucleophilic attack on organic substrates generally proceed with greater ease and efficiency using the phase transfer technique. It would appear that this method is particularly applicable to humic studies since the oxidation and alkylation reactions in common use for humic structural analysis belong to this class.

(a) Oxidation.

Phase transfer of anions such as permanganate, chromate and hypochlorite(95) into non-polar media produces highly active oxidising systems whose activity depends upon the anion and the solvent. It would be expected that such systems would be more effective than the aqueous oxidising media normally used for humic degradation and they would have the added advantage that, because of the relative ease with which activity can be modified, it should be possible to confer high specificity and selectivity upon the oxidation process.

(b) Reduction

Phase transfer methods can also be developed for the reductive cleavage of ethereal linkages in humic acid. It has been shown that ethers can be cleaved using borohydride/alkyl halide systems and that the BH_4^- anion can be transferred from an aqueous to an organic phase using a tetraalkylammonium salt(92).

(c) Alkylation

Alkylation, silylation and acylation procedures are widely used in humic studies to block acidic - OH and other active hydrogen functions and generate more soluble and usually more manageable products. Hydroxyl and amino functions react with aqueous base in the presence of a phase transfer catalyst to give anions which alkylate readily with electrophiles such as alkyl-halides. It has been demonstrated that such alkylation can also be effected using clay-supported phase transfer catalyst. The advantages offered by this technique are greater ease of phase separation together with shorter reaction times. The ease of phase separation could be advantageous when dealing with complex mixtures from humic reactions. In general it would appear that by means of phase transfer protection of active groups could be achieved more efficiently and under milder conditions(93,94. Liotta(95) developed phase transfer methods for the selective alkylation and ether cleavage of coals. In the alkylation reaction the coal was suspended in THF (or toluene) and a sufficient amount of a 40% aqueous solution of tetrabutyl ammonium hydroxide to neutralise the acidic hydroxyls was added, the mixture was stirred for some time and then methyl iodide was added. It was found that the reactions taking place could be described by the following equations:

(Coal) - OH + $(C_4H_9)_4$ NOH ------> (Coal) - $ON(C_4H_9)_4$ + H_20.

(Coal) - COOH + $(C_4H_9)_4$ NOH ------> (Coal) - $COON(C_4H_9)_4$ + H_20

(Coal) - $ON(C_4H_9)_4$ + CH_3 I ------> (Coal) - OCH_3 + $(C_4H_9)_4NI$

(Coal) - $COON(C_4H_9)_4$ + CH_3I ------> (Coal) - $COOCH_3$ + $(C_4H_9)_4NI$

The i.r spectra of the methylated coals showed no -OH stretching absorption in the 3400 cm^{-1} region indicating that methylation had occurred in a relatively efficient fashion. The methylated coals were substantially more soluble than the original coals in organic solvents and gave resolved and more easily interpretable i.r and nmr spectra.

5.2 THE USE OF PHASE TRANSFER CATALYSIS

It has been suggested (96) that the resistance of humic acids to chemical attack by many of the reagents arises from the fact that humic acids form a molecular aggregate in solution that must be diaggregated in order that specific functional groups in the humic acid molecules be exposed to chemical reaction.

To try and overcome the problems associated with aggregation and heterogenuity we decided to use the phase transfer method for the alkylation and as discussed previously tosylation of humic acids. Our purpose in alkylation was to try to make them more soluble in organic solvents.

The five humic acids were methylated using the Liotta(95) procedure described above. Since some difficulty was found in removing tetrabutyl ammonium hydroxide in the work up the use of another catalyst, tetrapropy-ammonium hydroxide was also investigated. Two humic acids (Aldrich, Fluka) were also ethylated using the Liotta procedure with ethyl iodide in place of methyl iodide to see if greater solubility could be achieved with larger alkyl groups.

The phase transfer methylated products appeared to be considerably more soluble than the original humic acids. Some showed up to 35% solubility when soxhlet extracted with chloroform. This high solubility suggested that the acids had been efficiently methylated and the hydrogen bonding interactions removed.

The i.r and n.m.r spectra of the methylated products showed bands arising from the phase transfer catalyst. The work up process for the removal of the phase transfer catalyst was repeated several times, and various alternative processes for removal of the catalyst were investigated. We found it impossible to remove the catalyst completely from these systems. It is difficult to determine the content of contamination by catalyst since alkyl bands of the catalyst overlap with alkyl bands from the humic materials in both the i.r and n.m.r. The amount of nitrogen in the humic acid is low and when present with phase transfer catalyst cannot be used as a measure of the phase transfer catalyst contamination content. It appears to us that the phase transfer catalyst forms complexes with the humic materials, possibly the nitrogen interacts with aromatic clusters, and because of this it is impossible to completely remove the catalyst from the humic acid. Solubility and elemental analysis of the methylated products are not very meaningful since the phase transfer catalyst content of the products is not known.

Infra red spectra of original humic acids show major absorption bands occurring at about 3400 cm^{-1} (OH stretching), 2900 cm^{-1} (aliphatic stretching), 1720 cm^{-1} (C = 0 stretching of COOH and ketones), 1620 cm^{-1} (aromatic C = C and/or H-bonded C = 0), and 1200 cm^{-1} (C - 0 and OH deformation of COOH).

Infra red spectra of methylated humic acids (figures 35 - 43), shows an increase in the intensity of the band due to C = 0 stretching at 1720 cm^{-1}, a reduction in OH stretching vibrations of COOH and OH groups in the 3600 to 3100 cm^{-1} region, and introduction of bands caused by C - H stretching and bending of added CH_2 groups at 2910 and 1440 cm^{-1}, respectively. In addition, absorption due to C - 0 stretching vibrations at 1250 cm^{-1} was sharpened because of methylation of acidic OH groups. Although much reduced, the bands in the 3600-3100 cm^{-1} region never completely disappear showing that the phase transfer catalyst methylation and other alkylation methods are not completely quantitative. Two mechanisms have been proposed(97-100) for the increased absorption at 1720 cm^{-1} when humic acids are methylated. The first is the conversion of COOH to the ester form (R-$COOCH_3$) enhancing the intensity of the existing C = O band, and secondly the elimination of ketonic C = O with subsequent shift in C = O absorption frequencies from about 1620 cm^{-1} to near 1700 cm^{-1}. The second mechanism implies an unusually large shift in C = O absorption frequencies (up to 80 cm^{-1}). In practice, both mechanisms may be operative, with any effect being overshadowed by ester formation. Intensification of the 1720 cm^{-1} band as a consequence of esterification is likely because the C = O of ester absorbs within a narrower range

Figure 30: I.r. Spectrum of Humic Acid (Aldrich)

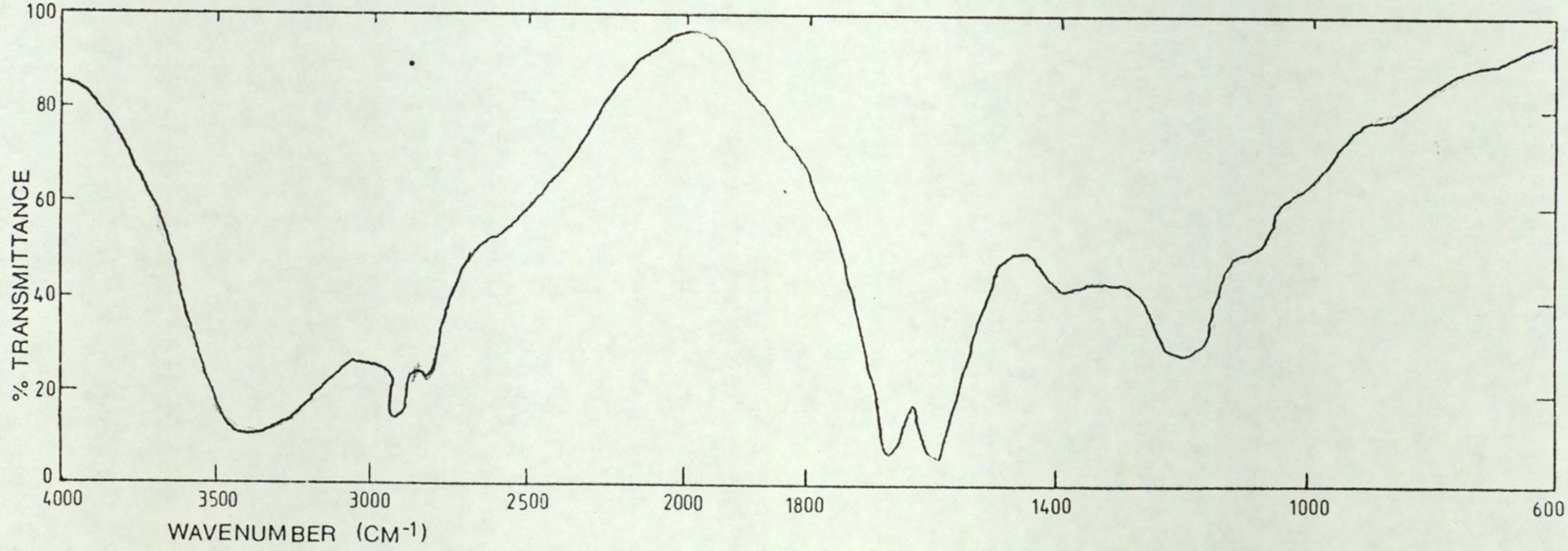


Figure 31: I.r. Spectrum of Humic Acid (Fluka)

