ORIGIN AND CHEMICAL NATURE OF SOIL ORGANIC MATTER

C. Saiz Jimenez

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PROEFSCHRIFT
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Prof.drs. P.A. Schenck
Toegevoegd promotor
Dr. J.W. de Leeuw

STELLINGEN

1. The statement that humic substances are not readily amenable to the pyrolysis technique is questionable.
   Gillam, A.H. and Wilson, A.M. (1985) Org. Geochem. 8:15-25.

2. The term "humic acid-like brown substance" is a demonstration of the need for clear definitions rather than a careful formulation.
   Mukai, H. and Ambe, Y. (1986) Atmos. Environ. 20:813-819.

3. Hatcher and Spiker point out that significant quantities of non-carbohydrate aliphatic structures (paraffinic structures) are present in terrestrial humin from peat and soil. Their suggestion that this material can arise from a.o. degraded lignin or protein-derived residues cannot be taken seriously.
   Hatcher, P.G. and Spiker, E.C. (1988) In: Humic Substances and their Role in the Environment. F.H. Frimmel and R.F. Christman eds. pp. 59-74, Wiley, Chichester.

4. The formation of humic and fulvic acids from carbonaceous materials such as charcoal and cinder, produced by the combustion of plants and subsequent weathering under natural conditions, is unlikely.
   Kumada, K. (1983) Soil Sci. Plant Nutr. 29:383-386.

5. The suggestion by Del Monte et al. that weddelite and whewellite on marble and limestones originate from the action of oxalic acid secreted by lichens is controversial.
   Del Monte, M., Sabbioni, C. and Zappia, G. (1987) Sci. Total Environ. 67: 17-39.

6. The attribution of the increase of Si and Al in the surface layers of some stone crusts to diatoms is a misinterpretation.
   Esbert, R.M. and Marcos, R.M. (1983) The stones of the cathedral of Oviedo and its deterioration (in Spanish). Colegio Oficial de Aparejadores y Arquitectos Tecnicos, Oviedo, 147 p.

7. The application of analytical pyrolysis in studies of weathered stones permits differentiation between biologically synthesized and anthropogenic compounds or between biological and anthropogenic crusts.

20 June 1988

C. Saiz Jimenez

C O N T E N T S

This thesis is based on the following publications:

Chapter 2 : C. Saiz-Jimenez and J.W. de Leeuw.
Organic Geochemistry 6 (1984) 287-293.

Chapter 3 : C. Saiz-Jimenez and J.W. de Leeuw.
Journal of Analytical and Applied Pyrolysis 9 (1986) 99-119.

Chapter 4 : C. Saiz-Jimenez and J.W. de Leeuw.
Organic Geochemistry 6 (1984) 417-422.

Chapter 5 : C. Saiz-Jimenez and J.W. de Leeuw.
Organic Geochemistry 10 (1986) 869-876.

Chapter 6 : C. Saiz-Jimenez, J.J. Boon, J.I. Hedges, J.K.C. Hessels and J.W. de Leeuw.
Journal of Analytical and Applied Pyrolysis 11 (1987) 437-450.

Chapter 7 : C. Saiz-Jimenez and J.W. de Leeuw.
The Science of the Total Environment 62 (1987) 115-119.

Chapter 8 : C. Saiz-Jimenez and J.W. de Leeuw.
Journal of Analytical and Applied Pyrolysis 11 (1987) 367-376.

Chapter 9 : C. Saiz-Jimenez, J.W. de Leeuw and G. Gomez-Alarcon.
The Science of the Total Environment 62 (1987) 445-452.

Chapter 10: C. Saiz-Jimenez, N. Senesi and J.W. de Leeuw.
Journal of Analytical and Applied Pyrolysis (submitted).

The publishers of Organic Geochemistry, Journal of Analytical and Applied Pyrolysis and The Science of the Total Environment are gratefully acknowledged for their permission to use the already published papers in this thesis.

SUMMARY

The results described in this thesis can be summarized as follows:

- Pyrolysis-gas chromatography and pyrolysis-gas chromatography-mass spectrometry are powerful analytical techniques to study the chemical nature of the soil organic matter fractions.

- The data obtained in this study reveal major contribution of polysaccharides, proteins, lignins, lipids and pollutant compounds in the different soil humic fractions (Chapter 3).

- Humic acids are characterized by an easily pyrolysable, acid hydrolysable and/or persulfate oxidisable moieties consisting of polysaccharides, proteins, lignins and a resistant moiety of a highly aliphatic nature (Chapters 7 and 8).

- The structures of the pyrolysis products encountered in the humic acids are also observed in more or less pure lignin preparations and in polysaccharides. Hence, there is no need to assume that the pyrolysable part of the humic acids consists of a condensed lignin-polysaccharide structure; a simple mixture of these two biopolymeric substances can explain the results satisfactorily. Further on, there is no direct need to assume that humic substances are generated by condensation reactions of lipids, carbohydrates, amino acids, etc. On the contrary, mixtures of more or less biodegraded biopolymers and originally present low molecular weight compounds explain the pyrolysis/evaporation data very well (Chapter 3).

- Fulvic acid fractions are made up of polysaccharide units or remains of these biopolymers with a varying contribution of lignins and/or lignin phenols, and fatty acids, as denoted by

the pyrolysis/evaporation products. The presence of polymaleic acid-like structures seems unlikely. The chemical composition of the different fulvic acid fractions is a consequence of the fractionation procedure followed (Polyclar or charcoal), as demonstrated in Chapters 2 and 3.

- Humins are similar to humic acids, except that they contain more polysaccharides (Chapter 3).

- Hymatomelanic acids cannot be considered as a true humic fraction, as they consist almost entirely of lipid compounds extractable from the humic acids. Consequently, it is to be expected that extraction of the intact soil with toluene-methanol, prior to fractionation will considerably reduce or completely eliminate the hymatomelanic acid fraction (Chapter 3).

- Because of the relatively high resistance of lignins to biodegradation and diagenesis, especially in anoxic environments, lignins are selectively preserved upon burial. However, in aerobic environments biodegradation of lignins by white rot fungi results in severe oxidation of the propenyl moiety (Chapter 4 and 6).

- Lignin and lignin derived products can be considered as characteristic biomarkers for terrestrial input. Softwood, hardwood and grass lignins can be differentiated in terms of their pyrolysis products. Pyrolysis data, wet chemical degradation and NMR data are in good agreement indicating that pyrolysis methods are useful for fast detailed characterization of woods and lignins (Chapters 5 and 6).

- The role of lignin in the formation of humic substances seems to be overestimated. Lignin and degraded lignin may be coextracted with humic acids or may be linked to humic substances, but they do not contribute significantly to the

resistant part of the soil humic acids. Other more resistant plant components such as the highly aliphatic biopolymer present in plant cuticles and suberin could represent important moieties of the humic acid structure (Chapters 8-10).

SAMENVATTING

De resultaten van het onderzoek beschreven in dit proefschrift kunnen als volgt worden samengevat:

- Pyrolyse-gas chromatografie en pyrolyse-gas chromatografie-massa spectrometrie zijn geschikte analytische technieken om chemische karakteristieken van gefractioneerd organisch materiaal uit bodems te bestuderen.

- De verkregen data geven aan dat er aanzienlijke hoeveelheden polysacchariden, proteïnen, lignines, lipiden en anthropogene verbindingen in de verschillende bodemhumusfracties aanwezig zijn (Hoofdstuk 3).

- Humuszuren worden gekenmerkt door polysaccharide-, proteïne- en lignine-brokstukken die via zure hydrolyse en/of persulfaat oxidatie kunnen worden verwijderd. Een residue met een duidelijk alifatisch karakter blijft over (Hoofdstukken 7 en 8).

- Vele structuren van de pyrolyse producten in de pyrolysaten van humuszuren worden ook aangetroffen in pyrolysaten van min of meer zuivere lignine preparaten en in polysacchariden. Daarom is er geen directe aanleiding om aan te nemen dat het pyrolyseerbare gedeelte van humuszuren een gecondenseerde lignine-polysaccharide structuur heeft; een mengsel van deze twee macromoleculaire componenten kan eenvoudig en afdoend de resultaten verklaren. Ook zijn er geen aanwijzingen dat humuszuren ontstaan zijn via condensatie van lipiden, suikers, aminozuren etc. In tegendeel, mengsels van min of meer gebiodegradeerde biopolymeren en oorspronkelijk reeds aanwezige laag moleculaire verbindingen verklaren de pyrolyse/evaporatie data zeer goed (Hoofdstuk 3).

- Fulvinezuur fracties blijken gebaseerd op de pyrolyse/evaporatie data te bestaan uit polysaccharide eenheden, al dan niet gedeeltelijk omgezet, met een variërende bijdrage van lignine en/of lignine fenolen en vetzuren. De aanwezigheid van polymaleïne zuurachtige structuren lijkt onwaarschijnlijk. De chemische samenstelling van verschillende fulvinezuur fracties wordt mede bepaald door de gevolgde fractioneringsprocedure (Polyclar of actieve kool) (Hoofdstukken 2 en 3).

- Humine fracties lijken veel op humuszuren, zij het dat ze wat meer polysacchariden bevatten (Hoofdstuk 3).

- Hymatomelaanzuren kunnen niet worden beschouwd als een "echte" humus fractie, daar zij nagenoeg geheel bestaan uit lipiden die uit humuszuren te extraheren zijn.
  Het kan dan ook verwacht worden dat bij een extractie van het integrale bodemmonster met b.v. tolueen/methanol voorafgaande aan de humusfractionering er geen of nagenoeg geen hymatolaanzuur fractie zal zijn (Hoofdstuk 3).

- Vanwege de relatieve resistentie van lignines ten aanzien van biodegradatie, in het bijzonder in anoxische milieus, worden lignines selectief aangereikt tijdens de vroege diagenese in bodems. In oxische milieus is dit in mindere mate het geval daar schimmels biodegradatie van met name de propenyl zijketen in lignines bevorderen (Hoofdstukken 4 en 6).

- Lignine en ligninederivaten kunnen worden beschouwd als karakteristieke "biomarkers" voor een terrestrische bijdrage aan het organische materiaal in sedimenten. Zacht hout-, hard hout- en graslignines kunnen worden onderscheiden aan hun pyrolyseproducten. Pyrolyse-, nat chemische degradatie- en NMR-data van recent en fossiel hout bleken in goede overeenstemming met elkaar; pyrolyse methoden zijn dus bruikbaar voor een

snelle en gedetailleerde karakterisering van hout en lignine (Hoofdstukken 5 en 6).

- De rol van lignine bij de vorming van humus lijkt overschat te zijn. Lignine en ligninederivaten kunnen na extractie terechtkomen in de humuszuur fractie. Ook als zij gebonden zouden zijn aan humus dragen zij echter weinig bij aan het resistente gedeelte van bodemhumuszuren. Andere, nog meer resistente plantenonderdelen zoals het alifatische biopolymeer in plantencuticulae en suberine kunnen belangrijke entiteiten vertegenwoordigen in de structuur van humuszuren (Hoofdstukken 8, 9, 10).

RESUMEN

Los resultados obtenidos en esta tesis pueden resumirse en las siguientes conclusiones:

- La pirólisis-cromatografía de gases y la pirólisis-cromatografía de gases-espectrometría de masas son técnicas analíticas adecuadas para el estudio de la naturaleza química de las distintas fracciones de la materia orgánica del suelo.

- Los resultados obtenidos en este estudio revelan que en las fracciones húmicas del suelo estan presentes, en mayor o menor cantidad, polisacáridos, proteinas, ligninas, lípidos y contaminantes (capítulo 3).

- Los ácidos húmicos se caracterizan por tener una mitad facilmente pirolizable, hidrolizable y oxidable, formada por polisacáridos, proteinas y ligninas, y una mitad, resistente a estos tratamientos, de naturaleza altamente alifática (capítulos 7 y 8).

- Las estructuras de los productos de pirólisis encontrados en los ácidos húmicos son las mismas que las observadas en muestras más o menos puras de ligninas y polisacáridos. Por tanto, no puede inferirse que la parte pirolizable de los ácidos húmicos tenga una estructura formada por ligninas y polisácaridos condensados; una simple mezcla de estos dos biopolímeros explicaría los resultados satisfactoriamente. Ademas, no puede asumirse que las sustancias húmicas se originen por reacciones de condensación entre lípidos, carbohidratos, aminoácidos, etc. Por el contrario, la existencia de mezclas de biopolímeros, transformados o degradados, y compuestos de bajo peso molecular explicarian los resultados obtenidos (capítulo 3).

- Las fracciones de ácidos fúlvicos estan constituidas por polisacáridos o restos de estos biopolímeros con una contribución variable de lignina y/o fenoles derivados de ligninas, así como lípidos, como se deduce de los resultados de la pirólisis. La presencia de estructuras del típo ácido polimaleico parece poco probable. La diferente composición química de las fracciones de ácidos fúlvicos es una consecuencia de los metodos de fraccionamiento seguidos (Polyclar o carbón activo) como se demuestran en los capítulos 2 y 3.

- Las huminas son similares a los ácidos húmicos aunque presentan un mayor contenido en polisácaridos (capítulo 3).

- Los ácidos himatomelánicos no pueden considerarse como fracción húmica, ya que estan compuestos casi enteramente de lípidos, extraidos de los ácidos húmicos. Consecuentemente, la extracción de un suelo con tolueno-metanol, antes del fraccionamiento, reducirá considerablemente o eliminará la fracción de ácidos himatomelánicos (capítulo 3).

- La lignina es selectivamente preservada en los sedimentos debido a su resitencia a la biodegradación y diagénesis, especialmente en ambientes anaeróbicos. Sin embargo, en ambientes aeróbicos, los hongos producen una importante oxidación en las cadenas laterales de la lignina (capítulos 4 y 5).

- La lignina y los fenoles derivados de lignina pueden considerarse como bioindicadores de aportes terrestres. Los distintos tipos de ligninas pueden diferenciarse mediante el estudio de sus productos de pirólisis. Los resultados obtenidos mediante pirólisis, degradaciones químicas y resonancia magnética nuclear coinciden, lo que demuestra que la pirólisis es una técnica analítica útil para una rápida caracterización de maderas y ligninas (capítulos 5 y 6).

- El papel de la lignina en la formación de sustancias húmicas parece haber sido sobreestimado. La lignina y sus productos de degradación pueden coextraerse junto a los ácidos húmicos, e incluso pueden estar unidos a las diferentes fracciones húmicas, pero no contribuyen significativamente a la mitad resistente de los ácidos húmicos del suelo. Otros componentes vegetales, dificilmente biodegradables tales como, por ejemplo, el biopolímero alifático presente en cutículas vegetales y suberina, pueden constituir una parte importante de la molécula húmica (capítulos 8-10).

C H A P T E R 1

Introduction

Humic substances:

Their definition. Humic substances have attracted the attention of soil chemists for over two centuries; in spite of intensive investigations, knowledge of the nature of these materials is still limited, however.

Part of the difficulties experienced are caused by inaccuracy and inconsistency of definitions used. Over the years the terms humus, soil organic matter and humic substances have been redefined continuously. The large number of definitions has lead to many confusions and is partly caused by attempts of many investigators to relate definitions of these materials with their structural features. Because these materials have a very complex, mainly macromolecular nature it is understandable that analytical data of all kinds related to partial structures present in these materials are interpreted in many different ways.

For a detailed historic review of these and other terms the reader is referred to Waksman (1936), Kononova (1966), Stevenson (1982) Aiken et al. (1985), Frimmel and Christman (1988). The most recent definitions after Stevenson (1982) and Aiken et al. (1985) are given in Tables 1 and 2.

Based on their solubility in alkali and acid, humic substances are usually divided into three main fractions: humic acids, which are soluble in dilute alkali but precipitate on acidification of the alkaline extract; fulvic acids which comprise that humic fraction which remains in solution when the alkaline extract is acidified; humins, which comprises humic fractions that cannot be extracted directly from the soil by dilute alkali or acid but by alkali only after HF/HCl treatment. A certain number of subfractions can also be obtained by solvent extraction or addition of electrolytes (Figure 1). Although the definitions of these humic fractions have undergone changes over the years as well they cause much less confusion because they are operationally defined without reference to their structural

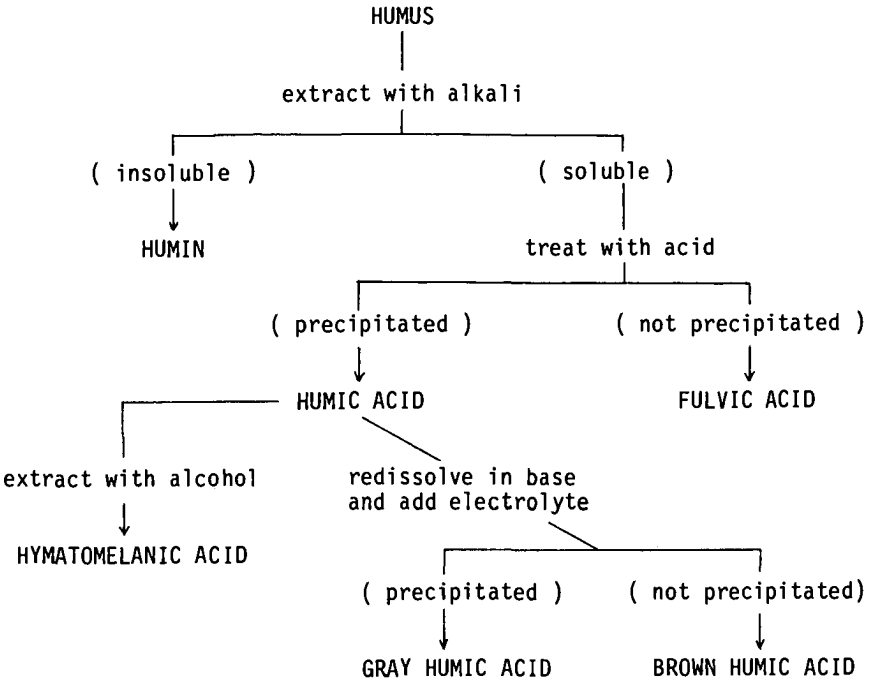
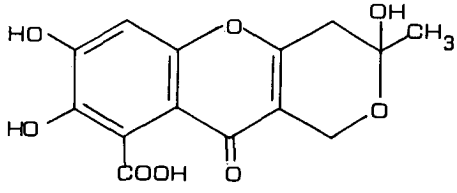


Figure 1. Scheme for the fractionation of humic substances, after Stevenson (1982)

properties. Figure 2 shows a guide to nomenclature of humic substances recently published by Thurman et al. (1988).

The classification schemes for defining components of humic substances have been questioned from time to time. As early as 1936, Waksman stated that it is not justified to draw conclusions on the chemical structures of various types of humic fractions obtained by a separation based upon solubility. In other words, humic acids, fulvic acids and humins are operationally defined terms as already indicated above.

Humic and fulvic acids obtained by different procedures or from different sources may differ significantly in their chemical composition (Saiz-Jimenez et al., 1979; 1986). Therefore, these fractions do not correspond to a unique chemical entity and they cannot be described in terms of precise chemical structure. These definitions have survived because of their practical utility, in spite of striking facts, as for instance, the existence of a well defined compound synthesized by Penicillium griseofulvum, P. flexuosum and P. brefeldianum with the structural formula



called fulvic acid (Turner, 1971).

Because of this vagueness, many controversies about humic fractions have originated over the years. However, whereas the fractionation scheme is arbitrary to some extent it has nonetheless been widely accepted because the fractions are in general more suitable for further analysis than unfractionated humic substances.

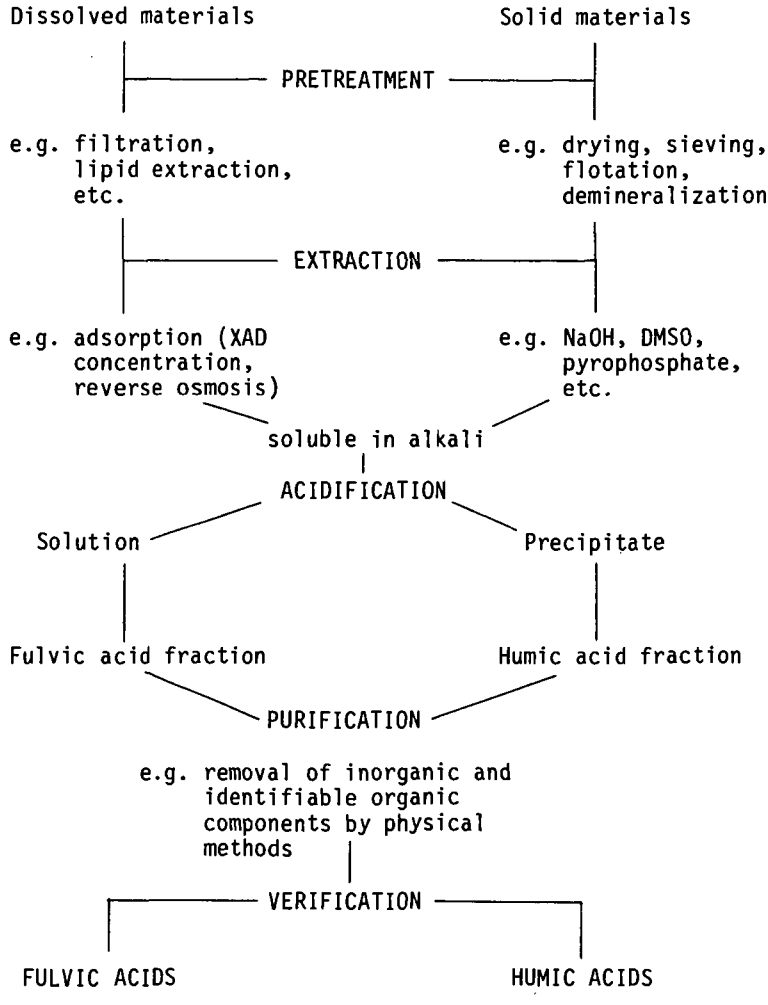


Figure 2. A guide to nomenclature of humic substances, after Thurman et al. (1988)

Humic substances:

Their origin. The term humus dates back to the time of the Romans, when it was used to designate the soil as a whole.

Wallerius first defined humus, in 1761, in terms of decomposed organic matter. The first historical reference to the isolation of a compound similar to that which became later known as humic acid dates to Achard, who, in 1786, extracted a brown substance from soil and peat, by using alkaline solutions. Upon adding sulphuric acid to the alkaline extract, he obtained a dark brown to almost black precipitate.

In 1804, de Saussure introduced the term humus, the latin equivalent of soil, to designate the dark-coloured organic matter in soil.

The first comprehensive study on the origin and chemical nature of humic substances was carried out by Sprengel (1837). Many of the procedures he developed for the preparation of humic acids became generally adopted, such as pretreatment of the soil with dilute mineral acids prior to the extraction with alkali.

The modern foundations of humus chemistry are attributed to Sven Oden (1919). Oden considered humic compounds as the light-brown to dark-brown substances of unknown constitution which are formed in nature by decomposition of organic matter through the actions of atmospheric agencies or in the laboratory by chemical reagents, and humic acids as those humic substances which show acid properties and thus form salts with strong bases.

Years before, Maillard (1912) suggested that humus is the product of a condensation reaction between carbohydrates and amino acids, in which microorganisms are not involved.

Eller (1921) stated that the oxidation of phenol, quinone and hydroquinone in an alkaline solution yields compounds similar to humic acids.

Beckley (1921) pointed out that the action of mineral acids on carbohydrates results in the formation of hydroxymethyl furfural, which on condensation gives rise to humus.

The most generally accepted theory is that humic substances are derived from lignin. According to this theory, lignin is

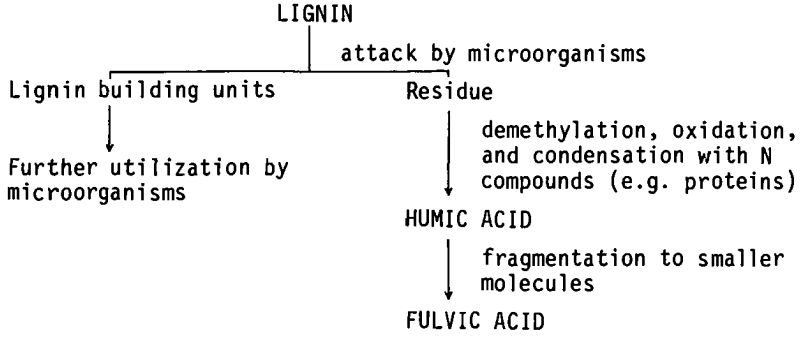


Figure 3. Schematic representation of the lignin theory of humus formation, after Stevenson (1982)

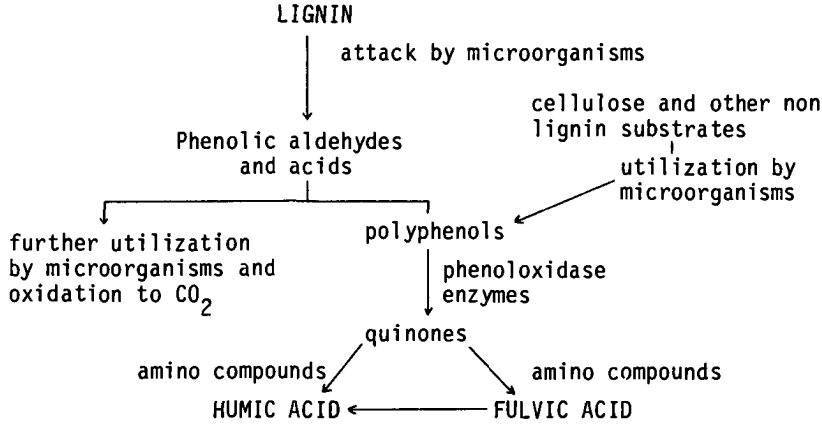


Figure 4. Schematic representation of the polyphenol theory of humus formation, after Stevenson (1982)

incompletely degraded by microorganisms and the residual moiety becomes part of the soil humus (Figure 3).