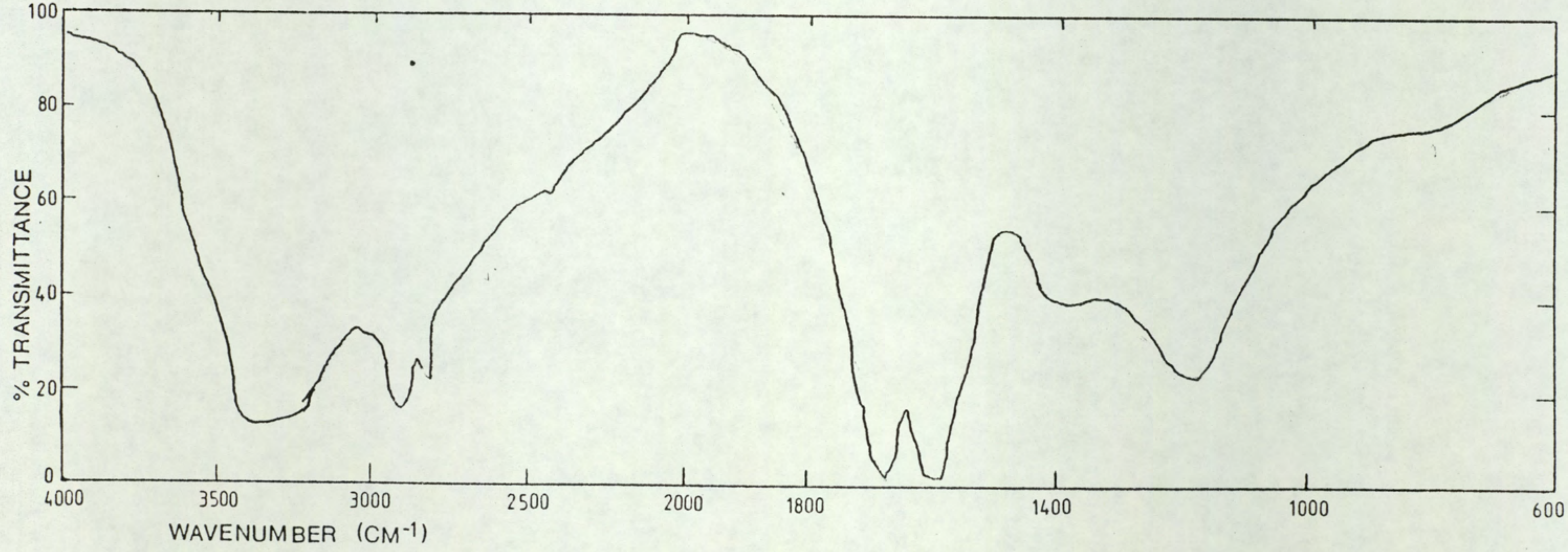


Figure 32_ I.r. Spectrum of Humic Acid (Lignite)

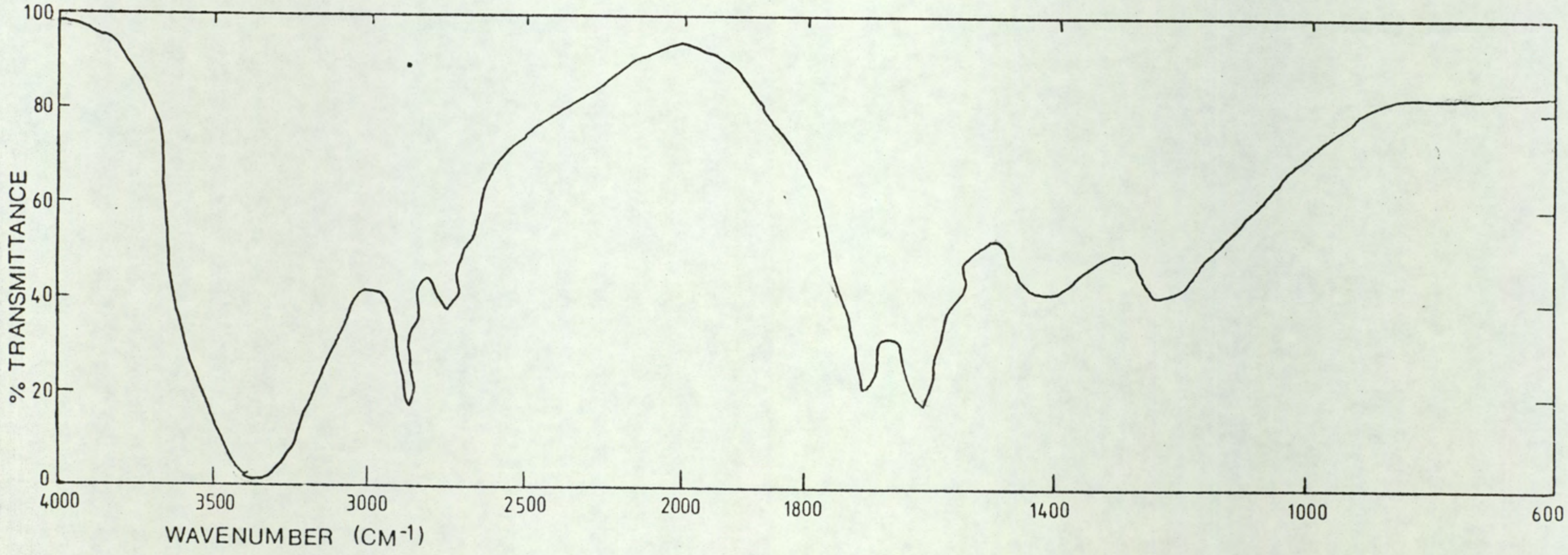


Figure 33: I.r. Spectrum of Humic Acid (Norfolk)

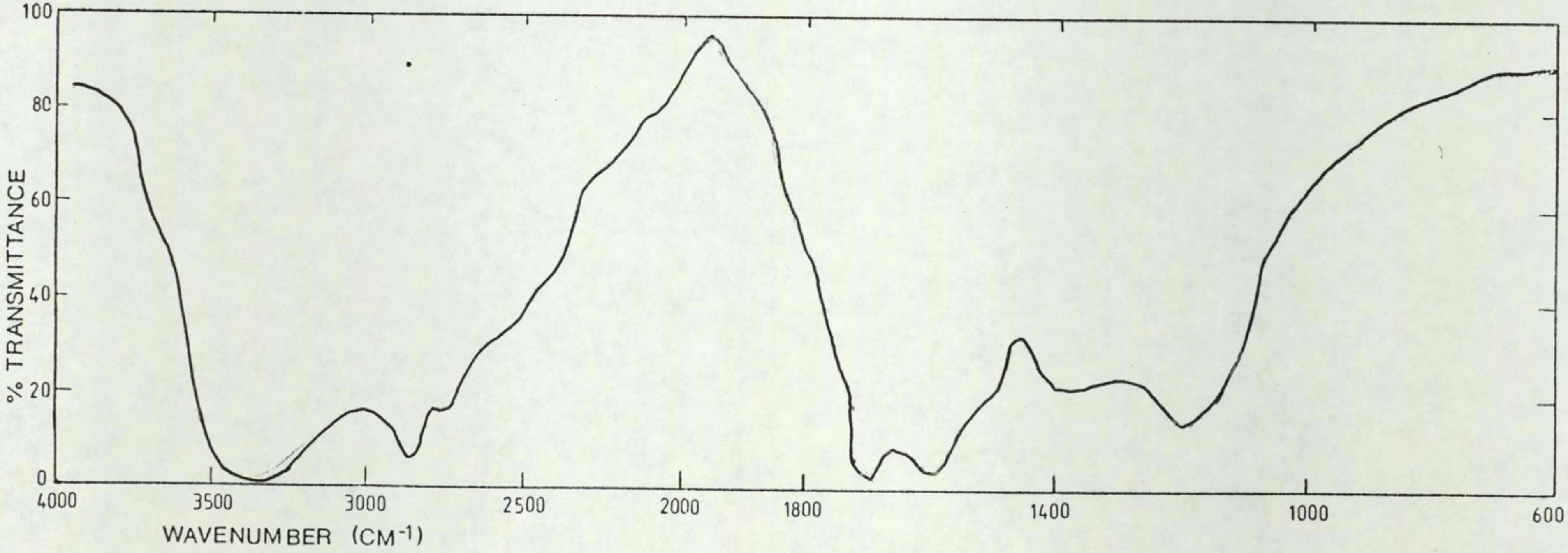


Figure 34: I.r. Spectrum of Humic Acid (Reading)

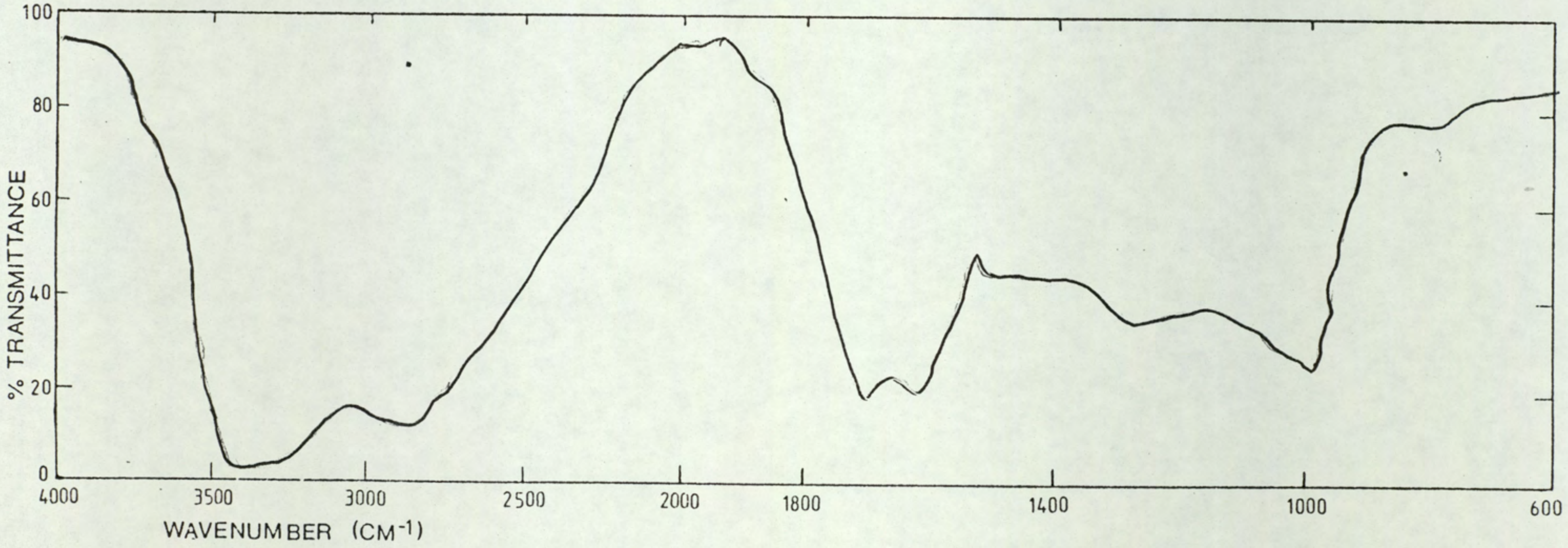


Figure 35: I.r. Spectrum of Methylated Humic Acid (Aldrich)