Wehmer reported in 1915 that, during fungal degradation of wood the lignocellulose is attacked, the cellulose is metabolized and the lignin in converted into humic substances.

Waksman (1936) stated that the lignin theory is substantiated by a number of facts, summarized as follows:

1. Decomposing wood and peat increase in lignin content with time.
2. Lignin, as opposed to cellulose, gives rise to aromatic transformation products. Humic acids are found to contain aromatic moieties as well.
3. Both lignin and humic acids contain methoxyl groups; the methoxyl content decreases with proceeding decomposition.
4. Both lignin and humic acids are acidic in nature; both can react with bases and both are characterized by their capacity of cation exchange, all be it to different degrees.
5. Both lignin and humic acids are insoluble in cold concentrated acids, the degree of insolubility increasing with proceeding decomposition.
6. When lignins are heated in aqueous alkaline solutions, they are transformed into methoxyl-containing humic acids, which does not hold for cellulose.
7. On oxidation under pressure, lignin gives rise to humic acids and finally to aromatic carboxylic acids, but cellulose is changed to other products.
8. Oxidation of brown coal under pressure gives benzenecarboxylic acids and no furancarboxylic acids.
9. Both lignin and humic acids are oxidized by oxidizing agents, such as permanganate and hydrogen peroxide.
10. Both lignin and humic acids are soluble in alkalis and precipitated by acids.
11. Both lignin and humic acids are partly soluble in alcohol and pyridine, depending also on the method of preparation; some lignin and some humus preparations are completely soluble in alcohol.

12. Both lignin and humic acids are decomposed with great difficulty or not at all by the great majority of fungi and bacteria.

Further, Waksman presumed that the nitrogen contained in humic acids resulted from a condensation of modified lignin with microbially derived proteins and other nitrogen-containing compounds.

These days, the so called polyphenol theory as put forward by Stevenson (1982) had become the more accepted one (Figure 4). It states that quinones of lignin origin, together with those synthesized by microorganisms polymerize either autooxidatively or by phenol oxidases in the presence of amino compounds to form humic macromolecules. Stevenson mentioned that because lignins are major plant constituents and relatively resistant to microbial decomposition they are sometimes considered to be the major, if not the primary, source of phenolic units in humic substances.

Humic substances:

Their chemical structure. The study of the chemical nature of humic substances has been hampered not only by their insolubility and macromolecular structure but also by the fact that they are a complex conglomerate of a variety of subunits and do not constitute a uniform molecule.

Much work has been devoted to the elucidation of the chemical structure of soil humic substances over a long period of time. Because of the complexity mentioned, degradative methods have been used with the aim of producing compounds that could be identified and whose structures could be related to those of the starting materials.

The degradative methods that have been used are mainly oxidations, reductions and hydrolyses. The oxidative reagents are often strong and could lead to significant alterations of the original building blocks and to the formation of artifacts. These artifacts are defined according to Norwood (1988) as identified

degradation product whose formation pathway is incorrectly interpreted leading the investigator to false structural inferences. This phenomenon has been demonstrated in the case of permanganate oxidation (Hayes and Swift, 1978). Recently, Martin et al. (1981) described persulfate as a mild oxidant which degrades about 50% of soil humic acids, leaving a residue that can easily be recovered and subjected to further analysis.

In general, much of the work on oxidative degradation is of limited value, because the structures of the reaction products only shed some light on those of the building blocks and not so much on their interconnections. The interpretation can even be incorrect because, in many instances, the naturally occurring units could be altered before or after their release from the macromolecular structure.

Reductive degradations e.g. by sodium amalgam have been successfully applied by Martin et al. (1974) to a diversity of soil humic substances and phenolic polymers. The method seems to work with samples with a certain number of aromatic ether linkages or with biphenyl structures where activating (hydroxy of methyl) substituents are ortho and/or para to the connecting bond (Hayes and Swift, 1978). Most of the compounds identified in humic substances are similar to those obtained by degradation of lignins or microbial phenolic polymers suggesting that these types of materials are also present in soil humic substances.

Hydrolytic procedures are effective in removing protein and polysaccharide constituents associated with humic substances. However, the rest of the molecule is not affected (Riffaldi and Schnitzer, 1973).

As a result of the identification of building blocks obtained by various degradative and non-degradative methods some model structures have been proposed for humic substances. Thus, Haworth (1971) concluded that the e.s.r. signal of humic acids is due to a complex aromatic core to which polysaccharides, proteins, simple phenols and metals are chemically and/or physically bound (Figure 5).

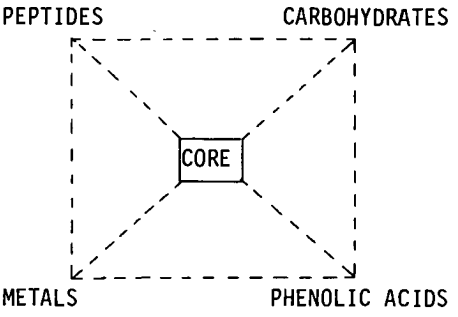


Figure 5. Diagramatic representation of a humic acid, after Haworth (1971)

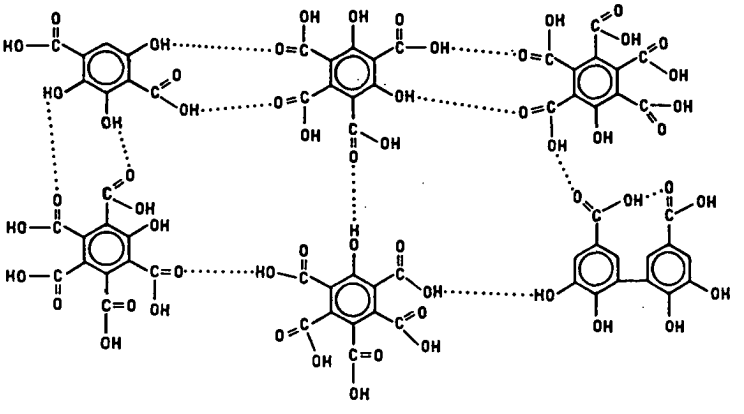


Figure 6. Structure of a fulvic acid, after Schnitzer (1978)

Based on degradative and non degradative methods and assuming that the major products thus obtained are building blocks, Schnitzer (1978) proposed
a model structure (Figure 6) in which the phenolic and benzenecarboxylic acids could be held together by hydrogen bonds, Van der Waal's forces, and π-bonding.

According to Stevenson (1982) humic substances consist of a heterogeneous mixture of compounds for which no single structural formula will suffice. Each fraction must be regarded as consisting of a series of molecules of different sizes, few having precisely the same structural configuration or array of reactive groups. As far as the structure of humic acids is concerned, contemporary investigators favour a "model" consisting of micelles of a polymeric nature, the basic structure of which is an aromatic ring of the di- or trihydroxy-phenol type bound by -O-, $-CH_2-$-NH-, -N=, -S- bridges and other groups. These structures may contain attached proteinaceous and carbohydrate residues.

Analytical pyrolysis.

Curie point pyrolysis in combination with either low voltage electron impact mass spectrometry or gas chromatography -mass spectrometry has been used for differentiating between microorganisms and to study the chemical nature of synthetic polymers, bio- and geopolymers (Meuzelaar et al., 1982).

Nagar et al. (1975) indicated that pyrolysis-mass spectrometry was promising for soil humus research. A more extensive study was made by Meuzelaar et al. (1977) by comparing humic acids from soils, peats and composted straw, with fungal melanins and lignins. Humic acids from different soils and peats and most of the fungal melanins gave similar pyrolysis mass spectra with typical ion series, related to proteins, polysaccharides and aromatic compounds. Furthermore, pyrolysis mass spectra of humic acids showed ion series typical of lignin (Haider et al., 1977).

Saiz-Jimenez et al. (1979) studied the different organic matter fractions present in a soil. The pyrolysis mass spectra of humic acid fraction showed prominent mass peaks related to polysaccharides, proteins and lignins. The spectrum of the humin fraction resembled those of the humic acid ones although peaks thought to originate from complex polysaccharides were more evident. The pyrolysis-mass spectrum of the polysaccharide fraction showed the characteristic pattern of the pyrolysate of a complex polysaccharide together with fragments from polymers of amino acids or amino sugars. The pyrolysis mass spectrum obtained from the fulvic acid fraction showed clear dissimmilarities to those of the humic acid fraction; signals from proteins as well as those related to phenols were low. Depending upon the isolation methods, pyrolysis mass spectra of fulvic acid preparations showed different suites of peaks related to polysaccharide and phenolic materials. Based on pyrolysis mass spectrometry hymatomelanic acid fraction gave the impression that it consisted of material rich in polysaccharides and lignins removable from humic acids by extraction with ethanol. This assumption was based on mass spectra with a limited mass range (m/z below 180). However, upon pyrolysis-gas chromatography-mass spectrometry analysis the hymatomelanic acid fraction was shown to consist mainly of lipid materials (see chapter 3).

Bracewell (1973) and Robertson (1977) have shown that pyrolysis-mass spectrometry enables the recognition of variations in humus type and differences of the genetic horizons in a soil profile. Pyrolysis-mass spectrometry has shown great promise in studies on the structures of soil organic matter and on the humification processes involved. This analytical approach can be used as a fingerprinting technique to clearly show similarities and dissimilarities between different fractions and samples. Preliminary tentative structural information is obtained as well.

To obtain more detailed insight into the structural composition of soil humic substances firmer identifications of pyrolysis products which reflect structural units present within the macromolecular matrix, are required. This can be achieved by

application of pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS).

Previous Py-GC-MS studies (Martin et al., 1977, 1979) have shown that common pyrolysis products of soil humic acids are aliphatic hydrocarbons and compounds related to polysaccharides, proteins, lignins. Further, Faix et al (1987) stated that pyrolysis of lignins gives similar results to those obtained by destructive (nitrobenzene oxidation) and non destructive (FTIR) methods.

Framework of the thesis.

In this thesis several soil organic matter fractions and composted humic acids are investigated in order to elucidate their composition and origin. Moreover, different types of lignins and woods and some of their degraded counterparts have been studied in an attempt to understand the complex structural changes occurring to woods and lignins by biotic and abiotic diagenesis.

In Chapter 2 the results of investigations of soil fulvic acids, a soil polysaccharide and polymaleic acid are presented. The soil fractions and the synthetic polymer were analysed by pyrolysis-gas chromatography-mass spectrometry in order to determine whether or not fulvic acids are mainly composed of polysaccharides and to what extent polymaleic acid-like structures occur in this humic fraction.

A detailed analysis of the soil organic matter fractions obtained from a representative soil, following the classical method of fractionation of humic substances, is summarized in Chapter 3. The data allow for an easy discrimination of these soil fractions based on the major characteristic series of pyrolysis products of each fraction.

Because lignin appears to be an important contributor to some of the soil humic fractions, a description of the pyrolysis products obtained from a spruce milled wood lignin, before and after fungal degradation, is presented in Chapter 4. The data

were also compared to those of a synthetic lignin and an industrial lignin.

Chapter 5 stresses the significance of lignin pyrolysis products as biomarkers. Pyrolysis permits a differentiation of the three types of lignins based on the presence or absence of characteristic phenols.

In Chapter 6 wet chemical and spectroscopic data of buried and present-day woods are compared with analytical pyrolysis data. The results obtained indicate that the pyrolysis data are in good agreement and are also complementary with the other data.

Because soil humic acids are complex mixtures of a broad variety of materials, acid hydrolysis was applied to remove the polysaccharide and proteinaceous moieties followed by solvent extraction of lipids. The residual humic acid fractions were pyrolysed and the significance of the greater part of the pyrolysis mixture, consisting of homologous series of straight chain alkanes, alk-1-enes and α,ω-alkadienes is discussed in Chapter 7. A more detailed study of the most resistant part of soil humic acids is described in Chapter 8. The residues obtained after acid hydrolysis and persulfate oxidation gave similar chromatograms of pyrolysis products, dominated by homologous series of aliphatic hydrocarbons, which might be related to the highly aliphatic biopolymers encountered in plant cuticles and suberins (Nip et al. 1986).

The chemical characterization and the pyrolysis data of the humic acid fraction of a sludge obtained from waste water of olive mills after disposal in lagoons is presented in Chapter 9. This and other wastes or composted materials are being used to fertilize agricultural soils. Chapter 10 describes some of the chemical and pyrolysis data of humic acids extracted from vermicomposts (manure composted by earthworms). The results show that lignin constitutes an important part of the organic matter present in the composted manures and can be isolated in the humic acid fraction.

TABLE 1

Glossary of terms after Stevenson (1982)

Terms	
Humus	Total of the organic compounds in soil exclusive of undecayed plant and animal tissues, their partial decomposition products, and the soil biomass
Soil organic matter	Same as humus
Humic substances	A series of relatively high-molecular-weight, brown to black coloured substances formed by secondary synthesis reactions. This term is used as a generic name to describe the coloured material or its fractions obtained on the basis of solubility characteristics. These materials are distinctive to the soil (or sediment) environment in that they are dissimilar to the biopolymers of microorganisms and higher plant (including lignin)
Nonhumic substances	Compounds belonging to known classes of biochemistry, such as amino acids, carbohydrates, fats, waxes, resins, organic acids, etc. Humus probably contains most, if not all, of the biochemical compounds synthesized by living organisms
Humic acid	The dark-coloured organic material which can be extracted from soil by various reagents and which is insoluble in dilute acid

Fulvic acid	The coloured material which remains in solution after removal of humic acid by acidification
Humin	The alkali insoluble fraction of soil organic matter or humus
Hymatomelanic acid	Alcohol soluble portion of humic acid.

TABLE 2

Glossary of terms after Aiken et al. (1985)

Terms	Definition
Humus	The organic portion of soil, brown or black in colour, consisting of partially or wholly decayed plant and animal matter, that provides nutrients to plants and increases the ability of soil to retain water. This term is not entirely synonymous with humic substances, although it is often used as a synonym.
Humic substances	A general category of naturally occurring, biogenic heterogeneous organic substances that can generally be characterized as being yellow to black in colour of high molecular weight, and refractory.
Humification	The process of fermentation of humic substances; generally the decomposition of organic material.
Humic acid	That fraction of humic substances that is not soluble in water under acid conditions (below pH 2), but becomes soluble at greater pH.
Fulvic acid	That fraction of humic substances that is soluble under all pH conditions.
Humin	That fraction of humic substances that is not soluble in water at any pH value.

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C H A P T E R 2

Pyrolysis-gas chromatography-mass spectrometry of soil polysaccharides, soil fulvic acids and polymaleic acid.

Pyrolysis–gas chromatography–mass spectrometry of soil polysaccharides, soil fulvic acids and polymaleic acid

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Abstract—Cryogenic Curie-point pyrolysis–gas chromatography–mass spectrometry has been applied to investigate the chemical composition of organic matter present in soils. Two soil fulvic acid fractions, a so-called soil polysaccharide fraction and polymaleic acid were analyzed. The soil polysaccharide fraction contains almost exclusively polysaccharides with major building blocks glucose, mannose and galactose. The soil fulvic acid fractions contain varying amounts of polysaccharides, lignins and lipids. Polymaleic acid structures were virtually absent in the podzol fulvic acid and absent in other soil organic matter fractions, indicating that these structures, previously suggested to be present in soil fulvic acids, do not play an important role.

Key words: pyrolysis, soil polysaccharides, soil fulvic acid, polymaleic acid, soil organic matter, humus

INTRODUCTION

The extraction by means of base and acid is a widely used method in soil chemistry to fractionate organic matter. The major fractions thus obtained are labelled humic acids (soluble in base, insoluble in acid), fulvic acids (both base and acid soluble) and humins (base insoluble).

Chemically spoken these fractions consist of well-defined compounds such as polysaccharides, proteins, lipids, lignins (nonhumic materials) and less well-defined complex polymeric organic matter (humic materials). It is thought that this humic material is a random condensation product of monomeric and oligomeric compounds released from decaying plants and animals and microbial cell components (Haider *et al.*, 1975).

In some cases it is possible, to some extent, to separate humin, humic, and fulvic acid fractions into humic and nonhumic components. For example, the so-called soil polysaccharide fraction is obtained from the fulvic acid fraction by adsorption on artificial polymers such as polyvinyl pyrrolidone Polyclar AT (Acton *et al.*, 1963).

The chemical nature of the humic components present in the humic acids, fulvic acids and humins have been investigated for about 100 years. However, it is still relatively unclear what the structural composition of these humic substances is. The major reason for this very likely is the difficult accessibility for chemical analysis due to the polymeric matrix.

Many investigators have used chemical degradation techniques in an attempt to overcome this problem (Schnitzer and Khan, 1972; Martin *et al.*, 1974, 1981). However, difficulties are being experienced in finding suitable degradative reagents and conditions which allow the isolation of structurally meaningful organic molecules, because the degradation reactions are either specific, resulting in low overall yields, or nonspecific, leaving uncertainty as to whether the products truly reflect the structure of the original material.

To avoid the problems encountered in chemical degradative methods we applied analytical flash pyrolysis to a selection of carefully chosen soil organic matter fractions. In this paper we report about the data obtained by pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) of a so-called soil polysaccharide, two fulvic acid fractions of different types of soils and polymaleic acid. This last sample was included in this study since recently some authors have considered polycarboxylic acids such as polymaleic acid as good model compounds for soil fulvic (Bracewell *et al.*, 1980) and humic acids (Wilson *et al.*, 1983).

EXPERIMENTAL

Typic Xerochrept soil

A brown soil, Typic Xerochrept according to the American Soil Taxonomy, was employed. The soil sample and the methods used for extraction and separation of the fulvic acid fraction have been described earlier (Saiz-Jimenez *et al.*, 1979). Briefly, the air-dried soil was extracted with a mixture of 0.1 M $Na_4P_2O_7$ and NaOH. The extract obtained after centrifugation was acidified with 0.1 N HCl. The resulting fulvic acid fraction was separated by adsorption on Polyclar AT into a soil polysaccharide and a purified fulvic acid fraction. The elemental composition and the pyrolysis mass analyses of these fractions have been reported before.

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Podzol soil

A podzol fulvic acid sample was kindly provided by Dr M. Schnitzer, Ottawa. The isolation and the chemical characteristics of this sample have been described elsewhere (Ogner and Schnitzer, 1971; Schnitzer, 1978).

Polymaleic acid

Polymaleic acid was prepared by the pyridine-catalysed homopolymerization of maleic anhydride as described by Braun and Pomakis (1974). The obtained product was further purified by cation-exchange resins (Amberlite IR 120, in H^+ form). The elementary composition of the sample was: C 46%, H 4%, N 0.5%, O (by difference) 49.5%.

Pyrolysis–gas chromatography–mass spectrometry

The Py–GC–MS analyses were carried out using a pyrolysis unit similar to the one described by Meuzelaar *et al.* (1975) modified for use at high temperatures (van de Meent *et al.*, 1980). The Curie temperature of the wires used was 510°C. The pyrolysis products were separated on a capillary glass WCOT column (28 m × 0.5 mm i.d.) coated with CP sil 5 (1.25 μm film thickness) held at 0°C for 5 min and subsequently temperature programmed to 300°C at a rate of 5°C min^{-1}. Helium was used as the carrier gas. The chromatograph (Varian Model 3200) was coupled to a Varian Mat 44 quadrupole mass spectrometer operated in the EI mode at 80 eV and with a cycle time of 2 s.

RESULTS

The reconstructed ion chromatogram traces of the pyrolysis mixture obtained from the soil polysaccharide, the two soil fulvic acids and the polymaleic acid are shown in Figs 1–4. The peak numbers in the figures correspond with the numbers mentioned in Table 1.

Pyrolysis products were identified by comparison of their mass spectra with literature data and with standards when available. Further, Py–GC–MS data obtained for well-defined polymers like amylose, cellulose, chitin, several proteins and peptides, and lignins allowed for a more detailed recognition of typical pyrolysis products (van der Kaaden *et al.*, 1983a,b, 1984; Saiz-Jimenez and de Leeuw, 1984; Boon *et al.*, in preparation).

Due to the great complexity of the pyrolysis mixture not all the individual compounds present could be separated by gas chromatography, which sometimes hampered firm identifications. Some products are tentatively identified on the base of mass spectrometric characteristics. In the Table 1 they are indicated with a question mark.

Soil polysaccharide

The pyrogram of the fraction obtained from the Polyclar AT eluate, the soil polysaccharide (Fig. 1), is almost exclusively made up of pyrolysis products of polysaccharides.

The major pyrolysis products encountered: furfural (35), methylfurfural (48), 4-hydroxy-5,6-dihydro-2-*H*-pyran-2-one (50), 4-hydroxy-6-methyl-5,6-dihydro-2-H-pyran-2-one (55), levoglucosenone (71), 1,4-dideoxy-*D*-glycero-hex-1-enopyranos-3-ulose (102), and levoglucosanes from galactose (109), mannose (113), and glucose (119) units, are well-known and considered to be very specific pyrolysis products from polysaccharides. In addition, several typical chitin pyrolysis products are present.

Podzol fulvic acid

The pyrogram of the podzol fulvic acid preparation (Fig. 2) mainly consists of pyrolysis products of polysaccharides, heavily oxidized lignin moieties and dialkyl phthalates. Major components are methylfuran (17), acetic acid (23), furfural (35), methylfurfural (48), levoglucosenone (71), characteristic polysaccharide pyrolysis products, and several dialkyl phthalates (124–127). Present also are lignin pyrolysis products such as guaiacol (70), vinyguaiacol (103), vanillin (108), acetoguaiacone (112), methyl vanillate (114) and vanillic acid (121).

Typic Xerochrept fulvic acid

The pyrogram of the purified acid sample (Fig. 3) shows as major peak phenol (51), guaiacol (70), vinylguaiacol (103) and a dialkyl phthalate (129). In addition, other conspicuous peaks correspond to methylfuran (17), benzene (22), toluene (31), furfural (35), methylfurfural (48), methylguaiacol (92), catechol (97), 2-6-dimethoxyphenol (104), C_{14} and C_{16} fatty acid (123, 132) and another dialkyl phthalate (125). Therefore, as stated also for the podzol fulvic acid, this preparation is predominantly composed of polysaccharides with considerable amounts of lignin moieties.

Polymaleic acid

The pyrogram of polymaleic acid (Fig. 4) exhibits major peaks for maleic anhydride (33), 2,3-dimethylmaleic anhydride (53), methyl hydrogen succinate (80), methyl hydrogen maleate (81), and an unknown compound, (105). 2-Cyclopenten-1-one (29), that has been reported by Bracewell *et al.* (1980) to be a major pyrolysis product of aliphatic polycarboxylic acids and polymaleic acid, was only present in trace amounts. Other pyrolysis products reported by Bracewell *et al.* (1980) for polymaleic acid, such as phenol and cresols, are not represent in our pyrolysate or are hardly distinguishable from the background level. Fatty acids may arise from contamination during the polymerization and/or purification process.

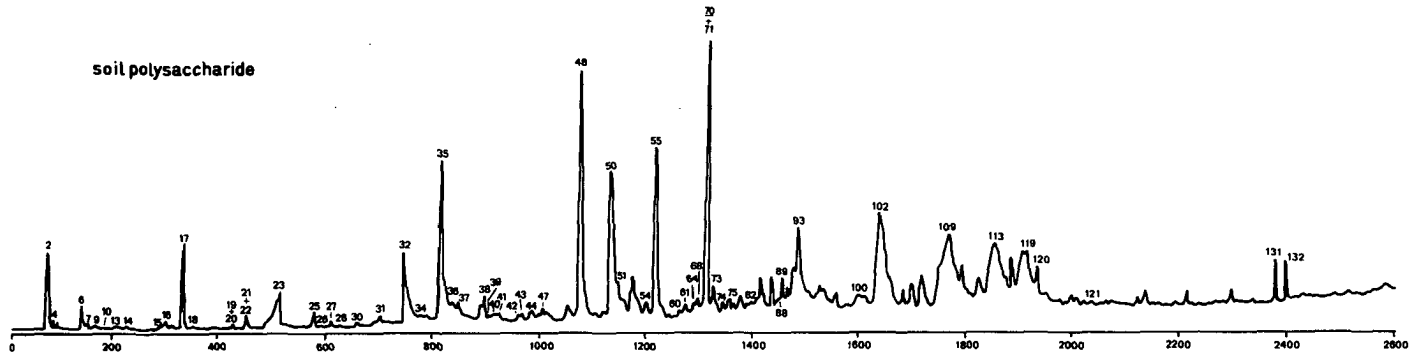

Fig. 1. Pyrogram of soil polysaccharide.