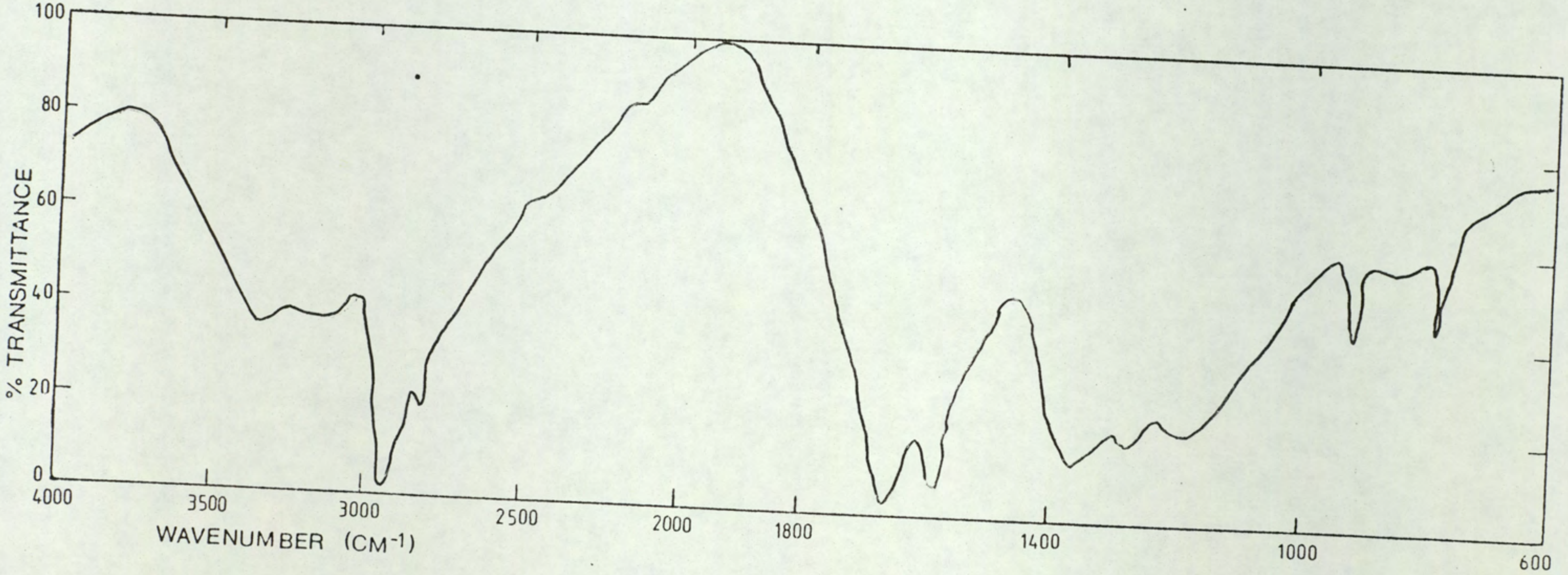


Figure 36: I.r. Spectrum of Methylated Humic Acid (Reading)

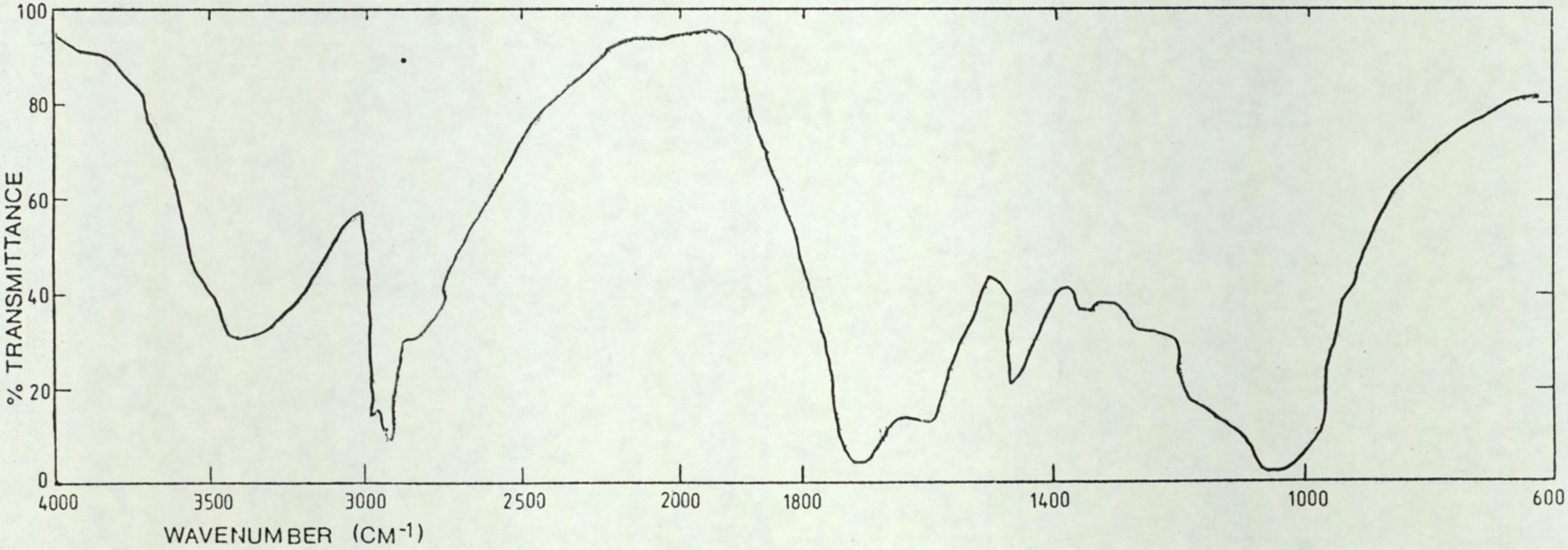


Figure 37: I.r. Spectrum of Methylated Humic Acid (Lignite)

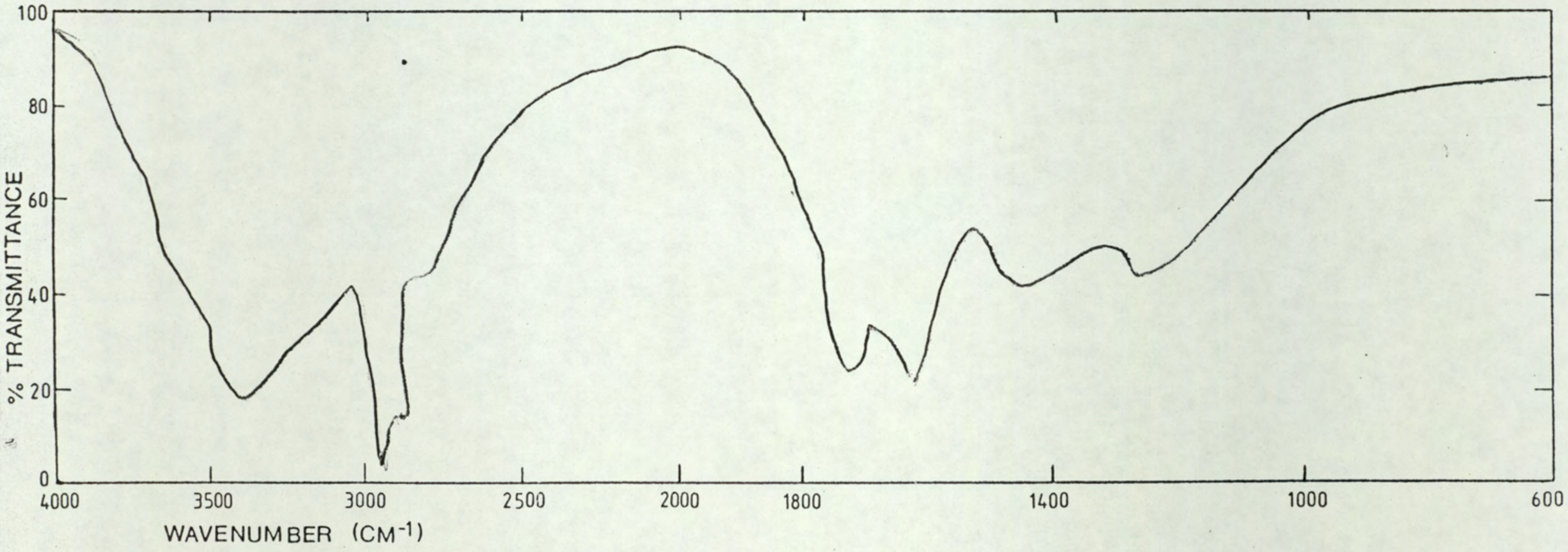


Figure 38: I.r. Spectrum of Methylated Humic Acid (Norfolk)

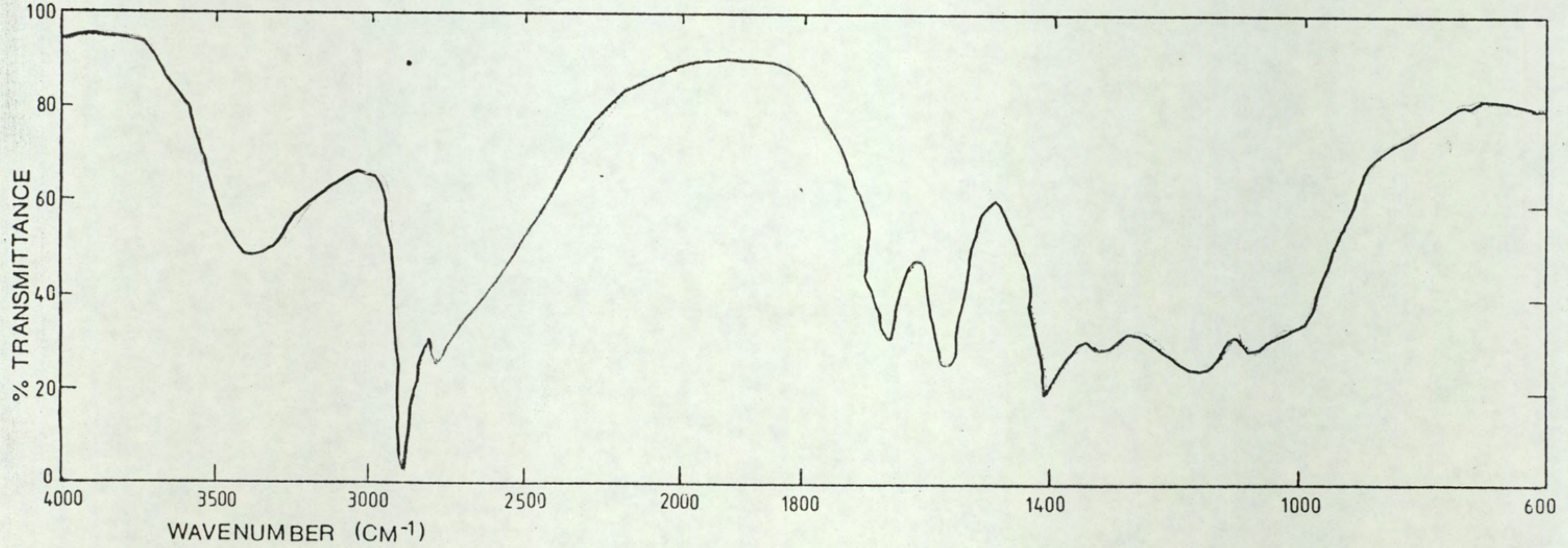
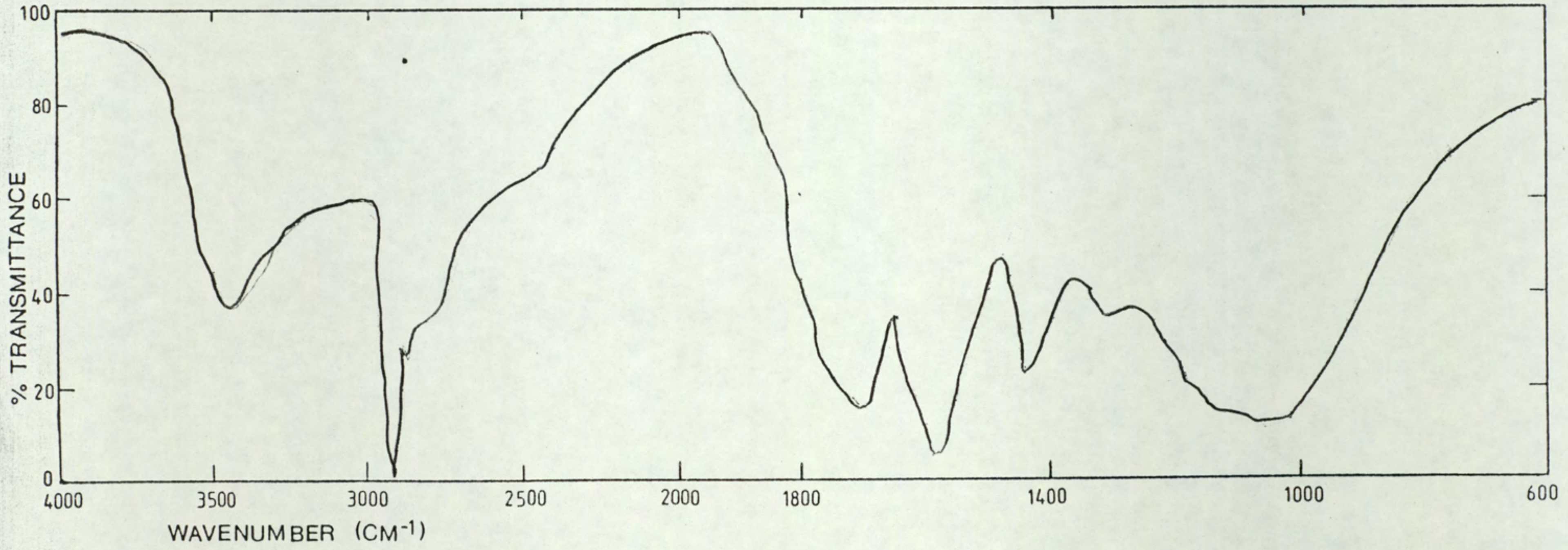


Figure 39: I.r. Spectrum of Methylated Humic Acid (Fluka)



than carboxylic acid, thereby giving a more pronounced maximum (101-102). Esters also show C = O absorptions at slightly higher frequencies, which explains the slight shifts observed for the methylated products.

Since concomitant changes occur in several major absorption bands when humic acids are methylated, direct comparisons in the 1660 to 1600 cm^{-1} region are difficult. Accordingly, considerable subjective error is encountered in ascertaining whether absorption in this region was reduced. Nevertheless, slight reduction is suggested

5.3 ^{13}C Nmr OF METHYLATED HUMIC ACID (ALDRICH).

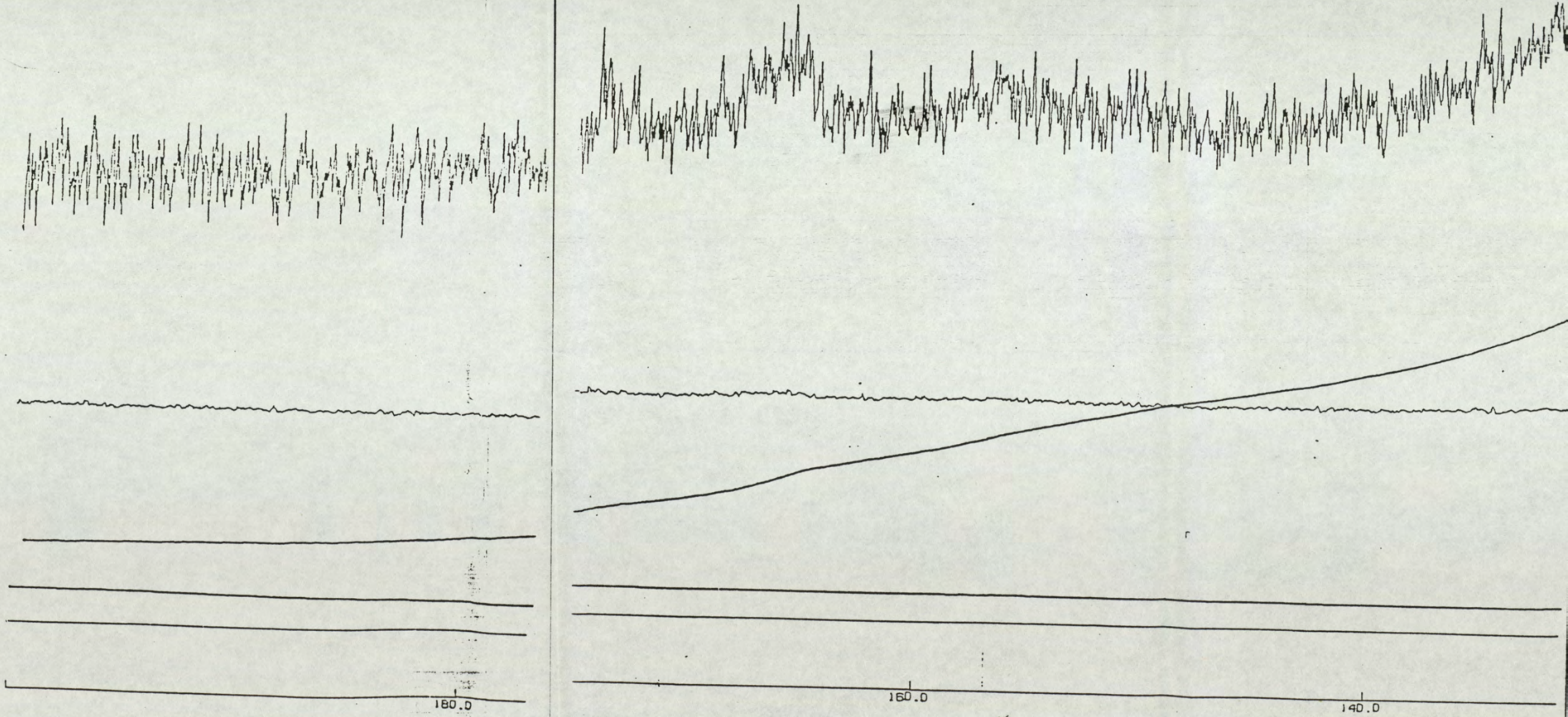
In order to enhance and separate the nmr signals of the hydroxyl functions from the broad featureless spectra of humic substances, we have methylated humic acids. Some of the products were sufficiently soluble to be analysed by ^{13}C nmr.