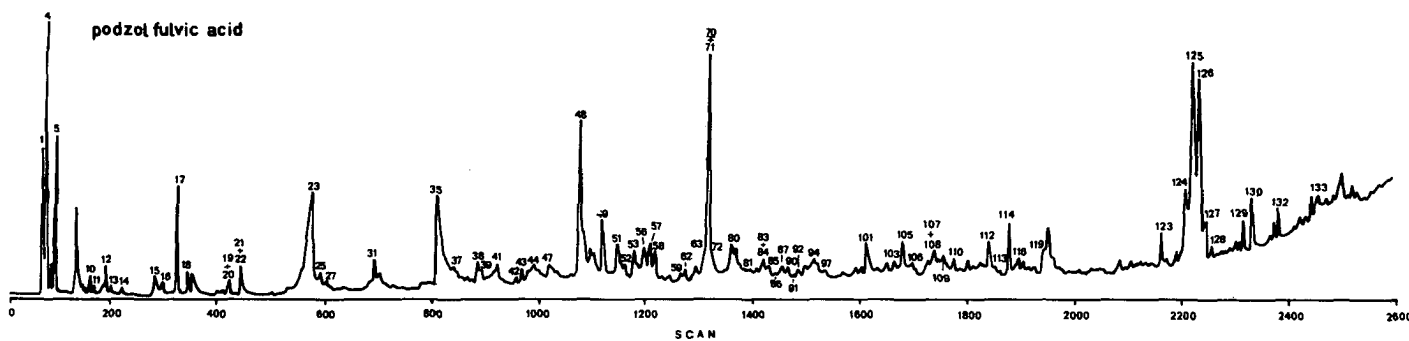

Fig. 2. Pyrogram of podzol fulvic acid. Underlined number indicates minor contribution to the peak.

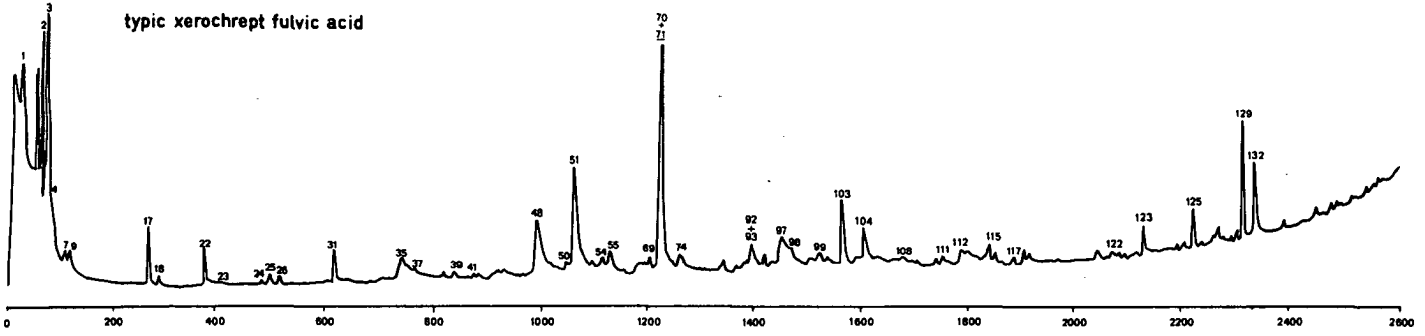

Fig. 3. Pyrogram of Typic Xerochrept fulvic acid.

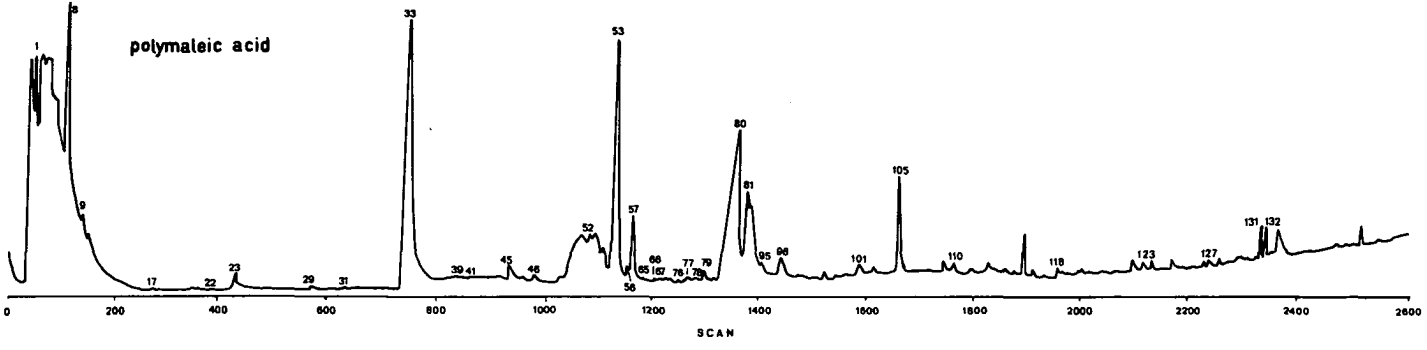

Fig. 4. Pyrogram of polymaleic acid. Underlined number indicates minor contribution to the peak.

Table 1. Pyrolysis products from soil polysaccharide, soil fulvic acids and polymaleic acid

Peak No.	Compound	Peak No.	Compound
1	Carbon monoxide	64	3-Hydroxy-6-methyl-3,4-dihydro-2*H*-pyran-2-one
1	Carbon dioxide	65	C_4-Alkylbenzene
1	Methane	66	C_4-Alkylbenzene
1	Ethane	67	C_4-Alkylbenzene
1	Hydrogen sulphide	68	Furan derivative
1	Propene	69	*p*-Cresol
1	Propane	70	Guaiacol
1	Methanol	71	Levoglucosenone
2	Sulphur dioxide	72	Methyl benzoate
3	Chloromethane	73	3-Hydroxy-6-methyl-2*H*-pyran-2-one
4	Methanethiol	74	3-Hydroxy-2-methyl-4*H*-pyran-4-one
5	Bromomethane	75	Chitin pyrolysis product
6	Butene	76	C_3-Alkylmaleic anhydride
7	Acetone	77	C_4-Alkylbenzene
8	Ethanol	78	C_4-Alkylbenzene
9	Furan	79	Ethyl methyl succinate
10	Iodomethane	80	Methyl hydrogen succinate
11	Ethanethiol	81	Methyl hydrogen maleate
12	Pentadiene	82	2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one
13	Cyclopentadiene		
14	2-Methylpropanal	83	C_3-Alkylphenol
15	2,3-Butadione	84	Methylindene
16	Pentane	85	C_4-Alkylbenzene
17	2-Methylfuran	86	C_4-Alkylbenzene
18	3-Methylfuran	87	Benzoic acid
19	3-Methylbutanal	88	C_4-Alkylbenzene
20	Hexene	89	Chitin pyrolysis product
21	2-Methylbutanal	90	C_4-Alkylbenzene
22	Benzene	91	Naphthalene
23	Acetic acid	92	Methylguaiacol
24	Ethylfuran	93	1,4-3,6-Dianhydro-α-*D*-glucopyranose
25	2,5-Dimethylfuran	94	Methylnaphthalene
26	2,4-Dimethylfuran	95	C_3-Alkylmaleic anhydride ?
27	2-Vinylfuran	96	Ethyl hydrogen succinate
28	*N*-Methylpyrrol	97	Catechol
29	2-Cyclopenten-1-one	98	Vinylphenol
30	2-Methylcyclopentanone	99	Ethylguaiacol
31	Toluene	100	Chitin pyrolysis product
32	Dihydropyran ?	101	Phthalic anhydride
33	Maleic anhydride	102	1,4-Dideoxy-*D*-glycero-hex-1-enopyranos-3-ulose
34	3-Furaldehyde	103	Vinylguaiacol
35	2-Furaldehyde	104	2,6-Dimethoxyphenol
36	Acetamide	105	Unidentified
37	Benzylalcohol	106	Eugenol
38	Furfuryalcohol	107	Methylphthalic anhydride
39	Ethylbenzene	108	Vanillin
40	3-Methylcyclopent-2-ene-1-one	109	Levoglucosane (galactose)
41	*m*-and/or *p*-xylene	110	Methylphthalic anhydride
42	Styrene	111	*trans*-Isoeugenol
43	*o*-Xylene	112	Acetoguaiacone
44	C_3-Alkylfuran	113	Levoglucosane (mannose)
45	Methylmaleic anhydride	114	Methyl vanillate
46	C_3-Alkylbenzene	115	Ethyl-2,6-dimethoxyphenol
47	α-Angelica lactone	116	(4-Hydroxy-3-methoxyphenyl)-propan-2-one
48	5-Methyl-2-furaldehyde	117	Vinyl-2,6-dimethoxyphenol
49	4-Oxo-pentanoic acid methyl ester	118	C_{12} Fatty acid
50	4-Hydroxy-5,6-dihydro-2*H*-pyran-2-one	119	Levoglucosane (glucose)
51	Phenol	120	Chitin pyrolysis product
52	C_3-Alkylbenzene	121	Vanillic acid
53	2,3-Dimethylmaleic anhydride	122	Acetosyringone
54	2-Hydroxy-3-methyl-2-cyclopenten-1-one	123	C_{14} Fatty acid
55	4-Hydroxy-6-methyl-5,6-dihydro-2*H*- pyran-2-one	124	Dialkyl phthalate
56	Dimethyl maleate	125	Dialkyl phthalate
57	Dimethyl succinate	126	Dialkyl phthalate
58	C_3-Alkylbenzene	127	Dialkyl phthalate
59	C_3-Alkylbenzene	128	C_{15} Fatty acid
60	5-(2-Hydroxyethylidene)-2(5*H*)-furanone	129	Dialkyl phthalate
61	2,3,4-Hexanetrione	130	C_{16} Fatty acid (branched)
62	2-Methylbutanoic acid methyl ester	131	Dialkyl phthalate
63	C_3-Alkylbenzene	132	C_{16} Fatty acid
		133	C_{18} Fatty acid

DISCUSSION

The products obtained after pyrolysis of the soil polysaccharide fraction very clearly show that this fraction consists almost exclusively of polysaccharides. Further, the soil polysaccharide fraction studied shows upon pyrolysis a similar pattern when compared with other well-defined plant polysaccharides (van der Kaaden *et al.*, 1983b).

The average composition of polysaccharides in inorganic soils showed that glucose, galactose and mannose are the dominant neutral sugars (Cheshire, 1977). It is noteworthy that the anhydrosugars from these units are clearly present in the pyrolysis products mixture. In addition, chitin pyrolysis products (van der Kaaden *et al.*, 1984) were present. Amino sugars, which account for up to 10% or more of the soil nitrogen and often form 5% of isolated soil polysaccharide (Cheshire and Anderson, 1975) are of widespread occurrence as structural components in micro-organisms and animals, and these seem the likely source of the soil material.

The obtained results show that Polyclar adsorption is an adequate method for isolating these polysaccharides, as already stated by Swincer *et al.* (1968) who reported that Polyclar proved to be the most useful of the materials tested for separating coloured materials from the polysaccharide in the fulvic acid solution. The question arises whether other so-called "soil polysaccharide" preparations previously studied by pyrolysis–gas chromatography (Martin, 1977) and isolated by acetone precipitation from the fulvic acid solutions are indeed mainly polysaccharides. In this context Acton *et al.* (1963) reported that the acetone precipitated fractions, after treatment of fulvic acid solutions with acetone, designated as microbial gum, has serious limitations as an indicator of soil polysaccharides because it has a high noncarbohydrate content and is not representative of the total carbohydrate constituents in soils. Further, the acetone soluble portion contained greater concentrations of polysaccharides than the precipitated gum portion.

The products present after pyrolysis of the podzol fulvic acid fraction mainly consist of polysaccharides and lignins, with a considerable contribution of phthalates. This observation disagrees with some studies of podzol fulvic acid fractions reported in the literature (Anderson and Russell, 1976; Schnitzer, 1978; Bracewell *et al.*, 1980). In some papers it has been stated, based on chemical degradation data, that the aromaticity of podzol fulvic acid fraction is about 70% (Schnitzer, 1977, 1978). However, it cannot be excluded, in our opinion, that the use of drastic oxidative reagents result in a severe breakdown of the nonaromatic moieties such as polysaccharides, resulting in a relative enrichment of aromatic structures.

Our results are much more in agreement with those of Hatcher *et al.* (1981) who found by CP–MAS ^{13}C NMR analyses an aromaticity of 35% in podzol fulvic acid fraction.

The presence of considerable amounts of dialkyl phthalates in this podzol fulvic acid is in agreement with the results of Ogner and Schnitzer (1970), who identified in this same preparation, after solvent extraction, several dialkyl phthalates. The origin of these phthalates, not shown in such high quantities in the other soil humic and nonhumic preparations (see Figs 1–4) is uncertain. Phthalates appear to be natural constituents of some plants and microorganisms (Peakall, 1975) and have been reported in a wide variety of substrates over a wide geographic area. Also, they may interact with fulvic acids during the extraction and purification procedures (Ogner and Schnitzer, 1970).

The pyrolysis products obtained from the Typic Xerochrept fulvic acid are mainly lignin and polysaccharide derivatives. Major differences with regard to podzol fulvic acid are the absence of aliphatic dicarboxylic acids and the minor amount of dialkyl phthalates.

The pyrolysis products obtained after the pyrolysis of polymaleic acid are related with the starting monomer. They are mainly maleic anhydride derivatives and aliphatic dicarboxylic acids. Bracewell *et al.* (1980) found 2-cyclopenten-1-one and 2,3-dimethyl maleic anhydride as major pyrolysis products from polymaleic acid. However, in our preparation 2-cyclopenten-1-one was a very minor pyrolysis product, maleic anhydride being the major one.

Bracewell *et al.* (1980) have reported that polycarboxylic and polymaleic acids can be regarded as model compounds for soil fulvic acids and water soluble soil organic polymers. Wilson *et al.* (1983) also consider that aliphatic polycarboxylic and polymaleic acids are important components of terrestrial and freshwater humic acids. This resemblance is not supported by our pyrolysis data. In fact, the differences among the pyrograms of polymaleic acid and the two soil fulvic acids are striking, and pyrolysis products directly derived from polycarboxylic and/or polymaleic acids represent a very minor part or are absent in fulvic acids. Also in our opinion, the short chain aliphatic dicarboxylic acids and their anhydrides present among the pyrolysis products of the podzol fulvic acid occur as such in the sample and may reflect metabolic products from plant origin and/or microbial activity.

CONCLUSIONS

(1) Pyrolysis–gas chromatography–mass spectrometry appears to be a powerful analysis technique to study the chemistry of soil organic materials, such as fulvic acids and polysaccharides.

(2) Soil fulvic acids, including podzol fulvic acid and the so-called soil polysaccharide fractions, are made up of polysaccharide units or remains of these biopolymers.

(3) In addition to polysaccharides, soil fulvic acids have varying contributions of lignins, dialkyl phthalates and fatty acids.

(4) A minor additional contribution of short chain aliphatic dicarboxylic acids and their anhydrides in the podzol fulvic acid pyrolysis mixture is observed. These compounds are thought to be present as such, or complexed by iron and aluminium in the so-called B_h horizon.

(5) The origin of these dicarboxylic acids in the podzol fulvic acid is uncertain. However, the presence of polymaleic acid-like structures, as suggested by Bracewell *et al.* (1980) and Wilson *et al.* (1983), seems unlikely in view of our results. A direct origin of the encountered dicarboxylic acids from metabolized higher plant constituents in these podzol soils is still a valid explanation for their presence as such.

(6) The abundant presence of maleic anhydride on one side and the virtual absence of 2-cyclopenten-1-one in our polymaleic acid pyrolysis mixture is in disagreement with Bracewell's data. Further investigations are needed to verify these contradictory results.

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C H A P T E R 3

Chemical characterization of soil organic matter fractions by analytical pyrolysis-gas chromatography-mass spectrometry.

CHEMICAL CHARACTERIZATION OF SOIL ORGANIC MATTER FRACTIONS BY ANALYTICAL PYROLYSIS–GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Curie-point pyrolysis–gas chromatography–mass spectrometry has been used to chemically characterize fulvic acid, humic acid, hymatomelanic acid and polysaccharide fractions of a representative soil. A detailed study of all the pyrolysis products was made. By comparing the results of this study with previous pyrolysis data for lignins, degraded lignins, polysaccharides, proteins, etc., we have been able to obtain more detailed information about the chemical composition of the pyrolysis products from different soil organic matter fractions. It is shown that fulvic acid fractions consist mainly of polysaccharide and/or carbohydrates and polyphenols, that humic acid and humin fractions are complex mixtures of several biopolymers such as polysaccharides, partially degraded lignins, peptides and lipids and that the hymatomelanic acid fraction represents mainly lipid materials.

INTRODUCTION

Plant residues constitute an important organic component of soils. Living, dying and dead tissues representing wide varieties of chemical substances undergo biochemical and chemical degradation reactions. The compounds produced in this way, the so-called humic substances, are very complex in nature and are thought to be more stable than the starting materials.

The classical method of fractionation of humic substances is based on their solubility in alkalis and acids. The major fractions thus obtained are

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humic acid, fulvic acid and humin. A further fractionation of humic acid into hymatomelanic acid, an ethanol-soluble fraction, is sometimes performed. In addition to humic substances, polysaccharides are quantitatively important compounds present in soil organic matter [1].

Numerous investigations have been undertaken to characterize the chemical structure of soil organic matter fractions by means of chemical degradation techniques [2]. More recently, pyrolysis–mass spectrometric studies of different humic fractions and related materials (e.g. fungal melanins, lignins, polysaccharides) have been reported. This analytical approach is used as a fingerprinting technique and clearly shows similarities and differences between different fractions present in soils [1,3–5].

However, to obtain an insight into the structural composition of the organic matter more firm identifications of the pyrolysis products, which reflect structural moieties present within the polymeric matrix, are required.

In this study we used Curie-point pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) to identify the pyrolysis products of the polysaccharide, fulvic acids, humic acid and humin and also the hymatomelanic acid fraction of a representative soil. By comparing the results of this study with those of previous Py–GC–MS studies of lignins, degraded lignins, polysaccharides and fulvic acids [6,7], we have been able to obtain more detailed information about the chemical composition of the pyrolysis products from different soil organic matter fractions.

EXPERIMENTAL

Soil sample

The soil sample used was obtained from the A_1 horizon of a brown soil (Typic Xerochrept) in the northern part of the province of Huelva, Spain.

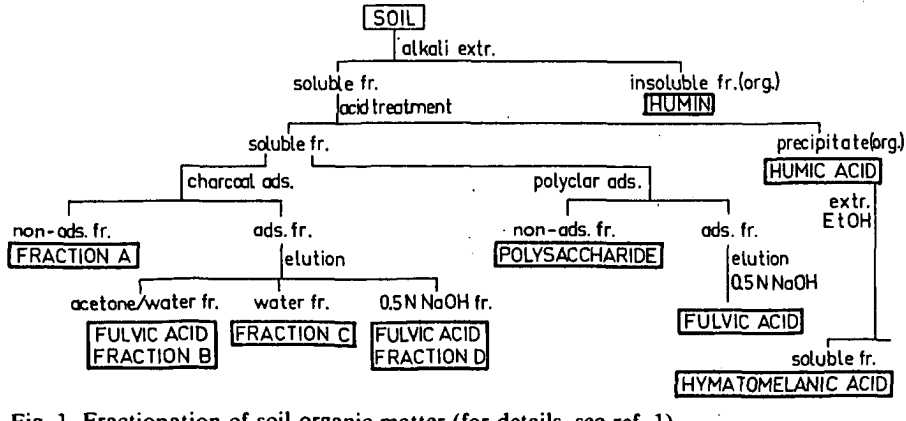


Fig. 1. Fractionation of soil organic matter (for details, see ref. 1).

TABLE 1

Elemental composition of soil organic matter fractions [1]

Fraction	C (%)	H (%)	N (%)	O (%)	Ash (%)
Polysaccharide	37.8	6.8	2.1	53.3	22.0
Humin	55.1	6.6	4.2	34.1	2.2
Humic acid	51.4	5.8	4.1	38.7	5.5
Hymatomelanic acid	57.8	7.5	1.1	33.6	2.5
Fulvic acid (Polyclar)	47.9	5.2	2.6	44.3	11.5
Fulvic acid (fraction B)	49.9	6.3	1.4	42.4	6.5
Fulvic acid (fraction D)	39.5	4.3	2.5	53.7	21.1

The altitude was 480 m and the vegetation consisted of an uncultivated prairie with gramineous plants, *Medicago* and *Trifolium*. The sample taken represented a depth of 0–10 cm and had a pH of 5.6 in water, a carbon content of 3.5% and a nitrogen content of 0.4%. The applied fractionation procedure for soil organic matter is described elsewhere [1]. For the reader's convenience we have included Fig. 1, which indicates the different fractions studies and the procedural pathways by which they were obtained. Table 1 shows the elementary composition of the isolated soil organic matter fractions on a dry and ash-free basis [1].

Pyrolysis–gas chromatography–mass spectrometry

The samples were suspended in methanol. One droplet of the suspension (10–20 μg of sample) was applied to a ferromagnetic wire with a Curie temperature of 510°C. The temperature rise time was about 0.15 s and the wire was held at the end temperature for 10 s.

The Py–GC–MS analyses were carried out using a pyrolysis unit similar to that described by Meuzelaar et al. [8] modified for use at high temperatures [9]. The pyrolysis products were separated on a capillary glass WCOT column (28 m × 0.5 mm I.D.) coated with CP-Sil 5 (1.25 μm film thickness) held at 0°C for 5 min and subsequently programmed to 300°C at a rate of 5°C/min. Helium was used as the carrier gas at a rate of 1.6 ml/min. The chromatograph (Varian Model 3200) was coupled to a Varian-MAT 44 quadrupole mass spectrometer operated in the electron impact (EI) mode at 80 eV and with a cycle time of 2 s.

RESULTS AND DISCUSSION

Pyrolysis products were identified by comparing their EI mass spectra with mass spectral libraries [10,11] and with mass spectra and GC retention times of standard compounds. Subsequently, Py–GC—MS data obtained

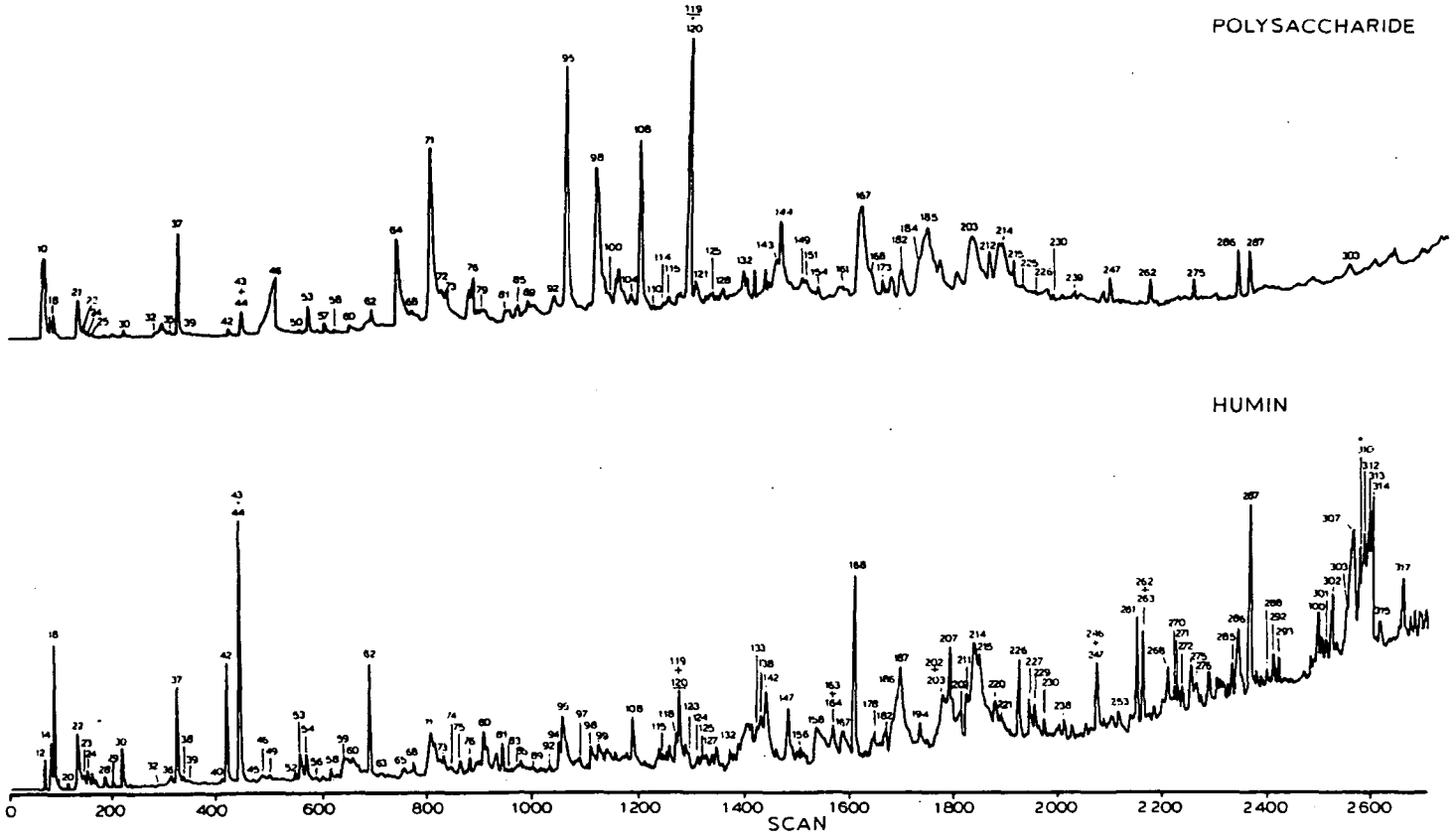


Fig. 2. Pyrolysis–gas chromatography–mass spectrometry of soil polysaccharide and humin. Peak identifications are given in Table 2. Underlined numbers indicate minor contributions to the peak.