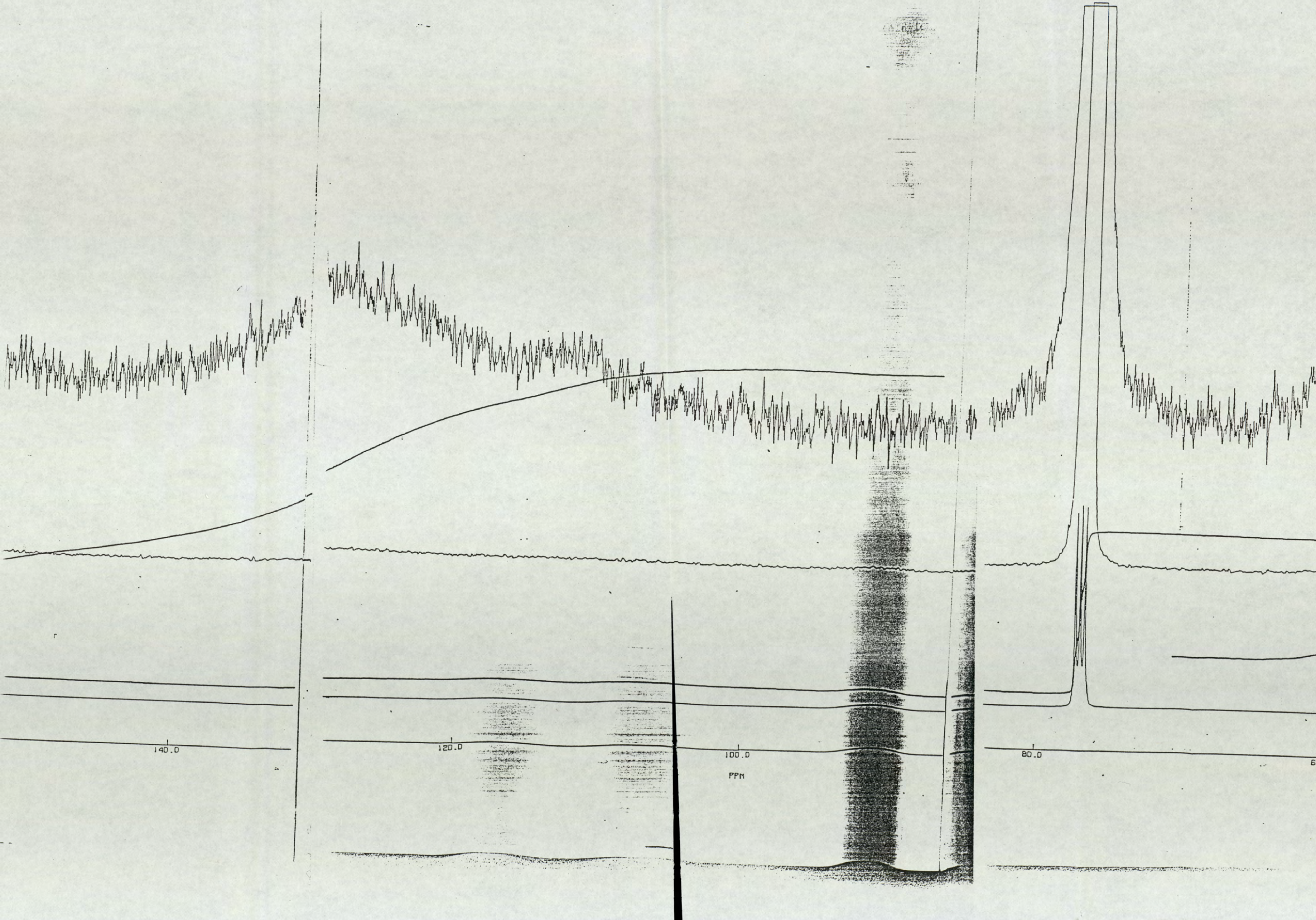
The ^{13}C nmr spectra were recorded on a Bruker instrument. The spectrum of the methylated humic acid (Aldrich) is weak and poorly resolved. The aliphatic region of O-55 ppm is difficult to interpret because considerable phase transfer catalyst contamination exists. However, peaks in the 50-60 ppm region indicate aliphatic $COOCH_3$ and $COCH_3$, also present at 52 ppm is a peak suggesting the presence of aromatic methyl esters. In addition to this a band in the 55-56 ppm region represents aromatic OCH_3, and in the 120-140 ppm region a band indicative of aromatic carbon, can be seen. Carbonyl carbon resonances are just detectable in the 160-180 ppm region, (see figure 40).

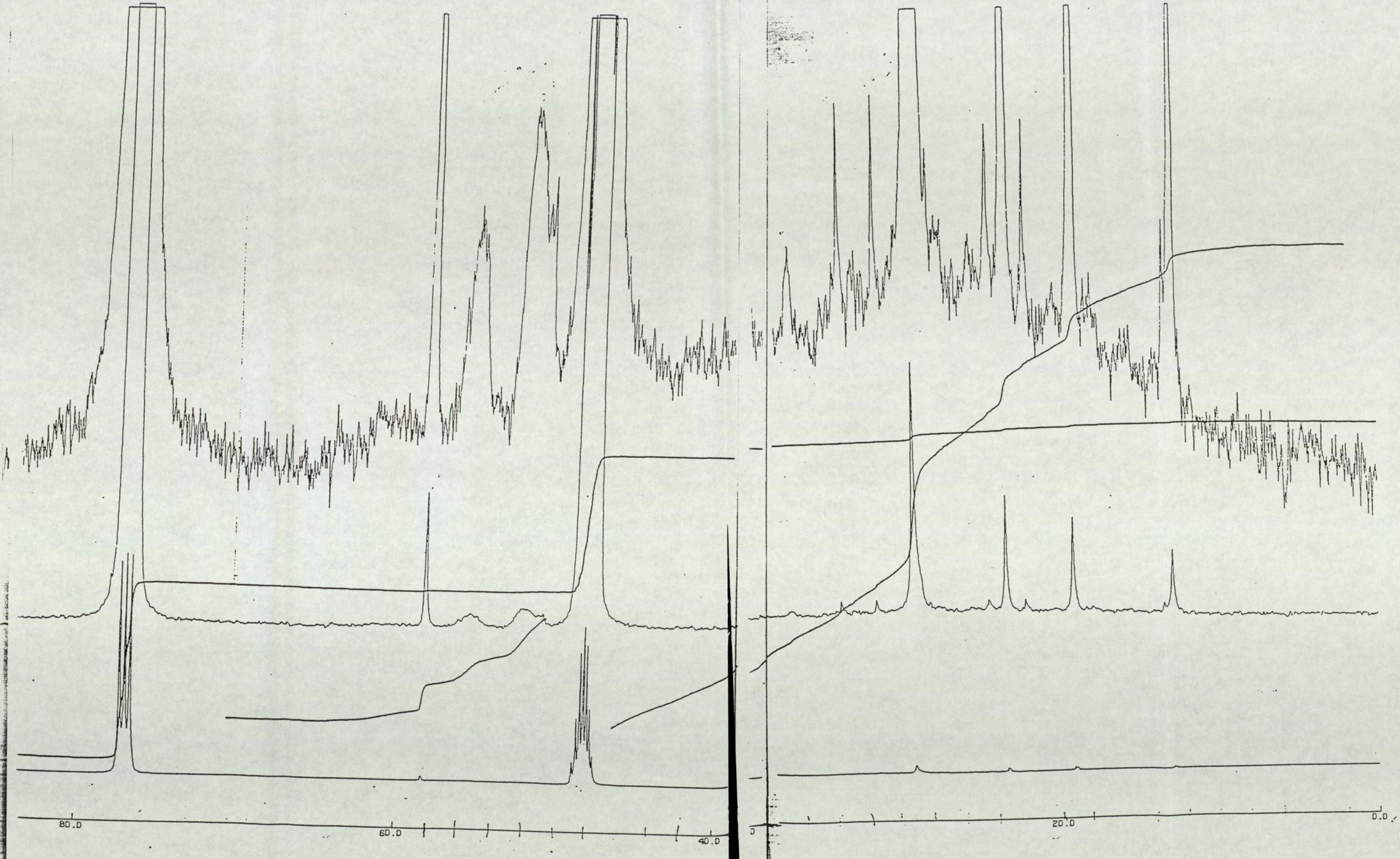
5.4 ^{1}H Nmr OF METHYLATED HUMIC ACID (ALDRICH)

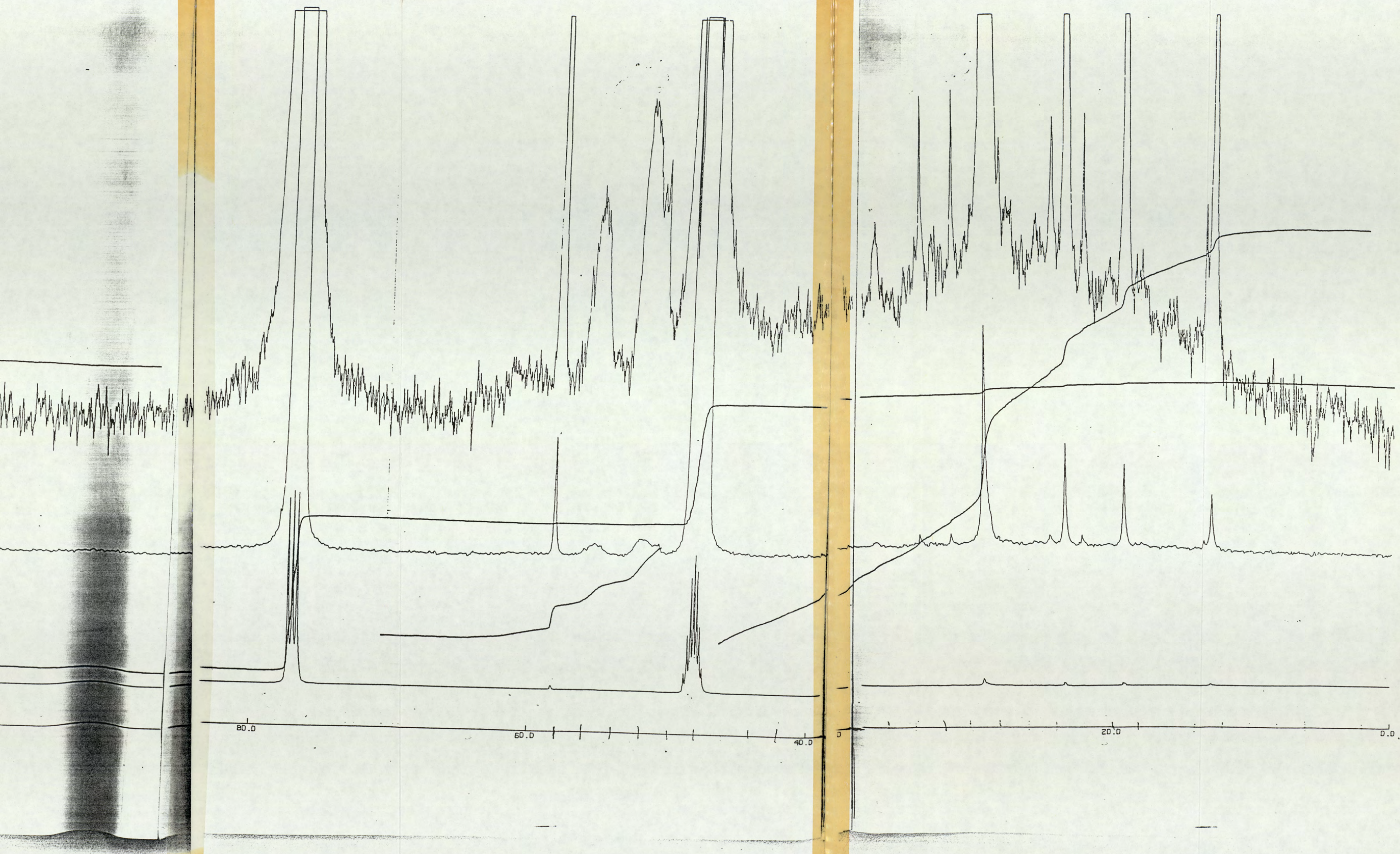
The ^{1}H nmr spectrum of methylated humic acid (Aldrich) shows the presence of aliphatic, and aromatic protons (chemical shift $\delta = 4.2$ ppm). Although

Figure 40: ^{13}C nmr Spectrum of Methylated Humic Acid (Aldrich)




140.0
120.0
100.0
PPM
80.0
6


80.0
60.0
40.0
0
20.0
0.0


80.0
60.0
40.0
20.0
0.0

i.r. analysis indicates that the phase transfer method is a very efficient method for the methylation of -OH functions, the disadvantage of using this method is that the catalyst cannot be completely removed from the products. It appears to be adsorbed onto the products. So although one obtains products which are sufficiently soluble in organic solvents to carry out nmr analysis, the nmr analysis is complicated by the presence of the catalyst in the system. The protons in the monomeric catalyst have much shorter relaxation times than the protons of the humic acids and even though only traces of the catalyst are present its absorptions swamp the humic absorptions.

CONCLUSIONS

Investigations have been carried out to evaluate the use of milder more specific reagents for the chemical degradation and derivatisation of humic acids. The alkylation reactions commonly used in the protection of the -OH functions in humic acids involve nucleophilic substitution reactions. It has been demonstrated previously that reactions involving anionic attack generally proceed with greater ease and efficiency using phase transfer conditions and in investigations we have found that methylation, ethylation and tosylation of humic acids proceeds very efficiently using tetraalkylammonium salts as phase transfer catalysts. The i.r. spectra of the alkylated products show only weak -OH absorptions and the products are relatively soluble in organic solvents as would be expected for materials which had lost their hydrogen bonding interactions.

Although the phase transfer method is very efficient and confers desirable properties in the humic materials there are disadvantages in the use of this method with these materials. It would appear that phase transfer catalysts complex with humic compounds and contaminate the products. It is impossible therefore to obtain reliable elemental and instrumental analyses on the products because they are complicated by the presence of the phase transfer catalyst. We would argue therefore, that there are 'pros' and 'cons' in the application of phase transfer methods to humic and coal type systems.

We have shown that it is possible to reduce even the carbonyl functions of humic acids with lithium aluminium hydride.

The alcoholic functions thus produced and those already present in the molecule can then be removed by tosylation and further reduction with lithium aluminium hydride. We have thus devised a relatively mild and specific route for the removal of most of the hydrogen bonding and dipolar interacting functional groups from humic acids.

Although a number of routes were examined we were unable to find a useful method for the removal of phenolic -OH functions from humic acids.

The application of highfield nmr to the study of humic materials was investigated briefly. Because these materials have such long relaxation times, it takes several hours of instrument time to obtain a reasonable spectrum, it was possible therefore to obtain only very few spectra since we did not have much instrument time available to us. We did obtain one very good spectrum which indicated that high field nmr is capable of revealing structural detail which cannot be obtained from low field instrumentation.

We also found that there was some conflict between the results obtained using 'solid' and solution nmr methods.

Our goal was to develop new approaches to the study of humic acids, we have gone some way towards this goal.

EXPERIMENTAL SECTION

E.1 GENERAL

E.1.1 Samples

Five humic acid samples were examined. Two were isolated from soils in Reading and Norfolk and were obtained by Dr A Young (University of Reading) and Dr M Hayes (University of Birmingham) respectively. The third sample was extracted from brown coal obtained from Dr A Gaines (University of London). The final two humic acids examined were commercially supplied by Aldrich and Fluka.

E.1.2 Extraction of Humic Acids

Samples were treated first with water to remove free low-molecular-weight carbohydrates, amino acids and other water soluble low-molecular-weight compounds, secondly, with a 1:1 (V/V) mixture of benzene and methanol to remove lipids, and thirdly, with 0.01 M HCl to dissolve carbonate minerals: Residues were then stirred overnight under nitrogen with an aqueous 0.5M NaOH solution (solution to sediment ratio >20:1). Soluble humic substances (humic acid and fulvic acid) were separated from the insoluble material (humin) by centrifugation. The NaOH solution was filtered through fibre-glass filters (0.5 µ pore size) to remove colloidal clays. The NaOH extraction of the soil was repeated but only small amounts of additional soluble humic material were obtained. The method of extraction used here is similar to that used by Schnitzer and Khan(101) and is the most common used method, having a high extraction efficiency. In order to precipitate humic acids, the filtered solution of soluble humic substances was then acidified to pH1 by addition of 6M HCl. These precipitates were separated from fulvic acids by centrifugation and washed with 0.1M HCl. Humic acids were then dried under vacuum at 60°C.

E.2 EXPERIMENTAL WORK RELATING TO CHAPTER 2

E.2.1 Elemental Analysis

The amount of carbon, hydrogen and nitrogen was determined by using a Carlo Erba auto analyser model 1106.V_20_5 was added to assist combustion in some cases. The values were corrected for ash contents measured by burning the samples at 960^{o}C. Oxygen was measured by difference.

Table (17)

Analysis of Humic Acids

Humic acid	Ash % dry basis	Analysis % d.a.f.)				
		C	H	N	S	O(diff)
Reading	4.0	53.0	3.8	0.8	2.2	40.2
Norfolk	0.89	55.3	4.8	3.5	1.2	35.2
Lignite	1.3	60.0	4.2	2.5	2.0	31.3
Aldrich	3.7	58.5	4.2	1	2.3	34.1
Fluka	7.8	54.0	4.4	0.8	2.2	38.6

E.2.2 Infra-red Spectroscopy

Infra-red spectra were obtained using a Perkin Elmer 559B spectrometer fitted with a 3600 Perkin Elmer data station. Solids were examined by the KBr disc method. All the humic acids showed bands at the following frequencies : 3400 cm^{-1} (hydrogen bonded OH), 2900 cm^{-1} (aliphatic C-H stretch), 1725 cm^{-1} (C=O of COOH , C=O stretch of ketonic carbonyl), 1630 cm^{-1} (C=C, hydrogen bonded carbonyl, C=C conjugated with COO^{-}), 1450 cm^{-1} (aliphatic C-H), 1400 cm^{-1}

(COO^-, aliphatic C-H), 1200 cm^{-1} (C=O stretch or OH deformation of CO_2H), 1050 cm^{-1} (silicate impurities).

E.2.3 Nmr Spectroscopy

Nuclear magnetic resonance spectroscopy of soluble humic acids was carried out on a Jeol FX90Q instrument. ^1H and ^{13}C nuclei were studied at 90MHz and 22.5MHz respectively. Proton spectra typically required 1000 pulses with up to a 4 second repetition time. ^{13}C spectra usually required at least 100,000 pulses for a reasonable result.

Solid state ^{13}C nmr at 50.3MHz was carried out on humic acid using a Bruker instrument at the University of East Anglia. Spectra were obtained using magic angle spinning (3KHZ), proton decoupling (60KHZ) and cross polarization (proton enhancement C.T. 3ms).

^1H nmr spectra were measured in deuterated dimethyl sulphoxide (DMSO-d_6) solution.

E.2.4 gc and gc/ms

Preliminary gc examination of humic acids and soluble degradation products was carried out on a Pye Unicam gas chromatograph fitted with a 1m 0.5cm (ID) SE30 column and flame ionisation detector. The oven temperature was programmed from 80°C to 250°C at a rate of 8°C/min with a delay of 4 mins. Samples giving good gc traces were further analysed using a computer integrated VGMM 12000 quadrupole mass-spectrometer fitted with a Pye Unicam 304 gas chromatograph. The same oven temperature program was used as in the preliminary study but this time a 30m SE30 glass capillary column (0.3mm I.D.) was used. This led directly into the source (maintained at 200°C) where components were ionised with a 70eV electron beam. Mass spectra were recorded every 2 seconds using a digital 2000 data system.

E.3 EXPERIMENTAL WORK RELATED TO CHAPTER 3

E.3.1 Reduction of Humic Acids

All five humic acids were treated with lithium aluminium hydride in different solvents under different conditions. The solvents used were:

1. Diethyl ether
2. Dimethyl diglyme
3. Tetrahydrofuran

E.3.2 Method 1

The humic acids (5.0g) and diethyl ether (250 cm^3)were placed in a 500 cm^3 flask equipped with a reflux condenser and $LiAlH_4$ (2.5g) was slowly introduced. The reaction mixture was refluxed for 3 days, cooled to room temperature, and filtered. The unreacted $LiAlH_4$ was destroyed by transferring the residue in small portions as rapidly as possible to a 2L beaker containing 200 cm^3 of water. The resulting mixture was acidified with 250 cm^3 of 2M HCl, and filtered. The product was washed with boiling water to remove the hydrochloric acid, then was dried under vacuum at 100°C overnight, i.r. spectra of the product taken as KBr discs were identical with those of the starting materials.