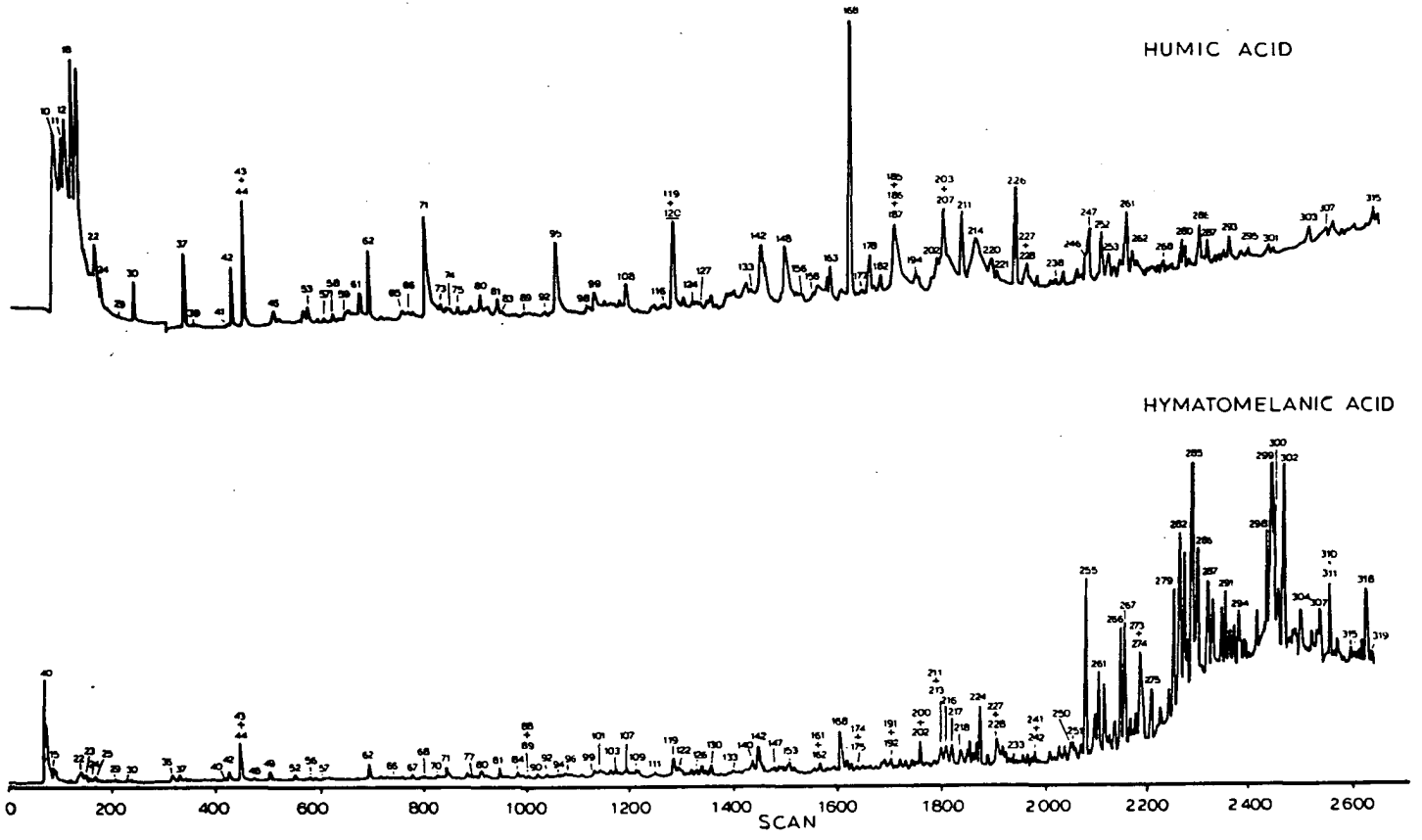
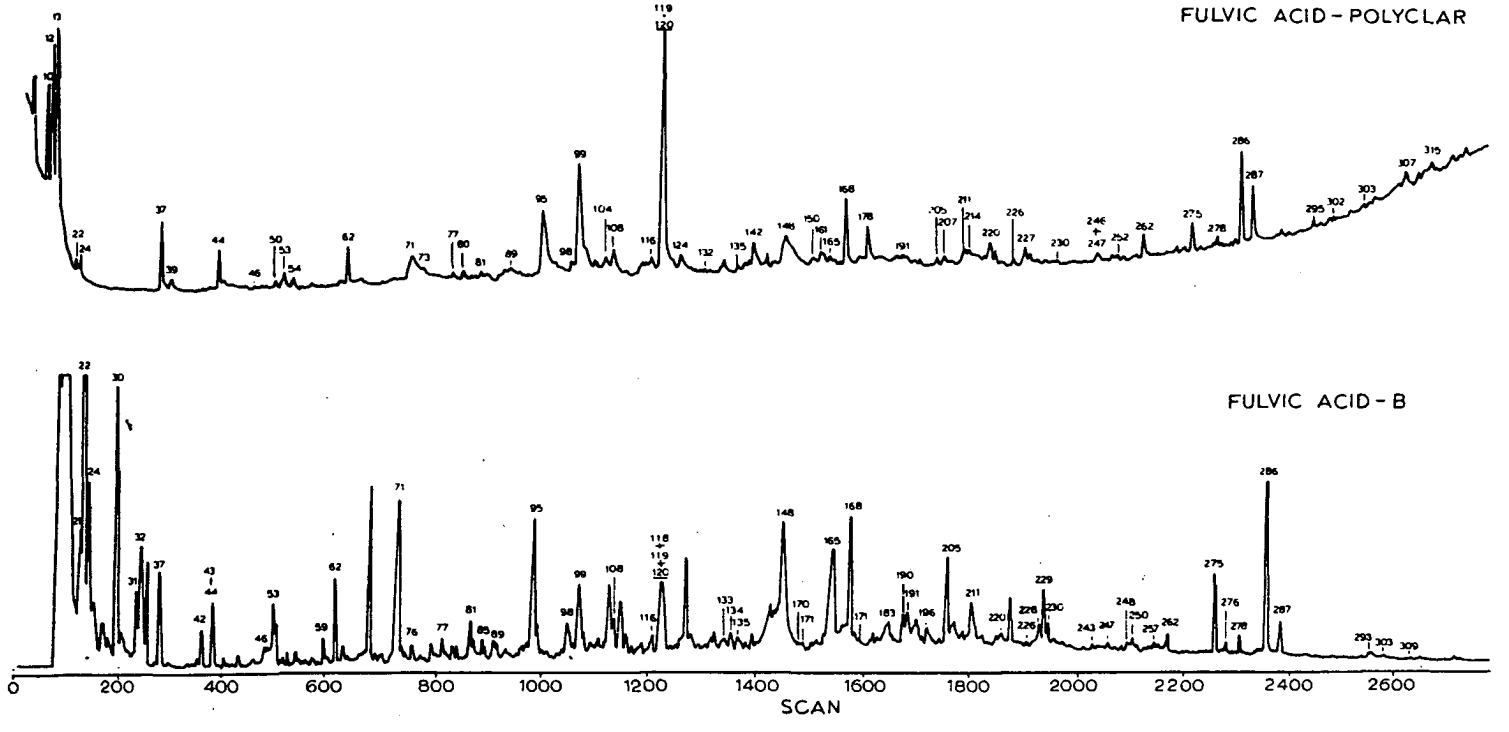


Fig. 3. Pyrolysis–gas chromatography–mass spectrometry of humic acid and hymatomelanic acid. Peak identifications are given in Table 2. Underlined numbers indicate minor contributions to the peak.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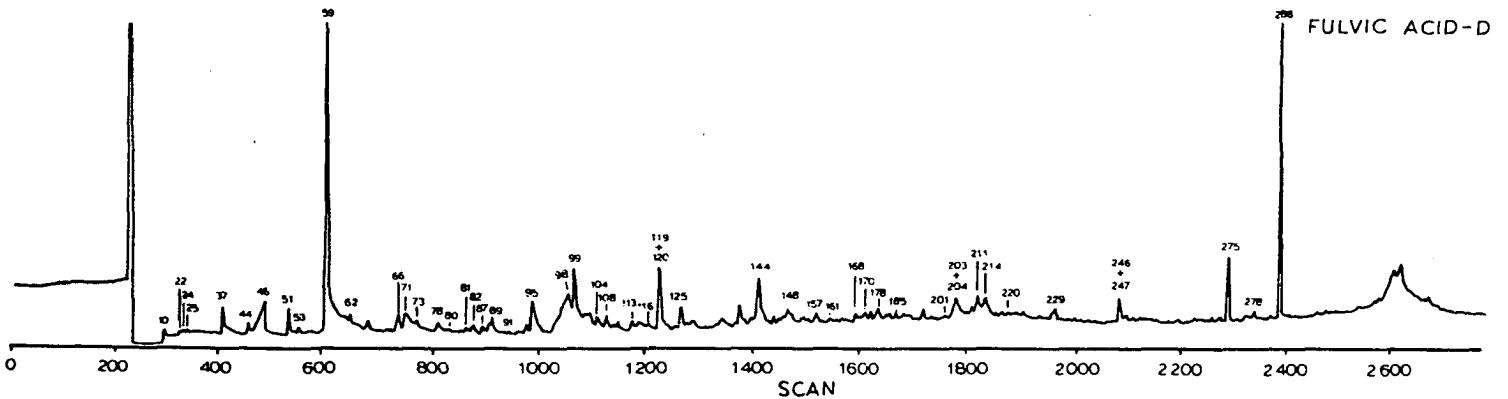

Fig. 4. Pyrolysis–gas chromatography–mass spectrometry of fulvic acid fractions. Peak identifications are given in Table 2. Underlined numbers indicate minor contribution to the peak.

TABLE 2

Pyrolysis products of soil organic matter fractions as identified by pyrolysis–gas chromatography–mass spectrometry

Peak No. *	Compound	Origin **
1	Carbon monoxide	
2	Carbon dioxide	
3	Methane	
4	Ethene	
5	Ethane	
6	Hydrogen sulphide	Pr
7	Propene	
8	Propane	
9	Methanol	Ps, Lg
10	Sulphur dioxide	
11	Hydrochloric acid	
12	Chloromethane	
13	Acetaldehyde	Ps
14	2-Methylprop-1-ene	
15	But-1-ene	
16	Buta-1,3-diene	
17	*n*-Butane	
18	Methanethiol	Pr
19	*trans*-But-2-ene	
20	3-Methylpent-1-ene	
21	2-Propenal	Ps
22	Acetone	Ps
23	Pent-1-ene	
24	Furan	Ps
25	Iodomethane	
26	*n*-Pentane	
27	Ethanethiol	Pr
28	*cis*-Pent-2-ene	
29	Cyclopentadiene	Ps
30	2-Methylpropenal	Ps
31	3-Buten-2-one	Ps
32	Butane-2,3-dione	Ps
33	2-Methylpentane	
34	Cyclopentane	
35	Methyldihydrofuran	Ps
36	Hex-1-ene	
37	2-Methylfuran	Ps
38	*n*-Hexane	
39	3-Methylfuran	Ps
40	Hexatriene	
41	Hexadiene	
42	3-Methylbutanal	Pr
43	2-Methylbutanal	Pr
44	Benzene	Ps, Pr, Lg
45	Thiophene	
46	Acetic acid	Ps
47	2-Methylcyclobutanone	Ps

TABLE 2 (continued)

Peak No. *	Compound	Origin **
48	Cyclohexane	
49	Cyclohexene	
50	Ethylfuran	Ps
51	1,4-Dioxan	
52	Hept-1-ene	
53	2,5-Dimethylfuran	Ps
54	2,4-Dimethylfuran	Ps
55	*n*-Heptane	
56	Dimethylfuran	Ps
57	Vinylfuran	Ps
58	N-Methylpyrrole	Pr
59	Pyridine	Pr
60	5-Methyl-2(5H)-furanone	Ps
61	Pyrrole	Pr
62	Toluene	Pr, Lg
63	Methylthiophene	
64	γ-Crotonolactone	Ps
65	Dihydropyran	Ps
66	Oct-1-ene	Lp
67	Oct-2-ene	Lp
68	3-Furaldehyde	Ps
69	*n*-Octane	Lp
70	*n*-C_5 Fatty acid methyl ester	Lp
71	2-Furaldehyde	Ps
72	Acetamide	Ps
73	Benzenemethanol	
74	Methylpyrrole	Pr
75	Methylpyrrole	Pr
76	Furfuryl alcohol	Ps
77	Ethylbenzene	Pr, Lg
78	Methylpyridine	Pr
79	3-Methylcyclopent-2-en-1-one	Ps
80	*m*- and/or *p*-xylene	Pr, Lg
81	Styrene	
82	2-Methylcyclopent-2-en-1-one	Ps
83	*o*-Xylene	Lg
84	Non-1-ene	Lp
85	C_3-Alkylfuran	Ps
86	Furancarboxylic acid	Ps
87	C_3-Alkylfuran	Ps
88	*n*-Nonane	Lp
89	α-Angelicalactone	Ps
90	*n*-C_6 Fatty acid methyl ester	Lp
91	Dimethylpyridine	Pr
92	α-Methylbenzenemethanol	
93	C_4-Alkylfuran	Ps
94	Benzaldehyde	Lg
95	5-Methyl-2-furaldehyde	Ps
96	C_3-Alkylbenzene	
97	C_3-Alkylbenzene	

TABLE 2 (continued)

Peak No. *	Compound	Origin **
98	4-Hydroxy-5,6-dihydro-2H-pyran-2-one	Ps
99	Phenol	Ps, Pr, Lg
100	Trimethylcyclopentenone	Ps
101	α-Methylstyrene	
102	Dec-1-ene	Lp
103	Iso-C_7 fatty acid methyl ester	Lp
104	2-Hydroxy-3-methyl-2-cyclopenten-1-one	Ps
105	C_3-Alkylpyridine	Pr
106	*n*-Decane	Lp
107	*n*-C_7 Fatty acid methyl ester	Lp
108	4-Hydroxy-6-methyl-5,6-dihydro-2H-pyran-2-one	Ps
109	Indene	
110	Furan-2,5-aldehyde	Ps
111	*o*-Cresol	Lg
112	C_3-Alkylbenzene	
113	C_3-Alkylbenzene	
114	5-(2-Hydroxyethylidene)-2H-furanone	Ps
115	Hexane-2,3,4-trione	Ps
116	3-Hydroxy-6-methyl-3,4-dihydro-2H-pyran-2-one	Ps
117	2-Furyl hydroxymethyl ketone	Ps
118	*p*-Cresol	Pr, Lg
119	Guaiacol	Lg
120	Levoglucosenone	Ps
121	3-Hydroxy-6-methyl-2H-pyran-2-one	Ps
122	*n*-C_7 Fatty acid	Lp
123	Ethylstyrene	Lg
124	3-Hydroxy-2-methyl-4H-pyran-4-one	Ps
125	3-Acetoxypyridine	Ps
126	Undec-1-ene	Lp
127	Benzyl cyanide	Pr
128	5-Hydroxy-2-methyl-4H-pyran-4-one	Ps
129	*n*-Undecane	Lp
130	*n*-C_8 Fatty acid methyl ester	Lp
131	Methylpentane-1,5-dioate	Lp
132	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Ps
133	Ethylphenol	Lg
134	C_4-Alkylbenzene	
135	C_4-Alkylbenzene	
136	C_2-Alkylphenol	Lg
137	C_4-Alkylbenzene	
138	$C_8H_9NO_2$ (chitin)	Ps
139	Naphthalene	
140	*n*-C_8 Fatty acid	Lp
141	C_2-Alkylphenol	Lg
142	Methylguaiacol	Lg
143	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	Ps
144	1,4:3,6-Dianhydro-α-D-glucopyranose	Ps
145	Methylnaphthalene	
146	Dodec-1-ene	Lp

TABLE 2 (continued)

Peak No. *	Compound	Origin **
147	Iso-C_9 fatty acid methyl ester	Lp
148	Vinylphenol	Lg
149	Resorcinol	Ps. Lg
150	2-Methylthiophenol	
151	Anhydrohexose	Ps
152	*n*-C_9 Fatty acid methyl ester	Lp
153	Methylhexane-1,6-dioate	Lp
154	5-(Hydroxymethyl)-2-furaldehyde	Ps
155	*n*-Dodecane	Lp
156	Amino acid dimer	Pr
157	1-Indanone	
158	Amino acid dimer	Pr
159	Methylthiophenol	
160	C_6-Alkylbenzene	
161	Ethylguaiacol	Lg
162	*n*-C_9 Fatty acid	Lp
163	Indole	Pr
164	$C_8H_9NO_2$ (chitin)	Ps
165	Phthalic anhydride	
166	Iso-C_{10} fatty acid methyl ester	Lp
167	1,4-Dideoxy-D-glycero-hex-1-enopyranos-3-ulose	Ps
168	Vinylguaiacol	Lg
169	Tridec-1-ene	Lp
170	Indan-1,3-dione	
171	1(3H)-Isobenzofuranone	
172	*n*-Tridecane	Lp
173	$C_7H_7NO_3/C_8H_{11}NO_2$ (chitin)	Ps
174	*n*-C_{10} Fatty acid methyl ester	Lp
175	Methylheptane-1,7-dioate	Lp
176	Amino acid dimer	Pr
177	Amino acid dimer	Pr
178	2,6-Dimethoxyphenol	Lg
179	Amino acid dimer	Pr
180	Amino acid dimer	Pr
181	Amino acid dimer	Pr
182	Eugenol	Lg
183	Benzenepropionic acid	Lg
184	$C_8H_9NO_2$ (chitin)	Ps
185	Levogalactosan	Ps
186	Amino acid dimer	Pr
187	Methylindole	Pr
188	*n*-C_{10} Fatty acid	Lp
189	Biphenyl	Lg
190	Methylphthalic anhydride	
191	Vanillin	Lg
192	Iso-C_{11} fatty acid methyl ester	Lp
193	Amino acid dimer	Pr
194	*cis*-Isoeugenol	Lg
195	Tetradec-1-ene	Lp

TABLE 2 (continued)

Peak No. *	Compound	Origin **
196	Methylphthalic anhydride	
197	Amino acid dimer	Pr
198	*n*-Tetradecane	Lp
199	*n*-C_{11} Fatty acid methyl ester	Lp
200	Methyloctane-1,8-dioate	Lp
201	4-Methyl-2,6-dimethoxyphenol	Lg
202	Amino acid dimer	Pr
203	Levoglucosane (mannose?)	Ps
204	Phthalide	
205	Dimethyl phthalate	
206	Amino acid dimer	Ps
207	*trans*-Isoeugenol	Lg
208	Amino acid dimer	Pr
209	Amino acid dimer	Pr
210	Ethyl-α-ethyl hexanoate	Lp
211	Acetoguaiacone	Lg
212	Trianhydro-2-acetamido-2-deoxyglucose ($C_8H_9NO_3$)	Ps
213	*n*-C_{11} Fatty acid	Lp
214	Levoglucosane (glucose)	Ps
215	$C_8H_9NO_3$ (chitin)	Ps
216	3,4-Dimethoxyacetophenone	Lg
217	Iso-C_{12} fatty acid methyl ester	Lp
218	Methyl vanillate	Lg
219	4-Ethyl-2,6-dimethoxyphenol	Lg
220	(4-Hydroxy-3-methoxy)phenylpropan-2-one	Lg
221	*n*-Pentadecane	Lp
222	Tributyl phosphate	
223	*n*-C_{12} Fatty acid methyl ester	Lp
224	Methylnonane-1,9-dioate	Lp
225	$C_8H_{11}NO_4$ (chitin)	Ps
226	4-Vinyl-2,6-dimethoxyphenol	Lg
227	*n*-C_{12} Fatty acid	Lp
228	Propioguaiacone	Lg
229	Diethyl phthalate	
230	Vanillic acid	Lg
231	Dihydroxydimethylbenzaldehyde	Lg
232	4-Allyl-2,6-dimethoxyphenol	Lg
233	Iso-C_{13} fatty acid methyl ester	Lp
234	Anteiso-C_{13} fatty acid methyl ester	Lp
235	Dianhydro-2-acetamido-2-deoxyglucose	Ps
236	Hexadecadiene	Lp
237	Hexadec-1-ene	Lp
238	*n*-Hexadecane	Lp
239	$C_8H_9NO_3$ (chitin)	Ps
240	*n*-C_{13} Fatty acid methyl ester	Lp
241	Methyldecane-1,10-dioate	Lp
242	Syringaldehyde	Lg
243	Tributyl phosphate	
244	*n*-C_{13} Fatty acid	Lp

TABLE 2 (continued)

Peak No. *	Compound	Origin **
245	Iso-C_{14} fatty acid methyl ester	Lp
246	4-*trans*-Propenyl-2,6-dimethoxyphenol	Lg
247	Biphenol	Lg
248	C_9-Alkylphenol	
249	Acetosyringone	Lg
250	C_9-Alkylphenol	
251	Heptadecadiene	Lp
252	4-Propyl-2,6-dimethoxyphenol	Lg
253	Heptadec-1-ene	Lp
254	*n*-Heptadecane	Lp
255	*n*-C_{14} Fatty acid methyl ester	Lp
256	Methylundecane-1,11-dioate	Lp
257	C_9-Alkylphenol	
258	4-Propenol-2,6-dimethoxyphenol	Lg
259	4-Propanol-2,6-dimethoxyphenol	Lg
260	C_9-Alkylphenol	
261	Prist-1-ene	Lp
262	*n*-C_{14} Fatty acid	Lp
263	Prist-2-ene	Lp
264	Anthracene	
265	10-Methyl-C_{14} fatty acid methyl ester	Lp
266	Iso-C_{15} fatty acid methyl ester	Lp
267	Anteiso-C_{15} fatty acid methyl ester	Lp
268	Octadec-1-ene	Lp
269	Methyl ferulate	Lg
270	*n*-Octadecane	Lp
271	Iso-C_{15} fatty acid	Lp
272	Anteiso-C_{15} fatty acid	Lp
273	*n*-C_{15} Fatty acid methyl ester	Lp
274	2,6-Dimethoxyphenyl-4-propionic acid	Lg
275	Diisobutyl phthalate	
276	*n*-C_{15} Fatty acid	Lp
277	10-Methyl-C_{15} fatty acid methyl ester	Lp
278	Dialkyl phthalate	
279	Iso-C_{16} fatty acid methyl ester	Lp
280	Phytadiene	Lp
281	Nonadec-1-ene	Lp
282	Iso-$C_{16:1}$ fatty acid methyl ester	Lp
283	Anteiso-$C_{16:1}$ fatty acid methyl ester	Lp
284	*n*-Nonadecane	Lp
285	*n*-C_{16} Fatty acid methyl ester	Lp
286	Dibutyl phthalate	
287	*n*-C_{16} Fatty acid	Lp
288	*n*-C_{16} Fatty acid ethyl ester	Lp
289	10-Methyl-C_{16} fatty acid methyl ester	Lp
290	Iso-C_{17} fatty acid methyl ester	Lp
291	Anteiso-C_{17} fatty acid methyl ester	Lp
292	Eicos-1-ene	Lp
293	*n*-Eicosane	Lp

TABLE 2 (continued)

Peak No. *	Compound	Origin **
294	*n*-C_{17} Fatty acid methyl ester	Lp
295	*n*-C_{17} Fatty acid	Lp
296	10-Methyl-C_{17} fatty acid methyl ester	Lp
297	Iso-C_{18} fatty acid methyl ester	Lp
298	$C_{18:2}$ Fatty acid methyl ester	Lp
299	$C_{18:1}$ Fatty acid methyl ester	Lp
300	$C_{18:1}$ Fatty acid methyl ester	Lp
301	*n*-Heneicosane	Lp
302	*n*-C_{18} Fatty acid methyl ester	Lp
303	*n*-C_{18} Fatty acid	Lp
304	10-Methyl-C_{18} fatty acid methyl ester	Lp
305	Iso-C_{19} fatty acid methyl ester	Lp
306	Anteiso-C_{19} fatty acid methyl ester	Lp
307	Dialkyl phthalate	
308	Docos-1-ene	Lp
309	*n*-Docosane	Lp
310	*n*-C_{19} Fatty acid methyl ester	Lp
311	Methylhexadecane-1,16-dioate	Lp
312	Terpenoid	Lp
313	Terpenoid	Lp
314	Terpenoid	Lp
315	*n*-C_{19} Fatty acid	Lp
316	Tricos-1-ene	Lp
317	*n*-Tricosane	Lp
318	*n*-C_{20} Fatty acid methyl ester	Lp
319	*n*-C_{20} Fatty acid	Lp
320	Dialkyl phthalate	
321	*n*-C_{21} Fatty acid	Lp
322	*n*-C_{22} Fatty acid	Lp

* Peak numbers as shown in Figs. 2—4.

** Ps = polysaccharide; Pr = protein; Lg = lignin; Lp = lipid.

for well defined polymers such as amylose [12], chitin [13], proteins and peptides [14] and lignins [6] allowed a detailed recognition of typical pyrolysis products.

The reconstructed ion chromatograms of the pyrolysis mixtures obtained from the soil organic matter fractions are shown in Figs. 2–4. The peak numbers in these figures correspond with the numbers mentioned in Table 2. Owing to the vast number of identified compounds in each pyrolysate (e.g., 175 in the hymatomelanic acid fraction), it was not possible to label all peaks in the chromatograms; therefore, only major peaks are indicated in the figures.

Most of the major compounds are well known pyrolysis products of biologically produced substances (polysaccharides, lignins, peptides, lipids, etc.). A number of compounds clearly indicate the presence of pollutants.

Polysaccharides

The compounds listed in Table 2 labelled Ps are pyrolysis products which are thought to be characteristic for polysaccharides. These products have been identified in the pyrolysates of cellulose, amylose and soil polysaccharide [7,12,15]. The abundant presence of anhydrosugars, pyranones and furans in the soil polysaccharide fraction indicates that this fraction consists almost entirely of hardly or non-biodegraded polysaccharides probably originating from residual plant polysaccharides and newly made microbial polysaccharides.

Compounds 185 and 203 have been tentatively identified as "levogalactosan" and "levomannosan". This identification is based on their mass spectral data (identical mass spectra when compared with levoglucosan), on the well known occurrence of galactose and mannose moieties in soil polysaccharides [16] and on the absence of these compounds in the pyrolysates of polyglucoses such as cellulose an amylose.

Compounds 116 (3-hydroxy-6-methyl-3,4-dihydro-2H-pyran-2-one) has been reported previously by Saiz-Jimenez and De Leeuw [7] in the pyrolysate of a soil polysaccharide. This compound is the same as that reported by Van der Kaaden et al. (ref. 12, peak 32) in pyrolysates of amylose.

The distribution pattern of the polysaccharide pyrolysis products encountered in the humin and humic acid fractions is similar to that observed in the soil polysaccharide fraction. In the fulvic acid B fraction the furans and to some extent the pyranones and levoglucosenone are clearly present. However, the anhydrosugars are hardly or not present. This might indicate that in this fraction there are monosaccharide moieties present in structures other than polysaccharides. A phenolic glycoside structure has been proposed for this fraction [17].

In the pyrolysis of carbohydrates, acidic conditions catalyse the formation of furans, in close analogy with the dehydration reactions of carbohydrates under aqueous acidic conditions, while alkaline conditions catalyse the breakdown of the sugar molecule to carbonyl compounds through reverse aldol condensation mechanisms [12]. In the absence of additives (as in this work), both types of reactions take place, but the ratio of furans to carbonyl compounds suggests slightly acidic conditions during pyrolysis. Because all fractions were obtained in the acidic form [1] and suspended in methanol to coat the ferromagnetic wires, it is understandable that the pyrolysis will preferentially produce furans over carbonyl compounds.

Cyclopentenones (compounds 79 and 82) have been reported in pyrolysates of soil organic matter [7,18]. Bracewell et al. [18] considered cyclopentenones as major pyrolysis products of aliphatic polycarboxylic acids and polymaleic acid and suggested that aliphatic polycarboxylic acids are important components in soil organic matter. This suggestion is not supported by previous pyrolysis data [7]. Further, cyclopentenones have been identified

as pyrolysis products from amylose [12] and are well known burned sugar aroma components [19].

Several pyrolysis products present in the polysaccharide and humin fractions (compounds 72, 125, 138, 164, 173, 184, 212, 215, 225, 235 and 239) have been found in pyrolysates of chitin [13]. It can be speculated that the chitin contribution in soil organic matter is mainly derived from fungi.

Lipids

It is very likely that a major part of the lipids encountered in the pyrolysates (peaks labelled Lp) are mainly the result of evaporation and are not generated from polymeric frameworks by pyrolysis. The lipid components are predominantly present in the hymatomelanic acid fraction obtained after ethanol extraction of the humic acid fraction. This also indicates that the lipids are mainly freely occurring components, which are easily extractable as such.

Aliphatic hydrocarbons have been detected in almost every plant, animal and microorganism examined, and therefore obvious sources of soil hydrocarbons are plant and animal residues and the soil microbial populations. However, it is improbable that animal residues contribute much directly to soil hydrocarbons. The series of *n*-alkanes and *n*-alkenes ranging from C_2 to C_{23} are encountered in a number of soil organic matter fractions and possibly originate from cuticle materials [20] and microbial populations [21].

Acyclic isoprenoid hydrocarbons such as prist-1-ene, prist-2-ene and phytadiene were identified in the humic acid, hymatomelanic acid and humin fractions. The phytyl side-chain of chlorophyll *a* is believed to be the source of phytadienes [22]. Recently, it has been reported that tocopherols are likely sources of pristenes, as both flash pyrolysis and thermal degradation of α-tocopherol yield prist-1-ene as a major pyrolysis product [23].

Morrison [24] has reported that many substances of a lipid nature, particularly of plant origin, are likely to be present in soils. Such substances would include tocopherols and porphyrins from higher plants and may accumulate as resistant remnants of plant residues undergoing humification. Further, Wagner and Muzorewa [25] considered that lipids extracted from soil organic matter may also be of microbial origin. Microbially synthesized products of a lipid nature in soil may become incorporated into soil humus without undergoing major degradative modifications.

The methyl esters encountered are thought to be procedural artifacts formed from the free fatty acids during the preparation of the pyrolysis samples using methanol as the suspension liquid.

Although the saturated and unsaturated straight-chain fatty acids are the main components in soil organic matter fractions, they are not very characteristic as almost any organism contains these fatty acids. The relatively abundant presence of iso-, anteiso- and 10-methyl fatty acids with chain

lengths ranging from C_{14} to C_{20} are highly characteristic for a microbial input of fungi and bacteria [26,27]. The α,ω-diacids might also be the result of bacterial degradation, although an origin from higher plant waxes cannot be ruled out [28].

There can be little doubt that steroids and terpenoids of various types occur in soils, but in spite of the abundance and variety of terpenoids in plants, there are only a few reports of their presence in soils [29]. Three compounds (312, 313 and 314) were tentatively identified as terpenoid hydrocarbons, based on their mass spectral fragmentation pattern (m/z 95, 109, 123, 149, 163, 191, 203, 207) [30]. These compounds (more detailed structures are as yet unknown) occur only in the humin and hymatomelanic acid fractions.

Lignins

The compounds listed in Table 2 labelled Lg are well known and characteristic pyrolysis products of lignins and degraded lignins [6]. The compounds identified show that they are contributions from grasses, higher plants and/or trees, as all three types of lignin building blocks (*p*-coumaryl, coniferyl and syringyl derivatives) are present.

Obviously, lignin is present in all fractions; however, in the soil polysaccharide and the hymatomelanic acid fractions the lignin contribution is minor. Substantial amounts of lignins are present in the humic acid, the humin and the fulvic acid fractions. The distribution of the lignin pyrolysis products indicates that the lignins are partly biodegraded, as the relative amounts of C_3-alkyl components are low and as carbonyl and carboxyl functional groups are clearly present [6].

Proteins and peptides

A number of pyrolysis products are of a protein origin and are labelled Pr in Table 2. Among them are the so-called "amino acid dimers", originating from valine, leucine and isoleucine pairs. This type of characteristic pyrolysis product, the structures of which are not yet completely known, are also encountered as major components in the pyrolysates of polyamino acids [14]. The peptides are present mainly in the humin and the humic acid fractions, indicating that the relatively high percentage of nitrogen as measured in these fractions (Table 1) originates from peptides.

Miscellaneous

A number of components present cannot be ascribed to well defined biopolymers, because they are not known as pyrolysis products of the studied biopolymers or they are not pyrolysis products at all.

Sulphur dioxide might originate from sulphonated materials (e.g., sulphonated polysaccharides). The sulphur content of different soil polysaccharide fractions ranges between 4 and 10% [31]. Occasionally, sulphur-containing compounds such as thiophenes and thiophenols have been identified in humic acid and fulvic acid fractions, respectively.

Chlorine compounds (hydrochloric acid and chloromethane) probably arise from the hydrochloric acid employed in the fractionation and purification procedures. At present, no explanation can be given for the presence of iodomethane and 1,4-dioxane.

Among the pyrolysis products some compounds considered as pollutants were identified. Dialkyl phthalates were the most prominent, especially in fulvic acid fractions. The origin of the pollutants may be diverse [32] and they interact with the soil organic matter fractions, either in the soil or during the extraction and fractionation procedures.

Nature of the soil organic matter fractions

Having discussed the origin of the pyrolysis product, it is now convenient to survey the main groups of compounds identified in each soil organic fraction.

Soil polysaccharide

The fraction obtained by the Polyclar filtration, called soil polysaccharide, consists almost entirely of polysaccharides. The major pyrolysis products encountered are well known and specific pyrolysis products of polysaccharides. Trace amounts of lignin pyrolysis products and fatty acids are also present.

Humin

The pyrolysate of this fraction consisted of a complex mixture. The main series of pyrolysis products encountered originate from polysaccharides and lignins. Lipids are significantly present, including alkanes, alkenes, fatty acids, terpenoids and pristenes. The presence of peptides is also evident. Pollutants are minor products.

Humic acid

The major pyrolysis compounds encountered are lignin derivatives. Polysaccharide products are also clearly present, whilst peptide pyrolysis products are less prominent. Lipids are minor components.

Hymatomelanic acid

This fraction consists almost entirely of lipids including alkanes, alkenes, acyclic isoprenoid hydrocarbons, fatty acids and aliphatic dicarboxylic acids. Lignin pyrolysis products are minor compounds.

Fulvic acid (Polyclar)

The initially retained fraction during the Polyclar filtration, called fulvic acid, contains mainly two series of pyrolysis products. Most abundant are the lignin pyrolysis products. A series of pyrolysis products originating from polysaccharides are also clearly present. Fatty acids and dialkyl phthalates are encountered in minor amounts.

Fulvic acid B (charcoal)

This fraction shows two main series of pyrolysis products originating from lignins and/or polyphenols and carbohydrates. Dialkyl phthalates are present, in addition to minor amounts of fatty acids.

Fulvic acid D (charcoal)

Major products in the pyrolysate of this fraction are dialkyl phthalates and pyridine. Lignin and carbohydrate pyrolysis products are less important than in the other fulvic acid fractions.

CONCLUSIONS

1. In the A_1 horizon of the studied Typic Xerochrept soil about 10% of the total organic matter is present as carbohydrate in the soil polysaccharide fraction [1]. In addition, various amounts of polysaccharide moieties are present in the humic fractions.

2. The structures of the pyrolysis products encountered in the humic acid fraction are also observed in more or less pure lignin preparations and in polysaccharides. Hence, there is no need to assume that the pyrolysable part of the humic acid fraction consists of a condensed lignin–polysaccharide structure; a simple mixture of these two biopolymeric substances can explain the results satisfactorily.

3. As already pointed out for the humic acid fraction, the pyrolysis data for the other humic fractions also indicate that there is no direct need to assume that humic substances are generated by condensation reactions of lipids, carbohydrates, amino acids, etc. On the contrary, mixtures of more or less biodegraded biopolymers and originally present low-molecular-weight compounds explain the pyrolysis data very well.

4. The chemical contents of the studied humic fractions are probably determined by the solubility of the individual components (e.g., polyphenolic substances such as lignins dissolve in base, but precipitate on acidification; most polysaccharides remain in aqueous solution; lipids do not dissolve in water but do so in ethanol; lipoproteins, glycolipids, glycoproteins, etc. [33] will end up in almost any fraction).

5. Our results indicate that hymatomelanic acid, a term introduced by Hoppe-Seyler in 1889, cannot be considered as a humic fraction, as it

consists almost entirely of lipid compounds extractable from the humic acid fraction. Probably extraction of the intact soil with toluene–methanol, for example, prior to fractionation would considerably reduce or completely eliminate the hymatomelanic acid fraction.

6. It is clear from the results obtained from the different fulvic acid fractions that the chemical composition of these fractions is a consequence of the fractionation procedure followed. These data are in agreement with previous observations [34].

7. Finally, the Py–GC–MS technique as applied in this study is a powerful method for chemically characterizing considerable amounts of soil organic matter.

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CHAPTER 4

Pyrolysis-gas chromatography-mass spectrometry of isolated, synthetic and degraded lignins.

Pyrolysis–gas chromatography–mass spectrometry of isolated, synthetic and degraded lignins

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Abstract—Curie-point pyrolysis–gas chromatography–mass spectrometry was applied to study the chemical structure of sound and fungus degraded, industrial and synthetic lignins. Pyrolysis products reflected in some detail the structural units present in the lignin polymer. Thus, sound spruce lignin yielded *trans*-isoeugenol coniferaldehyde and *trans*-coniferyl alcohol as major pyrolysis products. Biodegraded lignin yielded oxidized units, including vanillin, acetoguaiacone, methyl vanillate, propioguaiacone, vanilloyl methyl ketone and vanillic acid as major products. Kraft lignin also showed evidence of oxidation, although not as much as the biodegraded lignin. Major products from this industrial lignin were guaiacol, methylguaiacol, vinylguaiacol and homovanillic acid. Results indicated that synthetic lignin duplicates fairly well the structure of natural lignin. However, coniferylaldehyde and *trans*-coniferyl alcohol were the dominant products only from the synthetic lignin, indicating the presence of large amounts of coniferyl alcohol and coniferylaldehyde end groups.

Key words: pyrolysis, lignins, biodegradation, kraft lignin, DHP lignin, spruce lignin

INTRODUCTION

Lignins are phenolic polymers which occur as major components of vascular plants and hence can be considered as markers of land-derived organic matter in lacustrine and marine sediments.

Lignins are biosynthesized by an oxidative polymerization of three substituted cinnamyl alcohols: *p*-coumaryl-, coniferyl- and sinapyl alcohols (4-hydroxy-, 4-hydroxy-3-methoxy- and 4-hydroxy-3,5-dimethoxycinnamyl alcohol, respectively). The proportions of the three precursor alcohols differ between angiosperm and gymnosperm lignins (Adler, 1977).

Although a wide range of micro-organisms, especially fungi and bacteria, can decompose lignins to some extent, only certain higher fungi (Basidiomycetes) have been shown to decompose them extensively (Kirk and Fenn, 1982).

In soils, and probably also in sediments when present, lignins and/or their degradation products are thought to play an important role in the formation of humic substances (Saiz-Jimenez and de Leeuw, 1984).

Besides the natural input of lignin in soils and sediments *via* plant debris, an anthropogenic origin of lignin has to be considered. In fact, industrial lignins, produced as side-products in chemical pulping, are often discharged into the environment. As industrial lignins appear to be resistant to microbial decomposition in a variety of neutral and acidic, anoxic environments (Zeikus *et al.*, 1982), their accumulation in polluted areas has to be taken into account.

The chemical nature of the lignins has been studied by often tedious chemical degradation techniques such as nitrobenzene, permanganate, cupric oxide oxidation or acidolysis. However, the structure of lignins can be analysed rapidly by analytical flash pyrolytic methods (Martin *et al.*, 1979b; Obst, 1983; Schenck *et al.*, 1983). In this paper, pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) for sound, fungus degraded and industrially modified lignins is reported. Also, an artificial lignin synthesized *in vitro* from coniferyl alcohol using a peroxidase is included for comparative purposes. Because complex polymeric mixtures present in sediments and soils, coals, etc. are often studied by analytical pyrolytic techniques (Saiz-Jimenez *et al.*, 1979; Martin *et al.*, 1979a; van de Meent *et al.*, 1980; Schenck *et al.*, 1983) the understanding of the structures of pyrolysis products generated from different lignins will provide useful information about the origin of the lignin-related components most frequently identified in the various types of humic and other macromolecular materials. Moreover, a better understanding of the lignin structure after fungus mediated transformation can be obtained, and the structure of artificial lignin can be compared with that of natural lignins.

EXPERIMENTAL

Spruce milled wood lignin and lignin degraded by *Coriolus versicolor* (L. ex Fr.) Quel. were kindly

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provided by Dr T. Kent Kirk. Characteristics of the wood used, the extraction procedures and the isolation and the incubation methods are reported by Kirk and Chang (1974). Moreover, chemical characterization studies of both lignins have been reported (Kirk and Chang, 1975).

Indulin AT, a pine kraft lignin with an ash content less than 1%, was kindly provided by Dr J. Marton. Details of its chemical structure are described elsewhere (Marton, 1964; Marton and Marton, 1964).

The synthetic lignin, guaiacyl DHP (dehydrogenative polymerisate), was kindly supplied by Dr O. Faix. Its synthesis is described by Schweers and Faix (1973).

Pyrolysis–GC

The samples were suspended in methanol. One droplet of the suspension (~ 10–20 μg of sample) was applied to a ferromagnetic wire with a Curie temperature of 510°C.

The pyrolysis gas chromatographic analyses were carried out using a pyrolysis unit similar to the one described by Meuzelaar *et al.* (1975), modified for use at high temperatures (van de Meent *et al.*, 1980). Chromatographic separations were achieved with a capillary glass WCOT column (28 m × 0.5 mm i.d.) coated with CP sil 5 (1.3 μm film thickness) using a Packard Becker 419 gas chromatograph. Helium was used as the carrier gas.

Pyrolysis–GC–MS

Pyrolysis was performed as described above. The pyrolysis products were separated on a capillary glass WCOT column (28 m × 0.5 mm i.d.) coated with CP sil 5 (1.25 μm film thickness) held at a rate of 5°C min^{-1}. Helium was used as the carrier gas. The chromatograph (Varian model 3200) was coupled to a Varian Mat 44 mass spectrometer operated in the EI mode at 80 eV with a cycle time of 2 s.

RESULTS AND DISCUSSION

Pyrograms of the sound spruce lignin, the biodegraded spruce lignin, the industrial lignin and the synthetic lignin are shown in Figs 1–4.

The structural elucidation of the pyrolysis products (Table 1) was based on comparison of both retention time data and mass spectral data with those of standards, and with literature data.

The pyrogram of spruce lignin (Fig. 1) shows several major peaks, identified as 4-methylguaiacol (6), 4-vinylguaiacol (9), vanillin (12), *trans*-isoeugenol (15), coniferaldehyde (27) and *trans*-coniferyl alcohol (29). Coniferylaldehyde and coniferyl alcohol were not reported in previous Py–GC–MS studies (Martin *et al.*, 1979b; Philp *et al.*, 1982) of spruce lignin, but were recently identified from pine lignin by Obst (1983). Other prominent pyrolysis products identified in the spruce lignin pyrogram were guaiacol (4), eugenol (10), homovanillin (14), acetoguaiacone (16), propioguaiacone (22) and homovanillic acid (25). All of these pyrolysis products seem to reflect structural units present in the lignin polymer.

It is noteworthy the presence of methyl vanillate (19) among the pyrolysis products of lignins. Obst (personal communication) treated loblolly pine MWL with 1 N NaOH at 100°C, which would cleave all carboxylic esters. The pyrogram of the saponified MWL showed about the same amount of methyl vanillate as the untreated MWL. Therefore, it appears that methyl vanillate is an artefact produced during pyrolysis and does not represent methyl esters in lignin. Apparently vanillic acid is methylated during pyrolysis.

The distribution pattern of the pyrolysis products encountered in the fungus degraded lignin (Fig. 2) is very different from the pattern observed in the sound lignin (Fig. 1). Major components in the biodegraded lignin are guaiacol (4), acetoguaiacone (16), methyl vanillate (19) and vanilloyl methyl ketone (23). Other prominent peaks were identified as 4-vinylguaiacol (9), vanillin (12), propioguaiacone (22) and vanillic acid (24). The total absence of coniferaldehyde and *trans*-coniferyl alcohol, the relatively low intensity of 4-methylguaiacol, 4-vinylguaiacol and *trans*-isoeugenol and the presence of vanilloyl methyl ketone and vanillic acid among the pyrolysis products of the fungal degraded lignin is good evidence for oxidation of the C_3-alkyl chain at the C_α and C_β positions, and for cleavage in the side-chain, mainly between C_α and C_β. These conclusions based upon the pyrolysis data are in good agreement with those of Chen *et al.* (1982, 1983) who studied the fungal degradation of spruce lignins by other methods.

The pyrogram of kraft lignin (Fig. 3) shows significant differences from that of natural conifer lignin. Major peaks were identified as guaiacol (4), 4-methylguaiacol (6), 4-vinylguaiacol (9), *trans*-isoeugenol (15), and homovanillic acid (25). Other important pyrolysis products were identified as 4-ethylguaiacol (8), eugenol (10), vanillin (12), acetoguaiacone (16) and *trans*-coniferyl alcohol (29). The differences between the sound lignin and the kraft lignin are especially evident in the relatively high abundance of guaiacol, 4-methylguaiacol, 4-vinylguaiacol and homovanillic acid in the latter.

During delignification of wood in the kraft process, the lignin component is solubilized *via* degradation and ionization to free the fiber for the manufacture of paper. The lignin alteration is characterized by degradation of the side-chain, including a partial loss of C_γ atoms, β-guaiacyl ether bond cleavage, limited demethylation, and formation of stilbene structures from phenylcoumarans (Marton, 1964; Adler *et al.*, 1964; Lundquist *et al.*, 1977). These changes in the lignin structure are corroborated to some extent by the prominence of homovanillic acid together with the low intensities of coniferaldehyde and *trans*-coniferyl alcohol, in the pyrolysis mixture.

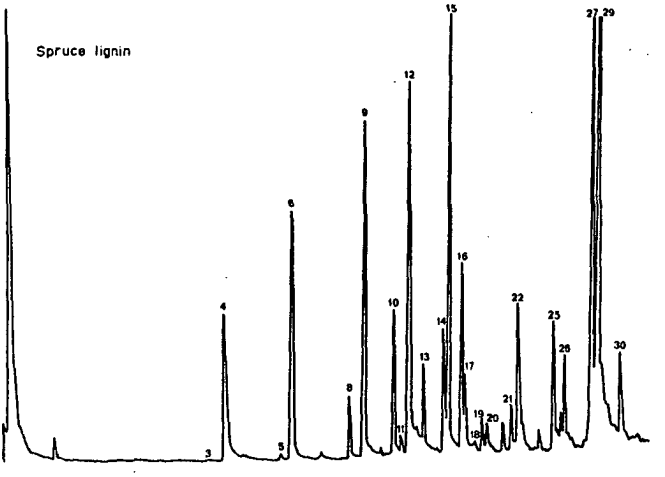

Fig. 1. Pyrogram of spruce milled wood lignin.