E.3.3 Method 2

The humic acids (5.0g) were refluxed with 2.5g of $LiAlH_4$ and 250 cm^3 of dimethyl diglyme for 3 days. The reaction mixture was cooled to room temperature and vacuum filtered. The unreacted $LiAlH_4$ was destroyed carefully by adding drops of water very slowly. The residue was then treated with 200 cm^3 of 2M HCl and washed several times with boiling water. The product was dried under a vacuum at 100°C overnight. I.r. spectra of the products taken as KBr discs were identical to those of the starting materials.

E.3.4 Method 3

The humic acids (5.0g) and 250 cm^3 of THF were placed in a 500 cm^3 flask equipped with a reflux condensor, 2.5g $LiAlH_4$ was then added slowly. The reaction mixture was refluxed for 6 days, cooled to room temperature and stirred for 6 days. The unreacted $LiAlH_4$ was destroyed. It was then filtered and washed with 250 cm^3 of 1M HCl and several times with water. The products were dried under vacuum at 100^{o}C overnight. The i.r spectra showed the elimination of carbonyl absorption at 1720 cm^{-1}, (C=O of COOH) and ketone, and an increase in 3400 cm^{-1} band (OH stretch).

E.3.5 Method 4

The humic acids (5.0g) and $LiAlH_4$ (2.5g) were suspended in 5.0 cm^3 of THF in a 100 cm^3 round bottom single neck flask, maintained under a nitrogen atmosphere and placed in an ultrasonic bath for 24h. The reaction was quenched and the products isolated, yields (4.5g). I.r. spectra show sharp peaks at 1600 cm^{-1} but there was no complete elimination of the 1720 cm^1 band (C=O stretch).

E.4 EXPERIMENTAL WORK RELATED TO CHAPTER 4

E.4.1 Tosylation of Humic Acids

All the five humic acids were tosylated, four methods were used, those methods are described below.

E.4.2 Method 1

The humic acid (2-10g) was suspended in 100-150 cm^3 of dry pyridine in a 250 cm^3 glass-stoppered conical flask. The reaction mixture was cooled to 0^{o}C and an excess of toluene sulphonyl chloride was added, this was achieved by the addition of tosyl chloride in a ratio of 8:1 by weight with respect to the humic acid. When the addition was complete the flask was placed in a refrigerator for the required reaction time. The progress of the reaction may be observed by the separation of pyridine hydrochloride as long needle like crystals (these crystals will not appear if the pyridine is wet). After the required reaction time the mixture was poured with stirring, into 300-400 cm^3 of iced water. After 15 mins additional stirring, the mixture was filtered and the residue was washed with water and then ether and finally dried under vacuum at 100^{o}C. Elemental analysis, (Table 18).

Table 18

Elemental Analysis of Tosylated Humic Acid

METHOD 1

Humic acid	C	H	N	S	O(diff)
Aldrich	43.1	3.3	0.9	7.3	45.4
Fluka	42.2	3.3	0.8	7.5	46.2
Lignite	50.1	3.5	2.0	8.0	36.4
Reading	40.0	2.5	1.1	8.1	48.3
Norfolk	44.0	3.2	2.5	5.2	45.1

E.4.3 Method 2

The humic acid (2-10g) was dissolved in 100-150 cm^3 of 10% aqueous sodium hydroxide contained in a 250 cm^3 conical flask. To this was added a solution of tosyl chloride in cold acetone (the tosyl chloride being in a ratio of 2.5:1 by weight with respect to the humic acid. The flask was then shaken for 15-20 mins, the mixture at first became warm and then on continued shaking cooled. The mixture was then left standing at room temperature for a particular reaction time, filtered and the residue washed with water then ether and finally dried under vacuum at 100^{o}C. Elemental analysis, (Table 19).

Table 19

Elemental Analysis of Tosylated Humic Acid

METHOD 2

Humic acid	C	H	N	S	O(diff)
Aldrich	44.0	5.4	0.8	4.4	45.4
Fluka	42.2	3.3	0.7	3.3	50.5
Lignite	50.0	3.6	1.9	2.9	41.6
Reading	55.0	2.5	1.1	3.3	38.1
Norfolk	58.0	3.2	2.5	2.2	34.1

E.4.4 Method 3

The humic acid, approximately 10.0g was suspended in 200 cm^3 of dry THF in a 500 cm^3 conical flask equipped with a magnetic stirrer and a nitrogen atmosphere. To this solution 80 cm^3 of tetrabutyl ammonium hydroxide 40% was added. When this addition was completed the mixture was left for 2h while stirring. Then approximately 20.0g of tosyl chloride was added, and the reaction mixture left stirring at room temperature for 24h. The mixture was filtered and the residue washed with water then ether and finally dried under vacuum at 100^{o}C. Elemental analysis, (Table 20).

Table 20

Elemental Analysis of Tosylated Humic Acid

METHOD 3

Humic acid	C	H	N	S	O(diff)
Aldrich	57.9	4.6	0.9	8.1	28.1
Fluka	58.0	4.6	0.8	9.1	27.5
Lignite	61.0	3.6	2.0	10.0	23.4
Reading	59.0	4.5	2.0	11.0	23.5
Norfolk	59.0	4.5	1.9	9.1	25.5

E.4.5 Method 4

The humic acid, approximately 10.0g was suspended in 100-150 cm^3 of THF and 50 cm^3 of 10% sodium hydroxide in a 250 cm^3 glass stoppered conical flask. This mixture was cooled to 0^{o}C and approximately 20.0g of tosyl chloride was added. When the addition was complete the flask was placed in a refridgerator for 12-24h. After this time the mixture was poured with stirring into 300-400 cm^3 of iced water. After 15 min of additional stirring the mixture was filtered and the residue was washed with water and ether and finally dried under vacuum at 100^{o}C. Elemental analysis, (Table 21).

Table 21

Elemental Analysis of Tosylated Humic Acid

METHOD 4

Humic acid	C	H	N	S	O(diff)
Aldrich	54.1	4.0	0.9	3.2	37.8
Fluka	55.1	3.9	1.0	4.4	35.6
Lignite	59.1	2.9	2.0	3.2	32.9
Reading	50.0	3.2	2.0	5.0	39.8
Norfolk	56.0	3.4	2.0	6.0	32.4

E.4.6 Reduction of Tosylated Humic Acids

The tosylated humic acid (4.0g) was suspended in 150 cm^3 of dry THF, lithium aluminium hydride (2.5g) was added and the reaction mixture was stirred for 3 days at room temperature. The excess of $LiAlH_4$ was destroyed by the drop wise addition of water, the reaction mixture was poured into 50% HCl and the residue washed with water several times. The product was dried under vacuum at 100^oC.

E.4.7 Acetylation of Humic Acids and Derivatives

All five humic acids, their $LiAlH_4$ reduced derivatives and the reduced tosylated samples were acetylated (with acetic anhydride in pyridine) using the method developed by Brooks et al(84).

$$ROH + (CH_3CO)_2O \longrightarrow CH_3COOR + CH_3COOH$$

$$H_2O + (CH_3CO)_2O \longrightarrow 2\ CH_3COOH$$

The excess anhydride was hydrolysed to acetic acid which was titrated with standard base.

E.4.8 The Determination of Hydroxyl Content

The humic acids (50-100 mg) were refluxed with a mixture of equal parts of pyridine and acetic anhydride (5 cm^3) for 2 to 3h under nitrogen. The mixture was cooled to room temperature and poured into water, then filtered under vacuum and washed thoroughly with water and dried over P_2O_5. The acetylated sample (50 mg) was refluxed with 25 cm^3 of 3M aqueous sodium hydroxide solution for 2h under nitrogen, 25 cm^3 6M sulphuric acid and 25 cm^3 distilled water were added and the products distilled using a splash head. The distillate was titrated with 0.1M sodium hydroxide using phenolphthalein as an indicator. The volume of the material in the distillation flask was maintained by adding distilled water in 25 cm^3 portions, A reagent blank was set up and the distillation continued until aliquots of the sample and the blank distillations gave identical titration values, then the acetyl content was calculated, (see Table 22 - 26).

E.4.9 Hydrogenation of Humic Acids

The reduced tosylated humic acid (2.0g) was tosylated again, mixed with Raney nickel catalyst (10.1g) and 200 cm^3 of ethanol and hydrogenated for a week. During that time it absorbed 300 cm^3 of hydrogen. When absorption of hydrogen was complete the solvent was evaporated and the residue was treated with sulphuric acid in order to dissolve the catalyst, then washed with water several times. The product was dried under vacuum at 100^oC. I.r. spectra of the product was identical with that of the starting material.