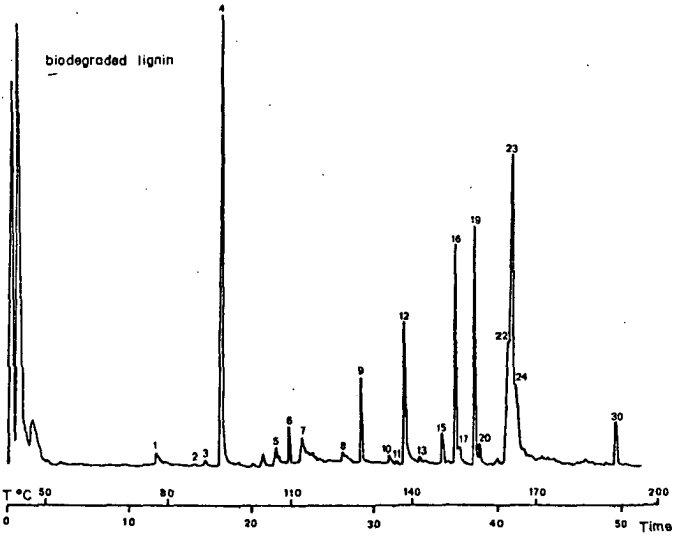

Fig. 2. Pyrogram of spruce milled wood lignin degraded by *Coriolus versicolor*.

Among the very volatile pyrolysis products of kraft lignin we encountered a number of organic sulphur compounds such as H_2S, SO_2, CH_3SH, C_2H_5SH and/or CH_3-S-CH_3, CH_3-S-S-CH_3 and CH_3-S-S-S-CH_3. These pyrolysis products are very probably generated from the sulphur-containing alkyl side-chain moieties present in the industrial lignin. This observation is in agreement with other work on kraft lignins (Marton, 1964).

The presence of characteristic sulphur-containing compounds, together with lignin pyrolysis products, can discriminate an anthropogenic origin of industrial lignins in recent sediments from a natural contribution of lignin or biodegraded lignins. Further,

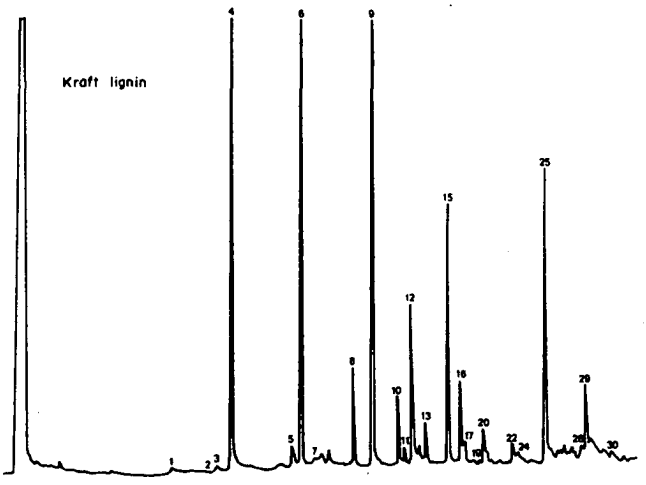

Fig. 3. Pyrogram of kraft lignin.

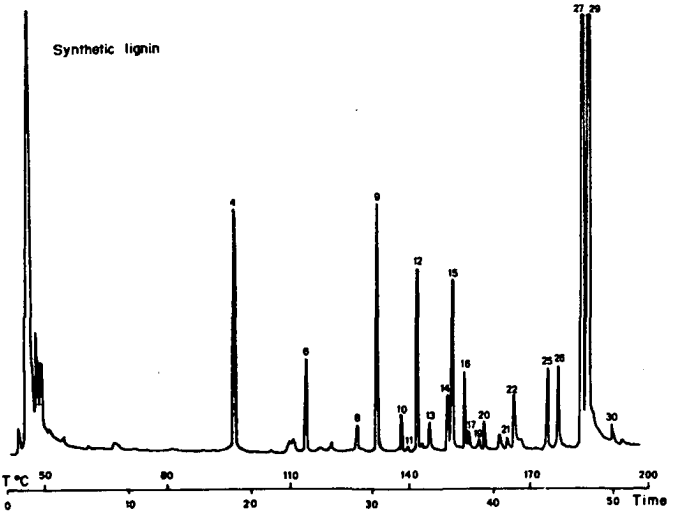

Fig. 4. Pyrogram of synthetic lignin.

characteristic lignin pyrolysis products most frequently identified in soil and aquatic humic substances and coals can be considered as polymeric biomarkers, indicating a plant origin.

Pyrograms of sound lignin (Fig. 1) and guaiacyl DHP (Fig. 4) are similar in that both have the same type of pyrolysis products. However, because the ratios of these products are different, it must be concluded that DHP and sound lignins are not identical, but merely similar. Compared to sound lignin the relative amounts of guaiacol (4), coniferaldehyde (27), and *trans*-coniferyl alcohol (29) are increased in DHP. Model DHP lignin as synthesized by the action of peroxidase on coniferyl alcohol

Table 1. Pyrolysis products from lignins

Peak No.	R_1	R_2	R_3	Remarks
1	OH			
2	OH	CH_3		
3	OH		CH_3	
4	OH	OCH_3		
5				Positional isomer of 6
6	OH	OCH_3	CH_3	
7	OH	OH		
8	OH	OCH_3	CH_2–CH_3	
9	OH	OCH_3	CH_2=CH_2	
10	OH	OCH_3	CH_2–CH=CH_2	
11	OH	OCH_3	CH_2–CH_2–CH_3	
12	OH	OCH_3	CHO	
13	OH	OCH_3	CH=CH–CH_3	*cis*
14	OH	OCH_3	CH_2–CHO	
15	OH	OCH_3	CH=CH–CH_3	*trans*
16	OH	OCH_3	CO–CH_3	
17				?
18	Cl	Cl	OCH_3	R_4=OCH_3
19	OH	OCH_3	COO–CH_3	
20	OH	OCH_3	CH_2–CO–CH_3	
21	OH	OCH_3	C_3H_5O	
22	OH	OCH_3	CO–CH_2–CH_3	
23	OH	OCH_3	CO–CO–CH_3	
24	OH	OCH_3	COOH	
25	OH	OCH_3	CH_2–COOH	
26	OH	OCH_3	CH=CH–CH_2OH	*cis*
27	OH	OCH_3	CH=CH–CHO	
28	OH	OCH_3	CH_2–CH_2–COOH	
29	OH	OCH_3	CH=CH–CH_2OH	*trans*
30				dialkyl phthalate

appears to be similar to certain conifer lignins. However, Kirk *et al.* (1975) have shown that in the synthetic lignin the relative amounts of coniferyl alcohol end groups are more abundant and that the natural lignin has a higher degree of cross-linking. These phenomena are supported by our pyrolysis data.

The dominant presence of coniferaldehyde and *trans*-coniferyl alcohol in the pyrogram of the DHP lignin point to a polymeric structure in which the original substrate (*trans*-coniferyl alcohol) is linked less firmly than in the natural lignin. In summary, we think that these synthetic lignins are very valuable models for the chemical study of many aspects of natural lignins.

CONCLUSIONS

(1) Pyrolysis–gas chromatography and pyrolysis–gas chromatography–mass spectrometry are powerful tools to rapidly chemically characterize isolated and synthetic lignins and lignin-derived polymers.

(2) Lignins and lignin-derived products can, therefore, be considered as polymeric biomarkers, less prone to external influences than extractable biomarker molecules.

(3) Biodegradation of lignin by white-rot fungi results in severe side-chain oxidation. These recognizably oxidized polymers are present among the building blocks of humic substances (Saiz-Jimenez and de Leeuw, 1984).

(4) An anthropogenic origin of lignin products in recent sediments, e.g. kraft lignin from papermills can be recognized and can be discriminated from a natural contribution of lignins or biodegraded lignins, based on pyrolysis data.

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CHAPTER 5

Lignin pyrolysis products: Their structures and their significance as biomarkers.

Lignin pyrolysis products: Their structures and their significance as biomarkers

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Abstract—Pyrolysis in combination with gas chromatography and mass spectormetry was used to characterize softwood, hardwood and grass lignins as well as the corresponding synthetic dehydropolymers. The method permitted differentiation of the three types of lignins. Softwood lignins yielded exclusively guaiacyl derivatives, coniferaldehyde and coniferyl alcohol being major compounds. Hardwood lignins gave rise to guaiacyl and syringyl derivatives, among which syringaldehyde, coniferyl alcohol and sinapyl alcohol were the most prominent. Grass lignins, represented by bamboo lignin, yielded *p*-vinylphenol as major compound. In addition, other guaiacyl and syringyl pyrolysis products were identified. The results indicate that guaiacyl and syringyl compounds are unique pyrolysis products of lignins and woods. Because of the relatively high resistance of lignins these pyrolysis products can be considered as characteristic biomarkers for terrestrial plant input.

Key words: milled wood lignins, dehydrogenative polymers, gymnosperm lignins, angiosperm lignins, grass lignins

INTRODUCTION

Lignins form an essential component of the woody stems of arborescent gymnosperms and angiosperms in which their amounts range from 15% to 36%. Lignins are not, however, restricted to arborescent plants, but are found as integral cell wall constituents in all vascular plants including the herbaceous varieties.

Investigations on the chemistry of lignin have shown it to be a substance of great structural complexity. Its structure is based on molecular units of the phenylpropane type. The complexity of the lignin molecule arises in part from the manner in which the C_6–C_3 units are linked to each other (and in part from the fact that these units are not chemically identified).

Lignins are polymeric products arising from an enzyme-initiated dehydrogenative polymerization of three primary precursors: *trans*-coniferyl, *trans*-sinapyl and *trans*-*p*-coumaryl alcohols.

The proportions of the three precursor alcohols differ between angiosperm and gymnosperm lignins. Gymnosperm lignin is made up by *trans*-coniferyl alcohol units, angiosperm lignin is constituted from *trans*-coniferyl and *trans*-sinapyl alcohol units and grass lignin contains all three alcohols as building blocks.

Living, dying and dead plants containing lignins are incorporated into soils and sediments, undergoing biochemical and chemical degradation reactions. Aerobic degradation of lignocellulose is slow since the presence of the lignin moiety severely decreases cellulose decomposition and because lignin is not the best microbial energy source. The absence of significant wood decomposition under anaerobic conditions is consequence of the requirement of oxygen for microbial degradation (Zeikus *et al.*, 1982). Therefore, in soils lignins are slowly biodegraded by certain fungi and bacteria, and partly degraded lignins and lignin phenols may contribute to the formation of humus and are present in humic fractions (Saiz-Jimenez and de Leeuw, 1985), while in anaerobic wetlands and sediments of marine and freshwater environments, lignocellulosic materials are better preserved.

Lignin derivatives have been identified in soil organic matter fractions (Saiz-Jimenez and de Leeuw, 1984a, 1985), buried woods (Hedges *et al.*, 1985), fossil woods (Sigleo, 1978; Hatcher *et al.*, 1982) and kerogen (Habermehl and Hundrieser, 1983). Because lignin components are relatively stable upon diagenesis, they can be considered as specific biomarkers for terrestrial plant input.

In previous work, Saiz-Jimenez and de Leeuw (1984b) demonstrated that isolated, synthetic, biodegraded and kraft lignins can be analysed by flash pyrolysis. Further, pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) data have shown a high correlation between pyrolysis products and the lignin units from which they arise (Martin *et al.*, 1979; Obst, 1983). Therefore, it is possible to discriminate between gymnosperm and angiosperm lignins as original contributors to sediments and soils.

In this paper, Py–GC–MS of three types of isolated and synthetic lignins is reported. This study of soft-

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wood, hardwood and grass lignins and the characterization of their pyrolysis products will improve our present knowledge of the chemical structure of this biopolymer and will provide useful information when comparing sound, biodegraded, fossil or buried woods.

EXPERIMENTAL

Samples

Two spruce milled wood lignin samples (MWL) were provided by Dr T. Kent Kirk (MWL-1) and by Dr O. Faix (MWL-2). Beech, aspen and bamboo MWL samples were supplied by Dr O. Faix. MWL samples were isolated according to Björkman (1956).

Dehydrogenation polymers (DHPs) were synthesized using *trans*-coniferyl, *trans*-sinapyl and *trans*-coumaryl alcohols in fixed proportions to reproduce the three types of lignins. They were kindly supplied by Dr O. Faix. Further information on these samples is reported by Kirk and Chang (1984), Faix and Besold (1978), Martin *et al.* (1979) and Saiz-Jimenez and de Leeuw (1984b).

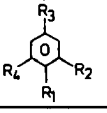
Pyrolysis–GC and pyrolysis–GC–MS

Details of the methods have been described in a previous paper (Saiz-Jimenez and de Leeuw, 1984b). Briefly, pyrolysis was performed with ferromagnetic wires having a Curie temperature of 510°C (pyrolysis time 10 sec) and pyrolysis products were separated on a capillary glass WCOT column (28 m × 0.5 mm i.d.) coated with CP sil 5 (1.25 μm film thickness) held at 50°C for 3 min and subsequently programmed to 250°C at a rate of 3°C min^{-1}. He was used as a carrier gas.

RESULTS

Pyrograms of the MWL and DHP samples are shown in Figs 1–8. In this study we concentrated on

Table 1. Pyrolysis products from lignins



Peak No.	R_1	R_2	R_3	R_4	Remarks
1	OH				
2	OH	CH_3			
3	OH		CH_3		
4	OH	OCH_3			
5	OH		CH_2—CH_3		
6					Positional isomer of 7
7	OH	OCH_3	CH_3		
8	OH		CH=CH_2		
9	OH		CH_2—CH=CH_2		
10	OH	OCH_3	CH_2—CH_3		
11	OH		CH=CH—CH_3		*cis*
12	OH	OCH_3	CH=CH_2		
13	OH	OCH_3		OCH_3	
14	OH	OCH_3	CH_2—CH=CH_2		
15	OH		CHO		
16	OH	OCH_3	CH_2—CH_2—CH_3		
17	OH	OCH_3	CHO		
18	OH	OCH_3	CH=CH—CH_3		*cis*
19	OH	OCH_3	CH_3	OCH_3	
20	OH	OCH_3	CH_2CHO		
21	OH	OCH_3	CH=CH—CH_3		*trans*
22	OH	OCH_3	CO—CH_3		
23	OH	OCH_3	COO—CH_3		
24	OH	OCH_3	CH_2—CH_3	OCH_3	
25	OH	OCH_3	CH_2—CO—CH_3		
26	OH	OCH_3	CH=CH_2	OCH_3	
27	OH	OCH_3	CO—CH=CH_2		
28	OH	OCH_3	COOH		
29	OH	OCH_3	CO—CH_2—CH_3		
30	OH	OCH_3	CH_2—COOH		
31	OH	OCH_3	CH_2—CH=CH_2	OCH_3	
32	OH	OCH_3	CH_2—CH_2—CH_3	OCH_3	
33	OH	OCH_3	CHO	OCH_3	
34	OH	OCH_3	CH=CH—CH_3	OCH_3	*cis*
35	OH	OCH_3	CH=CH—CH_2OH		*cis*
36	OH	OCH_3	CH_2—CHO	OCH_3	
37	OH	OCH_3	CH=CH—CH_3	OCH_3	*trans*
38	OH	OCH_3	CO—CH_3	OCH_3	
39	OH	OCH_3	CH=CH—CHO		
40	OH	OCH_3	CH=CH—CH_2OH		*trans*
41	OH	OCH_3	CH_2—CO—CH_3	OCH_3	
42	OH	OCH_3	COO—CH_3	OCH_3	
43	OH	OCH_3	CO—CH=CH_2	OCH_3	
44	OH	OCH_3	CO—CO—CH_3	OCH_3	
45	OH	OCH_3	CH_2—CH_2—CH_2OH	OCH_3	
46	OH	OCH_3	CH=CH—CHO	OCH_3	
47	OH	OCH_3	CH=CH—CH_2OH	OCH_3	*trans*

Note that the numbering of the ring atoms is different from the numbering used in Fig. 9.

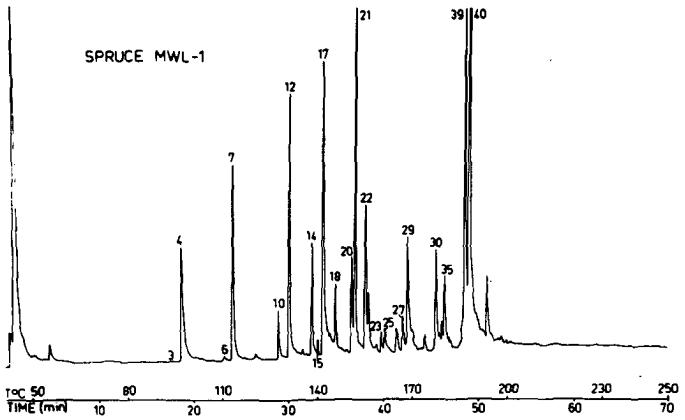


Fig. 1. Pyrogram of a *Picea engelmanii* milled wood lignin.

the phenolic pyrolysis products because they are highly representative of each lignin sample. Other products, like furan derivatives, which sometimes are present in the samples, and low molecular weight aromatic hydrocarbons such as benzene, toluene, styrene, alkylbenzenes, etc., are not discussed since they are minor pyrolysis products.

The structural elucidation of the pyrolysis products (Table 1) is based on comparison of both retention time and mass spectral data with those of standards and with literature data.

Milled wood lignins

Figure 1 shows the pyrogram of a spruce lignin (MWL-1). Major peaks are identified as 4-methylguaiacol (**7**), 4-vinylguaiacol (**12**), vanillin (**17**), *trans*-isoeugenol (**21**), coniferaldehyde (**39**) and *trans*-coniferyl alcohol (**40**). Other prominent pyrolysis products enountered are guaiacol (**4**), eugenol (**14**), homovanillin (**20**), acetoguaiacone (**22**), propioguaiacone (**29**) and homovanillic acid (**30**).

The other spruce lignin (MWL-2) analysed (Fig. 2) shows a high similarity with the MWL-1 sample. The presence of a few additional furan derivatives and the decrease of the peaks corresponding to coniferaldehyde (**39**) and *trans*-coniferyl alcohol (**40**) are noted. Moreover, vanillic acid (**28**) is only present in this sample.

Differences between the two spruce samples analysed are expected to some extent because interspecies variation and variations within a single plant (e.g. normal and compression wood) lignins have been reported (Sarkanen and Hergert, 1971). Further, differences may also be ascribed to slight modifications in the analytical isolation procedures, although basically the same procedure was used in both laboratories.

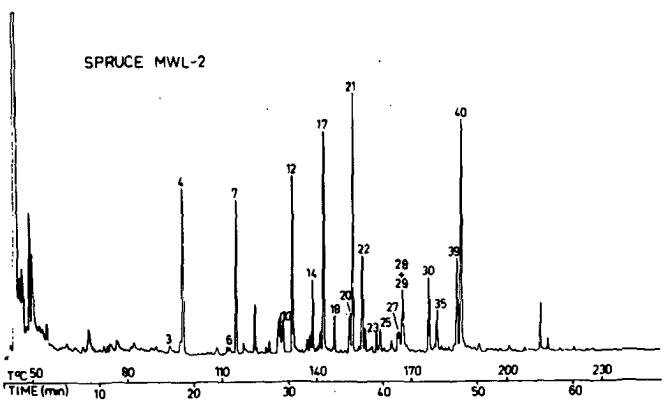


Fig. 2. Pyrograms of a *Picea abies* milled wood lignin.

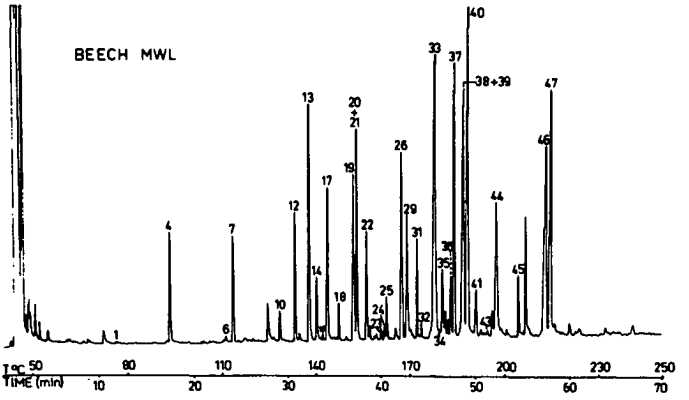


Fig. 3. Pyrogram of *Fagus silvatica* milled wood lignin. Contribution of acetoguaiacone (**20**) and acetosyringone (**38**) to the peaks are lesser than their corresponding coeluted compounds. A minor amount of dialkyl phthalate contributes to peak 46. The contribution of the identified compound to peak 44 is minor as compared with a syringyl derivative of MW 210 and unknown structure.

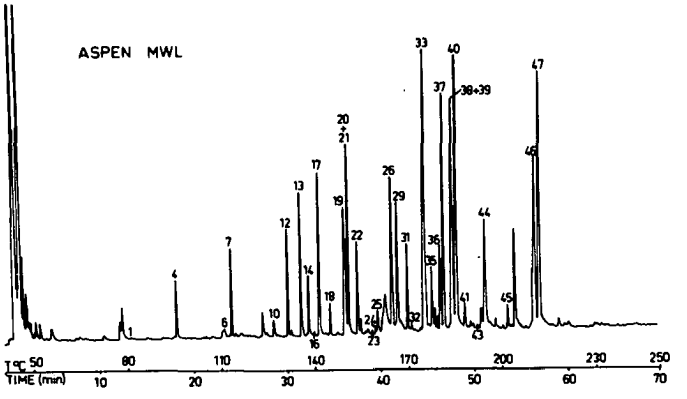


Fig. 4. Pyrogram of a *Populus* sp. milled wood lignin. Captions as in Fig. 3.

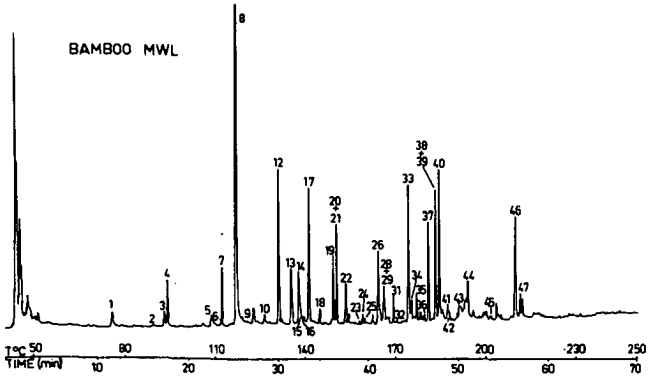


Fig. 5. Pyrogram of a *Bambusa* sp. milled wood lignin. Captions as in Fig. 3.

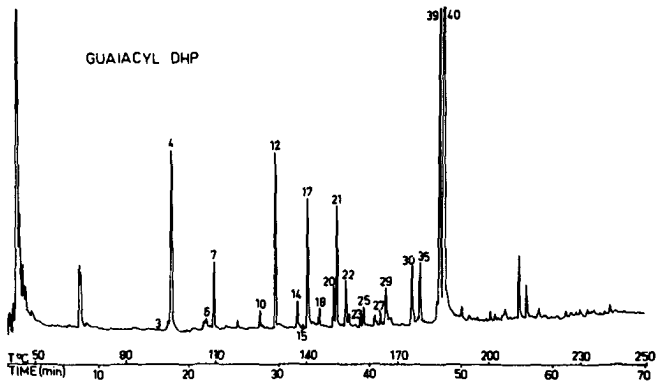


Fig. 6. Pyrogram of a guaiacyl dehydropolymer.

The pyrogram of beech MWL (Fig. 3) shows major peaks for 2,6-dimethoxyphenol (**13**), *trans*-isoeugenol (**21**), 4-vinyl-2,6-dimethoxyphenol (**26**), syringaldehyde (**33**), *trans*-4-propenyl-2,6-dimethoxyphenol (**37**), coniferaldehyde (**39**), *trans*-coniferyl alcohol (**40**), sinapaldehyde (**46**) and sinapyl alcohol (**47**). Prominent pyrolysis products are also guaiacol (**4**), 4-methylguaiacol (**7**), 4-vinylguaiacol (**12**), vanillin (**17**), 4-methyl-2,6-dimethoxyphenol (**19**), acetoguaiacone (**22**), propioguaiacone (**29**) and 4-allyl-2,6-dimethoxyphenol (**31**).

The pyrogram of an aspen MWL (Fig. 4) is quite similar to those of beech lignin and only a few differences are found in peak intensities.

The pyrogram of bamboo MWL is shown in Fig. 5. In addition to typical guaiacyl and syringyl pyrolysis products *p*-hydroxyphenyl compounds, as known to occur in grass lignins, are observed. The major pyrolysis product identified is *p*-vinylphenol (**8**). Other important compounds are 4-vinylguaiacol (**12**), vanillin (**17**), *trans*-isoeugenol (**21**), syringaldehyde (**33**), *trans*-4-propenyl-2,6-dimethoxyphenol (**37**), coniferaldehyde (**39**), *trans*-coniferyl alcohol (**40**) and sinapaldehyde (**46**).

Dehydrogention polymers

Guaiacyl DHP (Fig. 6) is similar to spruce MWLs in that both have the same type of pyrolysis products. However, the ratios of these products are different because compared to natural lignins the relative amounts of guaiacol (**4**), coniferaldehyde (**39**) and *trans*-coniferyl alcohol (**40**) are increased. This indicates differences between the natural and synthetic lignins probably in the proportion of linkages.

The pyrogram of the guaiacyl-syringyl DHP is displayed in Fig. 7. Major compounds are identified as 2,6-dimethoxyphenol (**13**), syringaldehyde (**33**), coniferaldehyde (**39**), *trans*-coniferyl alcohol (**40**), sinapaldehyde (**46**) and sinapyl alcohol (**47**). Other important compounds are 4-vinylguaiacol (**12**), vanillin (**17**) 4-methyl-2,6-dimethoxyphenol (**19**) *trans*-isoeugenol (**21**), 4-vinyl-2,6-dimethoxyphenol (**26**),

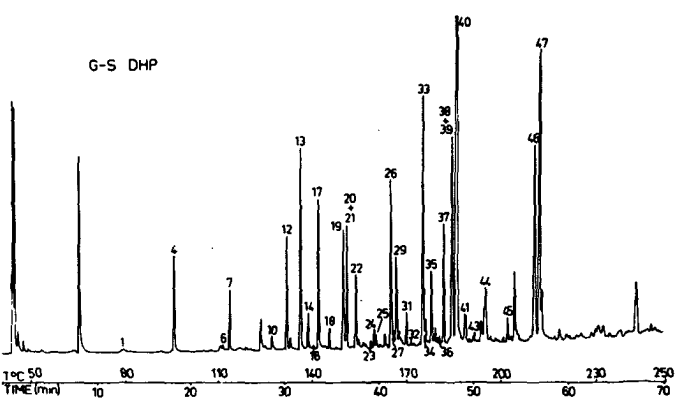


Fig. 7. Pyrogram of a guaiacyl-syringyl dehydropolymer. Captions as in Fig. 3.

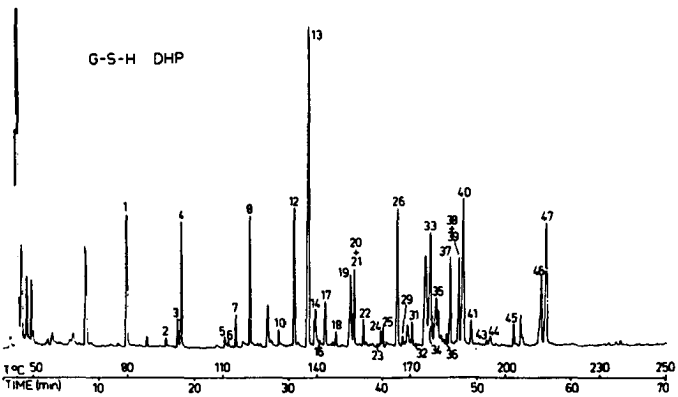


Fig. 8. Pyrogram of a guaiacyl-syringyl-*p*-hydroxyphenyl dehydropolymer. Captions as in Fig. 3.

and *trans*-4-propenyl-2,6-dimethoxyphenol (**37**). As for the guaiacyl DHP, the guaiacyl-syringyl DHP contains the same pyrolysis products as the natural lignin, but peak intensities are different, especially for *trans*-coniferyl alcohol.

The guaiacyl-syringyl-*p*-hydroxyphenyl DHP pyrogram (Fig. 8) shows a very different pattern with regard to bamboo MWL. The major phenolic compound identified is 2,6-dimethoxyphenol (**13**). Other prominent compounds are phenol (**1**), guaiacol (**4**), *p*-vinylphenol (**8**), 4-vinylguaiacol (**12**), 4-vinyl-2,6-dimethoxyphenol (**26**), syringaldehyde (**33**), *trans*-coniferyl alcohol (**40**) and syringyl alcohol (**47**). In this case the synthetic lignin does not reproduce well the main linkages present in the bamboo MWL, as indicated by the very different relative amounts of pyrolysis products.

To further substantiate the possible pyrolysis mechanisms of *p*-coumaril alcohol precursors, a *p*-hydroxyphenyl DHP was also studied. Among the phenolic compounds *p*-cresol and *trans*-*p*-propenylphenol are abundant pyrolysis products. Other important compounds are phenol, *p*-ethylphenol, *p*-vinylphenol, *p*-propylphenol, *cis*-*p*-propenylphenol and *p*-coumarylalcohol. Most of them are also encountered in guaiacyl-syringyl-*p*-hydroxyphenyl DHP. The *p*-coumaryl alcohol peak is lower than any of the identified compounds in the pyrolysate of *p*-hydroxyphenyl DHP, which might explain the difficulty of identifying this compound in bamboo MWL and its synthetic counterpart.

DISCUSSION

Pyrolysis products vs *lignin units*

Lignins are thought to be composites of two relatively well defined structural moieties: the so-called "bulk-polymer" characterized by a large number of free C_3-side chains and the "end-wise polymer", in which the β–O–4 linkage is dominant (Sarkanen, 1971). The identification of coniferaldehyde and coniferyl alcohol as major pyrolysis products in soft and hardwood lignins and their synthetic counterparts indicate that their presence is related with definite and relatively abundant structures. From all possible lignin substructures, two types, those with free end groups and those with arylglycerol–β–ether linkages appear to be the most likely candidates to explain the origin of these products. In fact, from units with free end groups the coniferyl derivatives will originate through cleavage of a C–O bond. Most probably they are released from their association with the lignin molecule through the cleavage of a β–O–4 linkage (Fig. 9). In the case of ether structures, the cleavage of two or more C–O bonds is involved. C–O bonds are the relatively weakest linkages present among the structural units of lignins, which could explain the abundance of the encountered major coniferyl derivatives. Both types of the substructures mentioned might account for about 50% of the natural lignins and perhaps more in the synthetic ones.

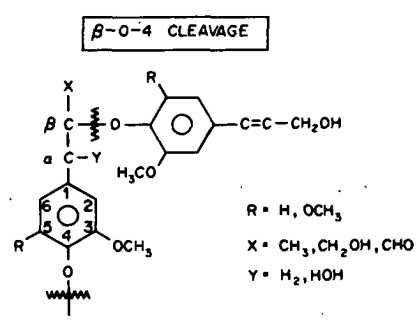


Fig. 9. Possible major types of lignin substructures thought to release coniferaldehyde/sinapaldehyde and coniferyl alcohol/sinapyl alcohol upon pyrolysis. Note that the numbering of the ring atoms in this figure is different from the numbering used throughout the text and in Table 1.

The occurrence of sinapaldehyde and sinapyl alcohol also points to the presence of free end groups in syringyl substructures. In this case β–O–4 bonds are not so abundant because the dual O-substitution at positions 3 and 5 (Fig. 9) forms a barrier against β–O couplings. Therefore, the β–β coupling might be more important among syringyl units (Sarkanen, 1971).

The relatively lower intensities of sinapyl alcohol as compared to coniferyl alcohol in the aspen and beech MWL sample might be due to this more pronounced β–β type of linkage, since these lignins are built up from almost equal amounts of coniferyl and sinapyl alcohols.

It is noteworthy that no hydroxyphenyl or syringyl compounds were encountered in the pyrolysates of softwood lignins, since Leopold and Malmström (1952) reported *p*-hydroxybenzaldehyde and syringaldehyde as minor components in the nitrobenzene oxidation of conifers.

Pyrolysis of bamboo MWL produces a rather unique suite of compounds (vinylphenol, propenylphenols, *p*-hydroxybenzaldehyde, etc.) that were not encountered in the softwood and hardwood lignins studied. Higuchi *et al.* (1967) reported that the larger proportion of *p*-hydroxybenzaldehyde produced in the alkaline nitrobenzene oxidation of grass lignins is formed from *p*-coumaric acid esterified with the lignin, and not from a *p*-hydroxyphenyl moiety in the lignin polymer itself. Further, these authors conclude that the main polymeric systems of the grass lignins are not very different from hardwood lignins. So, it is expected that the *p*-coumaric acid esterified with the lignin, upon pyrolysis, will yield the decarboxylated pyrolysis product *p*-vinylphenol.

Milled wood lignins vs *dehydrogenation polymers*

Milled wood lignins are considered to be, in many respects, almost identical with the original lignin, although they represent only 30–50% of the total lignin components, and generally contain small amounts of associated carbohydrates. The major differences between natural and synthetic lignins is the increase of coniferyl alcohol and aldehyde in the DHP samples, which indicates that an important moiety of the dehydropolymers is composed of the so-called "bulk polymer". In this connection, Haider *et al.* (1985) reported that ^{13}C labelling at the C–γ position of the side chain in the coniferyl alcohol precursor causes an enhancement of the signal corresponding to C–γ of coniferyl alcohol units with a free chain, which was the most intense signal in the DHP spectrum. The same result was obtained with C–α and C–β when labelled at the corresponding positions.

Spectroscopic and chemical studies point out that DHPs contain essentially the same structural units as MWLs (Kirk *et al.*, 1975). Previous analytical pyrolysis (Saiz-Jimenez and de Leeuw, 1984b) demonstrated that spruce and synthetic lignins are similar in that both generate the same type of pyrolysis products. However, because the ratios of these products were different, it was concluded that MWL and DHP were not identical, but merely similar. Further, in this study it is shown that differences between hardwood lignins and their synthetic counterpart are also evident. However, the much larger differences observed between the bamboo MWL and the guaiacyl-syringyl-*p*-hydroxyphenyl DHP indicate that the complexity of the natural lignin is not reflected in the synthetic one, probably due to the fact that the ester linkage is not present in the synthetic dehydropolymers.

Present pyrolysis data vs *literature data*

The results presented in this paper agree relatively well with those of Obst (1983) who studied loblolly pine and white oak MWL by Py–GC–MS. However, the different pyrolysis system (Pyroprobe) and temperature (800°C) probably result in a higher fragmentation of subunits (e.g. demethylation, as indicated by the identification of catechols). Catechol was only found upon Curie-point pyrolysis in biodegraded and kraft lignins (Saiz-Jimenez and de Leeuw, 1984b), where the action of respectively fungi and chemicals cause a demethylation. In the investigation reported here no catechols were encountered.

Martin *et al.* (1979) studied the same MWLs as those reported here. Also, in this case the pyrolysis system (Pyroprobe) and temperature (700°C) were different. The distribution patterns of pyrolysis products were somewhat different from those reported here. The largest difference concerned their identification of vinylphenols in the spruce MWL (equivalent to our MWL-2). A detailed study of the two spruce and the beech and aspen MWL samples failed to recognize vinylphenols among the pyrolysis products. Therefore, it is possible that the pyrolysis conditions used by the authors caused fragmentation of guaiacyl units to give rise to vinylphenols. However, Obst (1983) using an even higher temperature also did not report vinylphenols in pine and oak MWL.

From these data it must be concluded that comparison of results, even obtained for the same samples using different analytical systems and pyrolysis temperature is difficult.

Philp *et al.* (1982) studied lignins using Curie-point pyrolysis with an end temperature of 610°C. In pine lignin, among other compounds, 1,4-dimethoxybenzene and 1,2,3-trimethoxybenzene were identified and in eucalyptus lignin the major compound was identified as 3,4-dimethoxyphenol. Neither of these compounds could be identified in softwood and hardwood lignins by Obst (1983) or in this study, as expected, because there are no parent structures present in lignins which can give rise to them upon pyrolysis. As suggested by Obst (1983) identifications might have been wrong owing to misinterpretation of library spectra. This could occur because many of the

lignin pyrolysis products are not present in most library files and the best match does not always give the right structure. Reinterpretation of the data is necessary before a comparison can be made.

Sigleo (1978) reported that wood polymers survive 200 million years of silicification and diagenesis in the Petrified Forest National Park (Arizona) while Habermehl and Hundrieser (1983) found typical lignin oxidation products in Messel kerogen, 50 million years old. Because the guaiacyl and syringyl derivatives are unique to lignins and woods, and obviously can be preserved for millions of years, lignins can be considered as macromolecular biomarkers. Unequivocal evidence for the presence of lignin and/or wood in fossil materials can be obtained by studying the pyrolysis products. Moreover, Py–GC–MS analysis can clearly distinguish the three types of lignins.

The presence of *p*-vinylphenol as major compound in the pyrolysates of lignocellulosic materials, in addition to guaiacyl and syringyl derivatives, points to grass lignin. Guaiacyl and syringyl derivatives, in absence of *p*-hydroxyphenyl compounds, direct to hardwoods, while the presence of guaiacyl units alone indicate that the lignin was biosynthesized by conifers. To further prove these statements and to obtain more information about the fate of buried wood components such as carbohydrate and lignin structures, a study of spruce, alder and oak woods deposited in coastal sediments is in progress.

Note added in proof

Unidentified peak before **10** in Figs 3, 4, 5, 7 and 8 is methoxydihydroxybenzene. Also peak before **46** is *as*-sinapyl alcohol.

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CHAPTER 6

Chemical characterization of recent and buried woods by analytical pyrolysis. Comparison of pyrolysis data with ^{13}C NMR and wet chemical data.

CHEMICAL CHARACTERIZATION OF RECENT AND BURIED WOODS BY ANALYTICAL PYROLYSIS

COMPARISON OF PYROLYSIS DATA WITH ^{13}C NMR AND WET CHEMICAL DATA

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ABSTRACT

In a previous study, selective degradation of carbohydrates versus lignin in buried woods was demonstrated by non-destructive spectroscopy (^{13}C NMR) and destructive wet chemical methods (acid hydrolysis and alkaline CuO oxidation). In this paper, spruce, alder and oak woods deposited in coastal sediments and their recent equivalents are characterized by pyrolysis–mass spectrometry and pyrolysis–gas chromatography–mass spectrometry. Pyrolysis data, wet chemical degradation and NMR data are in good agreement, indicating that flash pyrolysis methods are useful for fast detailed characterization of this type of organic matter.

Biomass; gas chromatography; lignin; mass spectrometry; polysaccharides; pyrolysis; wood.

INTRODUCTION

Woody tissues are the most abundant biomass components in terrestrial environments and important contributors to sedimentary and soil organic

matter. Aerobic degradation of wood is slow because lignin is a poor substrate for microorganisms and decreases the decomposition rate of associated polysaccharides. Lignins are relatively slowly biodegraded in soils by certain fungi and bacteria, and partly degraded lignins and lignin derived phenols may contribute to the formation of humus [1]. The absence of significant wood decomposition under anaerobic conditions is a consequence of the requirement of oxygen for microbial degradation. In anaerobic wetlands and in sediments of marine and freshwater environments, woody materials are better preserved and bacteria are thought to be the major agents of wood degradation with polysaccharides being the dominant nutrient source [2].

In a previous study [3] the selective degradation of carbohydrate versus lignin in buried wood samples was demonstrated by non-destructive spectroscopy (^{13}C NMR) and destructive wet chemical techniques (acid hydrolysis and alkaline CuO oxidation). In the present study, the same samples are characterized by pyrolysis–mass spectrometry (Py–MS) and pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS). A comparison is made between buried samples of spruce, alder and oak wood from coastal sediments with their recent living equivalents. Pyrolysis data are discussed versus chemical degradation and NMR data in order to determine the feasibility of pyrolysis methods to study the chemical structure of buried woods.

EXPERIMENTAL

Samples

Buried spruce (*Picea sitchensis*) and red alder (*Alnus rubra*) wood samples were taken 10 cm beneath the bark from large logs (0.5 m diameter) excavated about 6 m apart in a silty clay horizon along the bank of the Hoko river (near the Strait of Juan de Fuca, Washington State, U.S.A.). The deposit is contemporaneous with a 2500 year old archeological site. Buried white oak (*Quercus* spp.) wood was obtained at a sediment depth of 100 m in a drill hole on the continental slope of the Gulf of Mexico off the coast of Louisiana and is likely to be over 25,000 years old. Recent equivalents of the three buried woods were taken from trunk wood sections. Further details on the samples have been given elsewhere [3].

Curie-point pyrolysis methods

Three Curie-point pyrolysis methods were applied. Curie-point Py–MS using the FOMautopyms was used for fingerprinting analysis of the wood pyrolysates. A recent description of the method for analysis of coal and related materials is given by Tromp et al. [4].

The mass range of the mass spectrometer used in this study was m/z 20–220. The pyrolysis chamber and the expansion chamber were heated to 160 and 200 °C, respectively. The Curie-point temperature of the ferromagnetic wires was 610 °C. Samples were taken from suspensions in water.

Py–GC was performed in a reactor described by Van de Meent et al. [5]. The pyrolysis unit was mounted in the injection block of a Packard Becker 419 gas chromatograph or on a Varian 3700 in the case of the Py–GC–MS system. The pyrolysates were separated on a 30-m fused silica column (I.D. 0.26 mm) coated with DB-1701 (film thickness 0.25 μm). The GC oven was programmed from 0 to 300 °C at a rate of 3 °C/min. GC–MS was performed with a MAT 44 quadrupole mass spectrometer operated in the electron impact mode at 80 eV.

Multivariate analysis

Discriminant analysis was performed on the Py–MS data file, which consisted of six categories (samples) in quadruplicate. A modified ARTHUR package, specially adapted to accept Py–MS data, was used for this procedure [6]. The output consists of plots or maps which show the relative difference in composition of the samples. The variables which cause the discrimination are given in so-called discriminant functions.

RESULTS AND DISCUSSION

Wet chemical studies

The analyses of neutral sugars and lignin-derived phenols indicated that at least 90 and 98% of the initial total polysaccharides in buried alder and oak woods, respectively, have been degraded along with 15–25% of the lignin [3]. The buried spruce was almost unaltered. The observation that softwood is more resistant to decomposition than hardwood is in agreement with previous studies [7].

^{13}C NMR

^{13}C NMR data of these samples have been discussed elsewhere in detail [3]. The major results are summarized hereafter. The spectra of the recent woods and the buried spruce wood are dominated by resonances in the 60–110 ppm region assignable to α-cellulose and hemicellulose. In contrast, resonances corresponding to lignin strongly predominate in the spectra of the two buried hardwood. The spectra resemble those obtained by Hatcher et al. [8] for modern spruce and a 10,000 year old buried spruce wood, which in this case has suffered selective polysaccharide degradation.

Pyrolysis–mass spectrometry

Pyrolysis–mass spectra are fingerprints of the pyrolysates evolved from complex organic matter samples. Examples for wood and wood polymers have been shown by Windig et al. [9]. Most of the mass spectrometry information on the polysaccharide fraction in wood is present in the mass range m/z 20–126, whereas mass peaks for the lignins plot in the range m/z 110–210. Recent changes in the pyrolysis chamber of the FOMautopyms have improved the transmission of higher molecular weight materials which results in a more complete picture of the lignin pyrolysate compared to previous data by Meuzelaar et al. [10]. The chemical significance of the mass peaks of polysaccharides and lignin in low-voltage pyrolysis spectra has been studied with Py–GC–MS using photoionisation at 11.8 eV [11,12]. Although many mass peaks are in fact impure, some tentative conclusions may be drawn in the case of complex pyrolysates such as those of woods.

Py–mass spectra of recent and buried soft (spruce) and hard (oak) wood samples are shown in Figs. 1–4. It is immediately evident that major changes in the ratio of polysaccharides to lignin have taken place in the buried oak sample. These effects are not evident in the spruce sample, although a change in the composition of the polysaccharide fraction is indicated by the decrease in the relative intensity of the mass peaks in the m/z range 30–120. The relative pure mass peak m/z 114, abundant in both pyrolysates, is typical of xylans. The ratios of the abundant mass peaks m/z 57, 60 and 73 in the modern oak Py–mass spectrum (Fig. 3) resemble the distribution in levoglucosan [13], a pyrolysis product of cellulose.

Lignin appears to have undergone minor structural changes. The spectrum of spruce shows mass peaks indicative of a guaiacyl lignin with m/z

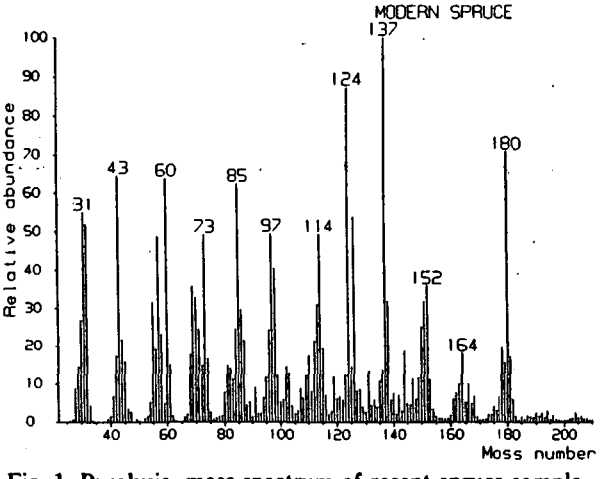


Fig. 1. Pyrolysis–mass spectrum of recent spruce sample.

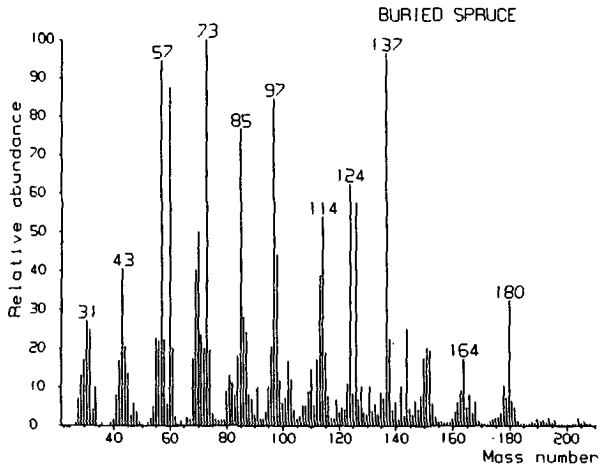


Fig. 2. Pyrolysis–mass spectrum of buried spruce sample.

180, 178, 166, 164, 152, 137 (a fragment ion peak) and 124. Oak lignin is a mixed guaiacyl–syringyl lignin in which *m/z* 210, 208, 196, 194, 182, 167 (a fragment ion peak) and 154 are indicative of the syringyl part of the macromolecule. The Py–MS profiles of the buried woods do not show any major changes in the ratios of these peaks, although the spectra of the buried hard woods show higher relative abundances for *m/z* 124, 164, 194 and a lower intensity of *m/z* 210.

Fig. 5 displays a map of the scores of the samples on the first and second discriminant functions, which show their relative difference in composition. The euclidian distance of the sample points is a measure of the difference in

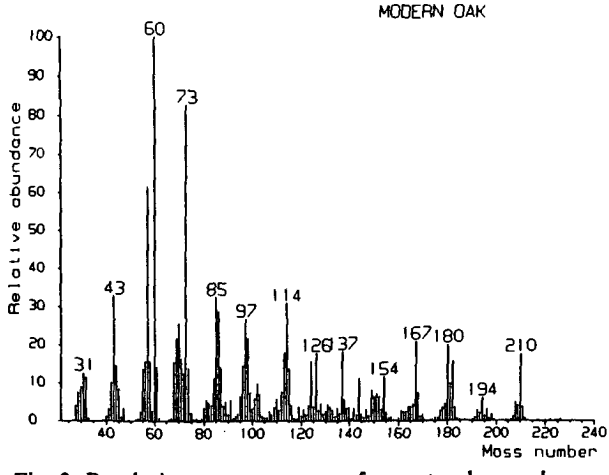


Fig. 3. Pyrolysis–mass spectrum of recent oak sample.

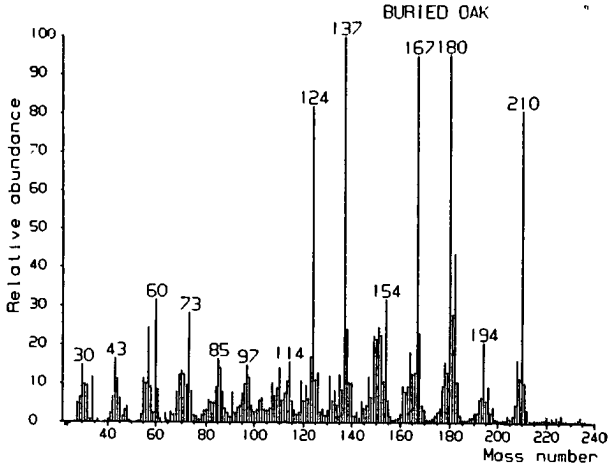


Fig. 4. Pyrolysis–mass spectrum of buried oak sample.

their Py–mass spectra. About 85% of the characteristic variance between the samples is explained by this D_1D_2 map. The reconstructed mass spectra of the first discriminant function (Fig. 6) shows that this axis of variance concerns the ratio of polysaccharides (mass peaks in D1 +) and lignins (mass peaks in D1 −). All recent woods plot high on D1 + (Fig. 6), whereas the buried woods, with the exception of spruce, plot towards lower polysaccharide and higher lignin markers (D1 −). The second axis of variance

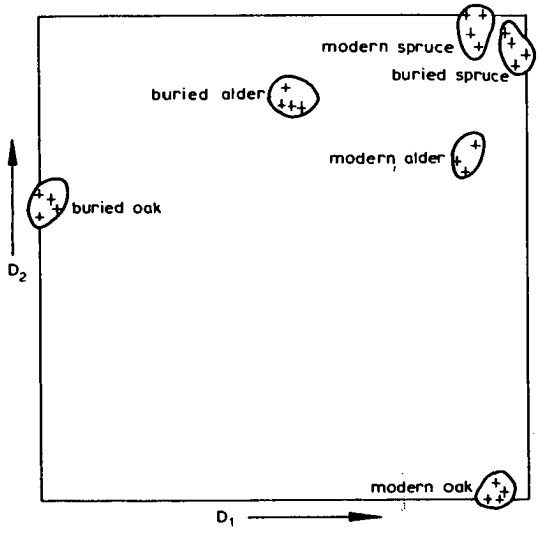


Fig. 5. D_1D_2 map with the scores of the samples.