Table 22

Acetyl and Hydroxyl Contents of Humic Acid and Derivatives (Aldrich)

Sample	Portions	0.1N NaoH	Acetyl Content	Hydroxyl Content
Humic Acid Aldrich	1	2.4		
	2	1.8		
	3	0.4		
	4	0.2	8 meq acetyl content/g of humic preparation	12.04 meq OH/g
	5	0.1		
	6	0.1		
Reduced Humic Acid	1	1.5		
	2	1.1		
	3	0.8	6.8 meq/g	9.50 meq OH/g
	4	0.5		
	5	0.3		
	6	0.1		
Reduced tosylated reduced Humic Acid	1	0.6		
	2	0.5		
	3	0.4	3 meq/g	3.4 meq OH/g
	4	0.2		
	5	0.1		
	6	0.1		

$$\text{Acetyl Content} = \frac{\text{Sample of titre - titre for blank x N base x 1000}}{\text{weight of sample in mg}}$$

$$= \text{meq acetyl content / g of humic preparation}$$

$$= \frac{\text{Acetyl Content}}{1-(\text{o.042 x Acetyl Content})} = \text{meq OH/g}$$

Table 23

Acetyl and Hydroxyl Contents of Humic Acid and Derivatives (Fluka)

Sample	Portions	0.1N NaoH	Acetyl Content	Hydroxyl Content
Humic Acid	1	2.0		
Fluka	2	1.4		
	3	0.7		
	4	0.5	7.8 meq/g	11.6 meq OH/g
	5	0.3		
	6	0.2		
Reduced Humic	1	1.5		
Acid	2	1.4		
	3	0.7	7.1 meq/g	10.12 meq OH/g
	4	0.6		
	5	0.3		
	6	0.2		
Reduced tosylated	1	0.7		
reduced Humic	2	0.6		
Acid	3	0.5	3.3 meq/g	3.83 meq OH/g
	4	0.4		
	5	0.2		

Table 24

Acetyl and Hydroxyl Contents of Humic Acid and Derivatives (Reading)

Sample	Portions	0.1N NaoH	Acetyl Content	Hydroxyl Content
Humic Acid Reading	1	2.8	12 meq/g	24.19 meq OH/g
	2	1.7		
	3	1.2		
	4	0.8		
	5	0.6		
	6	0.4		
	7	0.3		
Reduced Humic Acid	1	2.1	9.33 meq/g	15.32 meq OH/g
	2	1.8		
	3	0.9		
	4	0.6		
	5	0.4		
	6	0.2		
Reduced tosylated reduced Humic Acid	1	0.6	1.6 meq/g	1.7 meq OH/g
	2	0.3		
	3	0.2		
	4	0.1		

Table 25

Acetyl and Hydroxyl Contents of Humic Acid and Derivatives (Lignite)

Sample	Portions	0.1N NaoH	Acetyl Content	Hydroxyl Content
Humic Acid	1	2.1		
Lignite	2	1.4		
	3	1.1		
	4	0.7	7.6 meq/g	111.19 meq OH/g
	5	0.5		
	6	0.4		
	7	0.2		
Reduced Humic	1	1.4		
Acid	2	1.2		
	3	0.8	7 meq/g	9.91 meq OH/g
	4	0.6		
	5	0.4		
	6	0.2		
Reduced tosylated	1	0.7		
reduced Humic	2	0.5		
Acid	3	0.3	2.1 meq/g	2.30 meq OH/g
	4	0.2		

Table 26

Acetyl and Hydroxyl Contents of Humic Acid and Derivatives (Norfolk)

Sample	Portions	0.1N NaoH	Acetyl Content	Hydroxyl Content
Humic Acid	1	2.2		
Norfolk	2	1.7		
	3	1.0		
	4	0.6	9.5 meq/g	15.80 meq OH/g
	5	0.4		
	6	0.2		
Reduced Humic	1	1.6		
Acid	2	0.8		
	3	0.5	4.5 meq/g	5.54 meq OH/g
	4	0.2		
Reduced tosylated	1	0.9		
reduced Humic	2	0.8		
Acid	3	0.4	3.6 meq/g	4.24 meq OH/g
	4	0.3		
	5	0.2		

E.4.10 Hydrogenation of humic acid derivative prepared using sulphur- trioxide complex

The reduced tosylated humic acid (0.5g) was mixed with pyridine-sulphur trioxide complex in dry THF and was left stirring for 10d. Then the THF was removed and the residue was dissolved in 3.5g potassium hydroxide in 50 cm^3 of water, 5g of Raney nickel catalyst was added and the mixture was hydrogenated. After 3d about 250 cm^3 of hydrogen had been absorbed. The solid was filtered off and the residue was washed with water several times and with dilute sulphuric acid then dried under vacuum at 100°C . When i.r spectra using KBr discs were taken, there was no significant change in the spectra compared with the starting material.

E.4.11 Preparation of Diethyl Chlorophosphate

(3.2 cm^3, 0.032 mole) of triethylamine was added slowly to a cooled OC, stirred mixture of (26 cm^3, 0.20 mol) of diethylphosphite and (35 cm^3, 0.4 mole) of carbon tetrachloride, the temperature was maintained at 0°C for 15 minutes, then allowed to rise to room temperature and stirring continued for 3 hours. The reaction mixture was filtered, stripped completely of low boiling component at room temperature and vacuum distilled yield (20g) of colourless sweet-smelling oil. B.P. 88-9°/15mm., 80-1°/8mm.

E.4.12 Phosphorylation of Humic Acids

The Deithyl chlorophosphate (10.0g) was added to a mixture of (5.0g) of Humic acid (Aldrich) in 100 cm^3 of dry THF and 65 cm^3 of tetrabutylammonium hydroxide the reaction mixture was stirred for 48 hours, then filtered and the residue washed several times with water and dried under vacuum at 60°C. Yield (3.5g), elemental analysis C : 48.3% H : 5.6%, N : 1.3% O : 44.8. The elemental analysis are not reliable because of difficulty of removing the phase transfer catalyst. I.r. spectrum using KBr disc were taken, there was no significant change in the spectrum compared with the starting material.

E.4.13 Decarboxylation of Humic Acids

All five humic acids were decarboxylated using the method of Benkeser et al[103].

The humic acids (5.0g) were dissolved in 80 cm^3 acetonitrile and 30 cm^3 of $SiHCl_3$ was added. The mixture was cooled to 0^{o}C and tri-propyl amine (0.26 cm^3) was added slowly. After the amine was added, the mixture was heated to reflux for 24h, then 1l diethyl ether was added. The reaction mixture was filtered and the residue was refluxed with methanol (50 cm^3) for 1h., then 120g of potassium hydroxide was dissolved in 180 cm^3 methanol and 50 cm^3 water and added to the reaction mixture and refluxed for 48h. The mixture diluted with (600 cm^3) distilled water then 2M HCl was added to the aqueous solution and left standing overnight. Then the residue was washed several times with water and dried under vacuum at 100^{o}C. Yield (3.8g). Elemental analysis, (Table 27).

Table 27

Elemental Analysis of Dexarboxylated Humic Acids

Sample	C	H	N	S	O(diff)
Aldrich	54.5	3.0	1	4.5	37.0
Fluka	54.0	4.4	0.8	2.2	38.6
Lignite	58.0	4.2	2.5	2.3	33.0
Reading	53.0	3.8	0.8	2.2	40.2
Norfolk	55.3	4.8	3.5	1.2	35.2

E.4.14 Preparation of Tosyl Derivative of 2-Naphthol

2-Naphthol (10g) was mixed with 25 cm^3 of pyridine and 20g of p-toluene-sulphonyl chloride and heated on a water bath for 15 min. It was then poured into 250 cm^3 of cold water and stirred until the oil solidified. It was then filtered and washed with cold diluted hydrochloric acid (to remove pyridine), with cold diluted sodium hydroxide solution (to remove any 2-naphthol present) and then with cold water.

E.4.15 Hydrogenation of 2-Naphthol Tosylate

The tosylated 2-naphthol (10g) was dissolved in 120 cm^3 of absolute ethanol and 35g of Raney nickel catalyst was added. The mixture was then hydrogenated using the apparatus shown in Figure 41. When the absorption of hydrogen was complete after 16h , the catalyst was removed by centrifugation and washed thoroughly with alcohol. Isolation of the products was achieved by evaporation of the solvent and crystallisation. Yield (8g)(83).

E.5. EXPERIMENTAL WORK RELATED TO CHAPTER 5

E.5.1 Methylation of Humic Acids

Several methods of methylation were tested using the following reagents:

1. Methanol and concentrated sulphuric acid.
2. Dimethyl sulphate and anhydrous potassium carbonate in acetone, used widely for methylation of hydroxy anthraquinones.
3. Methyl iodide and tetrabutylammonium hydroxide (phase transfer catalyst).

E.5.2 Methylation by Method 1.

The humic acids (1.0g) were refluxed with dry methanol (50 cm^3)

containing concentrated sulphuric acid ($1cm^3$), overnight. After cooling, the mixture was poured into water ($150cm^3$) and the precipitated humic acids were filtered off washed and dried under vacuum at 100^oC, yield 0.8g. I.r spectra showed an increase in the intensity of the band due to C=O stretching at 1720 cm^{-1}, a reduction in the OH band in the 3600 cm^{-1} to 3100 cm^{-1} region, and the development of new bands due to C-H stretching of added CH_2 groups at 2920 cm^{-1}. In addition, absorptions due to C-O stretching vibrations in the 1250 cm^{-1} region were sharpened.

E.5.3 Methylation by Method 2.

The humic acids (2.5g) were refluxed for 24h with dimethylsulphate (28 cm^3) and anhydrous potassium carbonate (24.0g) in acetone (40 cm^3), moisture being excluded from the apparatus. The acetone was then removed under reduced pressure and the reaction mixture was acidified with 2M hydrochloric acid. The precipitated humic acids were filtered off, washed with water until free from chloride ions and dried under vacuum at 100^oC. Yield 2.2g. I.r. spectra showed absorption at weak 3400 cm^{-1} (OH stretching weak); 2940 cm^{-1} to 2800^{-1} (aliphatic C-H moderate) ; 1720 cm^{-1} (C=O, very strong); 1640 cm^{-1} (C=O of quinones, moderate); 1600 cm^{-1}, strong, (aromatic C-O or strongly bonded C=O strong); 1440 cm^{-1} (CH bending CH_3).

E.5.4 Methylation by Method 3.

The humic acids (5.0g) were suspended in dry THF (200 cm^3) after 2hrs tetrabutylammonium hydroxide (90 cm^3) was added and methyl iodide (20 cm^3) was added. The reaction mixture was stirred at room temperature, overnight. Volatile materials (THF and CH_3I) as well as some water were distilled off under vacuum at 60^oC. Tetrabutylammonium iodide has a moderate water solubility but was difficult to remove completely from the sample. The methylated product was finally dried under vacuum at 100°C, overnight. Yield (3.5g). I.r. spectra show the following decrease in a hydroxyl band of 3300-3600 cm^{-1}, an increase in the intensity of the aliphatic C-H bands 2800-3000 cm^{-1} and an intense carbonyl peak centered at 1600 cm^{-1}. This peak is also assigned to the aromatic carbon linkage. Elemental analysis of products from this method are not reliable since it is very difficult to remove the phase transfer catalyst.

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