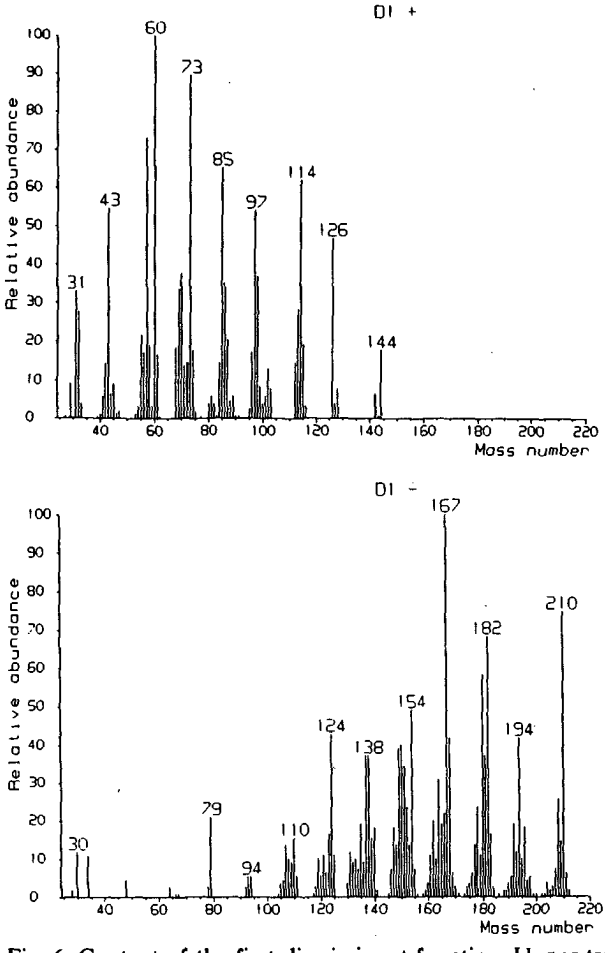


Fig. 6. Content of the first discriminant function. Upper trace, D1+; lower trace, D1−.

concerns the nature of the lignin, i.e. the ratio between guaiacyl and syringyl markers, which is evident from the reconstructed mass spectra of the second discriminant function (Fig. 7). The D2+ spectrum is indicative of guaiacyl units (*m/z* 180, 178, 152, 137, 124). The spectrum of D2− shows mass peaks for syringyl units (*m/z* 210, 194, 182, 167, 154) as well as peaks for levoglucosan (*m/z* 57. 60, 73).

Recent spruce and buried spruce score highest on D2+ because they contain only guaiacyl lignin. The modern oak scores highest on the syringyl side of D2. Recent and buried alder take intermediate positions, partly because of their guaiacyl/syringyl ratio and also because of the change in polysaccharide/lignin ratio in the fossil sample. The two hardwoods have undergone the strongest degradation of their woody tissues, as the euclidean

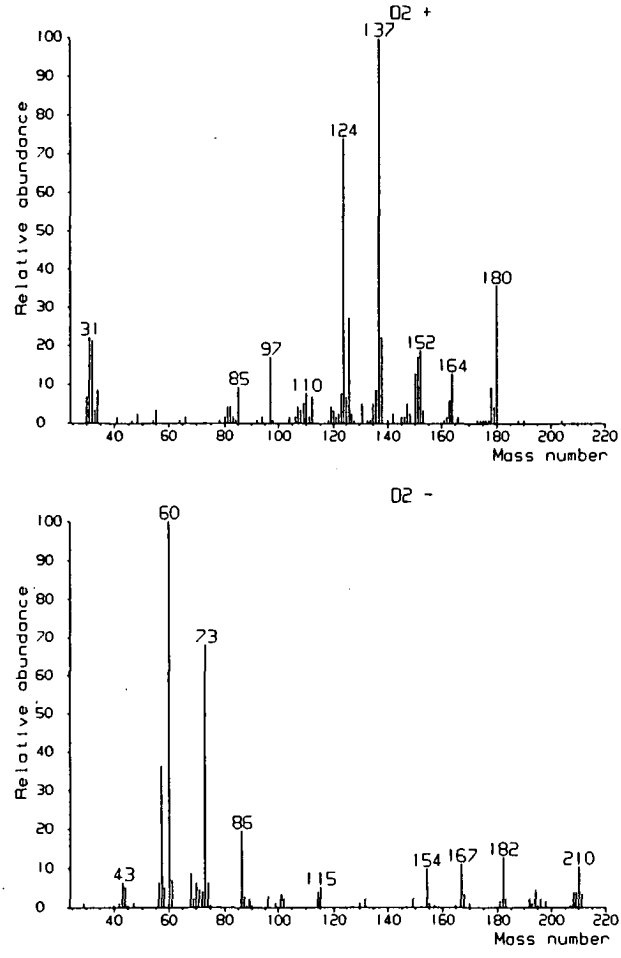


Fig. 7. Content of the second discriminant function. Upper trace, D2+; lower trace, D2−.

distance between recent wood and fossil material is substantial. The buried spruce sample plots close to the modern undecomposed equivalent, which means that the buried spruce sample is only slightly decomposed compared to the other samples. It is likely that the high levoglucosan evolution from the oak sample is a special characteristic, which may not be representative for all oak secondary xylem. These pyrolysis results are in full agreement with the previously obtained spectral and chemical degradative analyses.

Pyrolysis–gas chromatography–mass spectrometry

Py–GC traces of wood samples show the complexity of the pyrolysis product mixtures (Figs. 8–10). The identities of major and significant

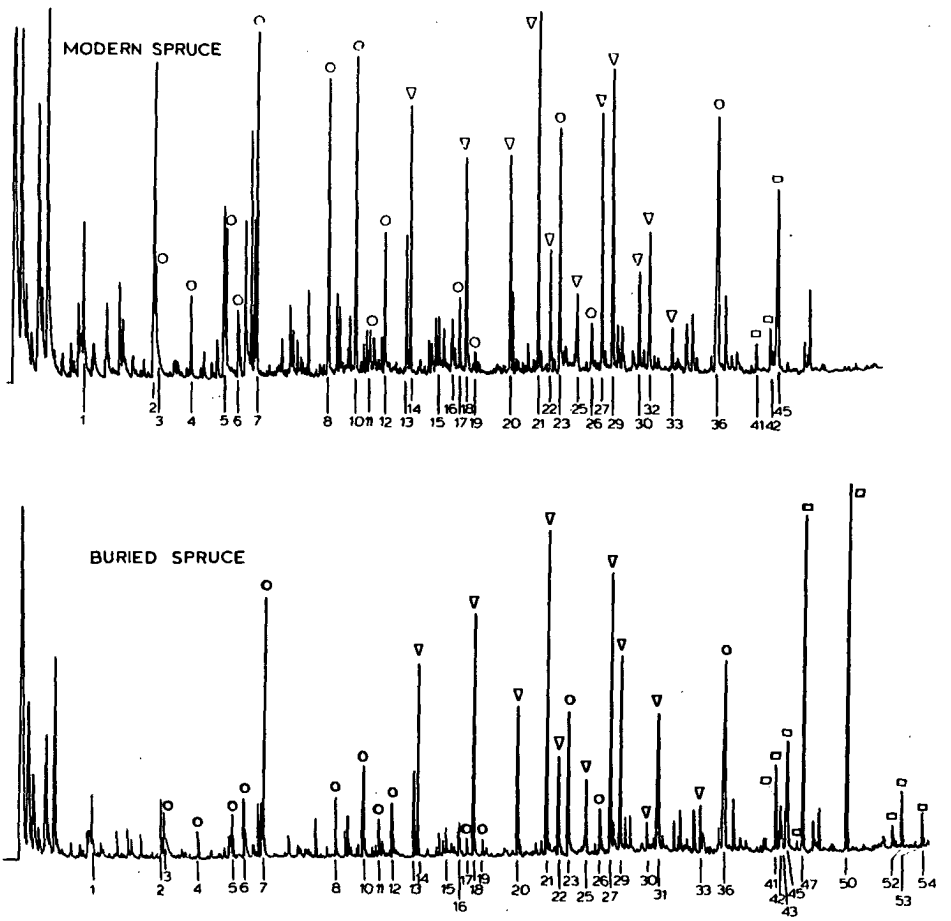


Fig. 8. Pyrolysis–gas chromatography trace of recent spruce sample (upper trace) and buried spruce sample (lower trace). The numbers correspond with the numbers in Table 1. ○: Polysaccharide pyrolysis products; ▽: guaiacyl lignin pyrolysis products; △: syringyl pyrolysis products; □: resin compounds.

compounds are given in Table 1. Recent woods yield high amounts of polysaccharide pyrolysis products such as 2-furaldehyde (7), α-angelicalactone (8), 5-methylfuraldehyde (10), 4-hydroxy-5,6-dihydro-2H-pyran-2-one (11) and levoglucosan (36). These compounds are yielded in drastically reduced amounts from the buried hardwoods, but much less so from the buried spruce wood. In comparison to the native woods the pyrolysis products of the guaiacyl lignin and syringyl lignin are relatively increased in the alder and oak buried woods, while in the spruce this increase is less significant.

The most prominent guaiacyl lignin pyrolysis products are methylguaiacol (18), vinylguaiacol (21), *trans*-isoeugenol (27) and vanillin (29). Among the

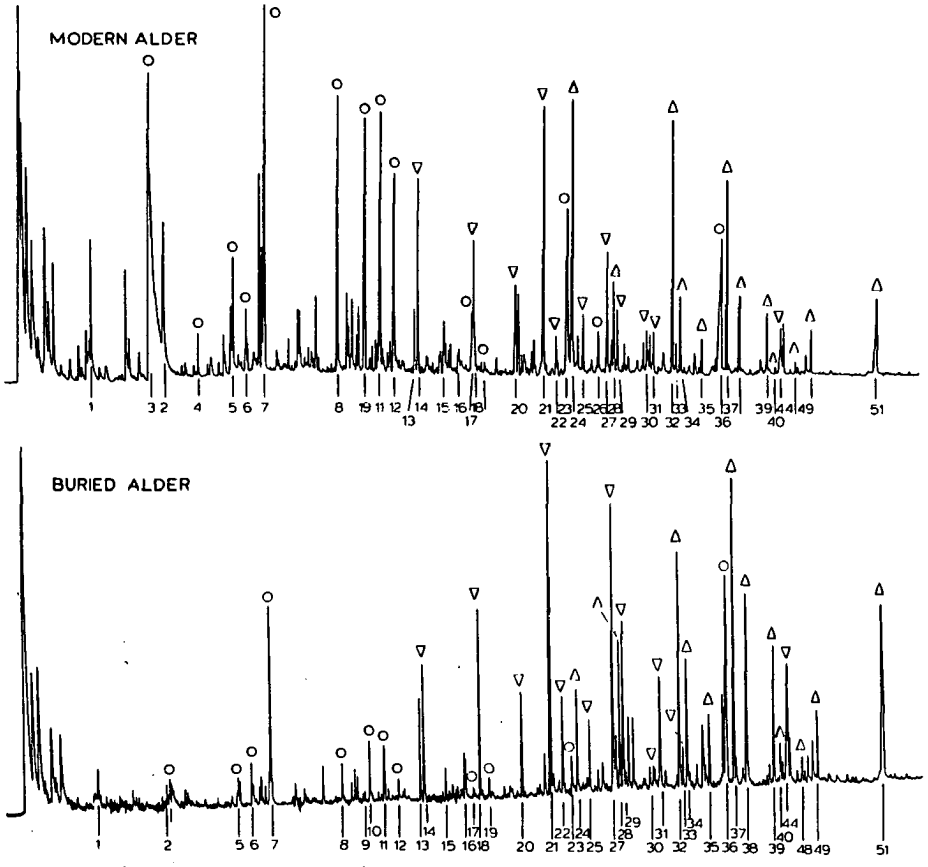


Fig. 9. Pyrolysis–gas chromatography trace of recent alder sample (upper trace) and buried alder sample (lower trace). See legend of Fig. 8 for significance of numbers and symbols.

syringyl lignin compounds, 2,6-dimethoxyphenol (24), vinyl-2,6-dimethoxyphenol (32), *trans*-propenyl-2,6-dimethoxyphenol (37) and syringaldehyde (38) predominate.

The recent and buried spruce woods are, however, different with respect to another type of compound, viz. resins. The buried spruce contained relatively large amounts of abietic acid derivatives, such as dehydroabietin (47) and *nor*-simonellite (50). This difference in resin content between the recent and the buried wood samples was not observed by NMR and chemical degradation [3]. Although not checked it is believed on the basis of previous studies [14] that the resin compounds are mainly present as such and simply evaporate during the rapid heating of the wire. The relatively different amounts of resin compounds are probably due to heterogeneity of the samples and not indicative of any relative enrichment of resin compounds upon burial.

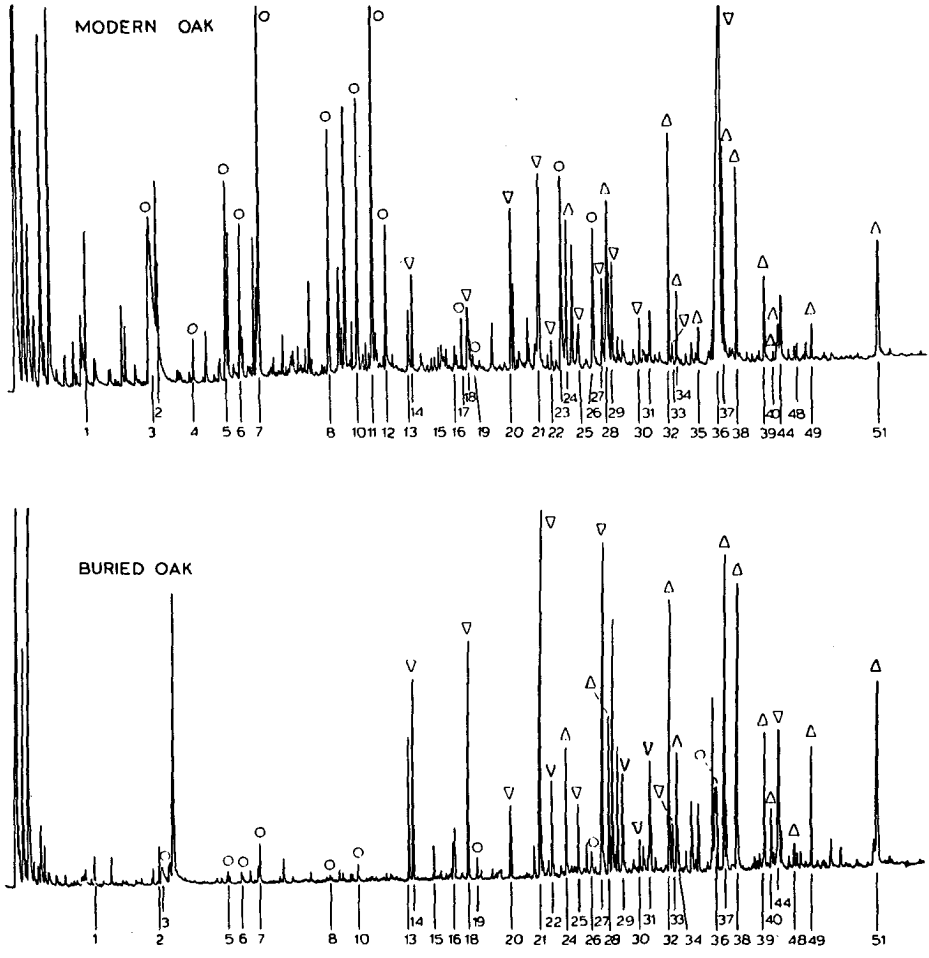


Fig. 10. Pyrolysis–gas chromatography trace of recent oak sample (upper trace) and buried oak sample (lower trace). See legend of Fig. 8 for significance of numbers and symbols.

Guaiacyl lignin (G) versus syringyl lignin (S) ratios have been calculated from the intensities of identified pyrolysis products (Table 2). Percentages of identified polysaccharide, lignin and resin pyrolysis and evaporation products were also calculated (Table 3). It is clear from the G/S ratios that during burial a selective loss of syringyl units occurs. Furthermore, the comparison of the percentages of different components indicates that polysaccharides decrease during burial in all woods: less in the spruce, but significantly in the oak sample. Guaiacyl lignin pyrolysis products remain almost constant in the buried spruce. The Py–GC–MS data thus are in good agreement with the Py–MS and discriminant analysis data.

Wet chemical and spectroscopic data versus analytical pyrolysis data

The study of chemical changes in woods after burial can be accomplished by wet chemical, spectroscopic or analytical pyrolysis methods. As already mentioned these three different approaches are in very good agreement with each other. Nevertheless there are a number of aspects to consider when comparing these different and partly complementary techniques.

Wet chemical degradations are lengthy, tedious, and often involve derivatization of reaction mixtures before analysis. Further, the hydrolysis and degradation reactions applied are far from complete, so the figures obtained are underestimates. It has been reported that CuO oxidation of guaiacyl lignin only yields 30% (w/w) of phenols [3]. These approaches do give, however, detailed information on the individual structural moieties present in the macromolecular assemblages.

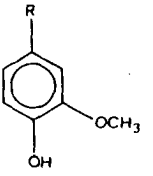
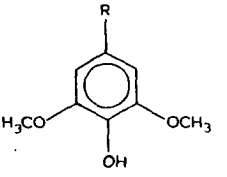
TABLE 1

Compounds identified in the pyrolysates of the recent and buried wood samples

No.	Compound	No.	Compound
1	Benzene	28	Methyl-2,6-dimethoxyphenol
2	Toluene	29	Vanillin
3	Acetic acid	30	Homovanillin
4	2-Methylfuran	31	Acetoguaiacone
5	Dihydropyran	32	Vinyl-2,6-dimethoxyphenol
6	3-Furaldehyde	33	Guaiacylpropan-2-one
7	2-Furaldehyde	34	Allyl-2,6-dimethoxyphenol
8	α-Angelicalactone	35	*cis*-Propenyl-2,6-dimethoxyphenol
9	α-Methylbenzylalcohol	36	Levoglucosane
10	5-Methylfuraldehyde	37	*trans*-Propenyl-2,6-dimethoxyphenol
11	4-Hydroxy-5,6-dihydro-2H-pyran-2-one	38	Syringaldehyde
12	2-Hydroxy-3-methyl-2-cyclopenten-2-one	39	(2.6-Dimethoxyphenyl)-ethanal
13	Phenol	40	(2,6-Dimethoxyphenyl)-ethanone
14	Guaiacol	41	19-*nor*-Dehydroabietane
15	*o*-Cresol	42	*nor*-Abietane
16	*p*-Cresol	43	Isomer of 41
17	Levoglucosenone	44	Coniferaldehyde
18	Methylguaiacol	45	Isomer of 41
19	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	46	19-*nor*-Abieta-4(18)8,11,13-tetraene?
20	Ethylguaiacol	47	Dehydroabietin
21	Vinylguaiacol	48	(2,6-Dimethoxyphenyl)-propanone
22	Eugenol	49	(2,6-Dimethoxyphenyl)-propanedione
23	5-(Hydroxymethyl)-2-furaldehyde	50	*nor*-Simonellite
24	2,6-Dimethoxyphenol	51	Sinapaldehyde
25	*cis*-Isoeugenol	52	Retene
26	1,4-Dideoxy-D-glycerohex-1-enopyranos-3-ulose	53	Dehydroabietane
		54	Simonellite
27	*trans*-Isoeugenol		

TABLE 2

Ratios of guaiacyl and syringyl pyrolysis products in the pyrolysates of the hardwood samples

R	G/S				Guaiacyl unit	Syringyl unit
	Modern alder	Buried alder	Modern oak	Buried oak		
-H	0.71	1.28	0.97	1.55		
$-CH_3$	1.43	1.29	0.69	1.48		
$-CH=CH_2$	1.06	1.38	0.84	1.41		
$-CH=CH-CH_3$	0.63	0.95	0.40	1.06		
-CHO	0.81	0.87	0.51	0.89		
$-CO-CH_3$	0.48	0.80	0.54	0.25		
-CH=CH-CHO	0.61	0.68	0.30	0.85		
Σ	0.83	1.03	0.62	1.08		

The solid-state ^{13}C NMR technique offers limited resolution of specific changes on the molecular level that might characterize wood degradation [3].

On the other hand the advantage of the ^{13}C NMR analysis is that the method is relatively speaking the most quantitative one.

Analytical pyrolysis methods are non-selective, can be applied to comparatively small samples (microgram level) in a highly reproducible way, and provide data at the molecular level of polysaccharides, lignins and resins without any pretreatment of samples. The application of multivariate analysis of Py–MS data to classify native and decomposed woods opens the possibility of evaluating large series of samples.

It has been suggested that wood characteristics have been preserved with relatively little morphological changes in some brown coals because of the conifer resins that protect the wood from extensive alteration [8].. The presence of diterpenoid compounds derived from abietic acid in the buried spruce wood supports this explanation. Although it was concluded from wet chemical analyses that the polysaccharide content in the buried wood is

TABLE 3

Percentages of identified polysaccharide and lignin pyrolysis products

	Polysaccharide	Guaiacyl lignin	Syringyl lignin	Resin (%) *
Modern spruce	49.3	50.7	–	5.9
Buried spruce	37.6	62.4	–	26.0
Modern alder	48.3	25.7	26.9	–
Buried alder	19.4	41.6	39.0	–
Modern oak	55.8	18.7	25.5	–
Buried oak	6.3	50.3	43.4	–

* Percentage of resin compounds with regard to polysaccharide + lignin pyrolysis products.

unaltered [3], the Py–MS and Py–GC–MS data indicate that some polysaccharide pyrolysis products decrease significantly and others do not. It appears probable that modifications in polysaccharide structure, possibly by changes in intermolecular bonding, are the reason. Further studies are however required to investigate this hypothesis.

An interesting fact is the absence in the buried wood samples of significant amounts of oxidized phenols (e.g. vanillic and syringic acids). These compounds reflect the aerobic fungal degradation of lignins and woods [15]. Because the degradation of woods in aerobic environments is an oxidative process requiring oxygen, the low amount of oxidized phenols in the buried wood samples might reflect an anoxic burial history.

ACKNOWLEDGEMENT

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C H A P T E R 7

Nature of plant components identified in soil humic acids.

NATURE OF PLANT COMPONENTS IDENTIFIED IN SOIL HUMIC ACIDS

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SUMMARY

The chemical structure of two acid hydrolysed soil humic acids was investigated using analytical pyrolysis. A large part of the pyrolysis mixture consisted of a homologous series of straight chain alkanes, alk-1-enes and α, ω - alkadienes probably derived from plant cuticles. The origin of other major components in the pyrolysate, phenols and aromatic hydrocarbons, is less clear.

INTRODUCTION

The origin of the humic acid fraction of soils is still unclear in spite of extensive investigations accomplished over the last years (ref. 1). Different origins have been proposed, based on the most recent theory or on the application of the latest analytical method. Thus, it has been proposed that soil humic acids are derived from lignins, microbial activity and synthesis of melanins, reaction between sugars and amino acids, etc. (ref. 1). Although there is no doubt that the components mentioned are involved in the formation of humus, recent investigations have demonstrated that lignin, sugar and protein moieties are peripheral or loosely attached to the so-called core of soil humic acid and can easily be removed by acid hydrolysis (ref. 2). The question arises what the structure and origin of the more resistant part is. This core is not or hardly affected by acid hydrolysis or mild oxidations but is extensively degraded upon drastic oxidation with permanganate (ref. 3).

This paper describes the chemical nature of the most resistant part of soil humic acids based on Curie-point pyrolysis-gas chromatography-mass spectrometric analyses. This technique has proved to be useful in the characterization of biopolymers and geopolymers (ref. 2, 4, 5).

METHODS

Two soil samples from the South of Spain were used in this study. One of them was a Typic Rendoll and the other a Typic Rhodoxeralft. The preparation of the humic acid fractions has been described previously (ref. 6). Elemental analyses and NMR spectra of the humic acid fractions have also been reported (ref.7). The

analytical pyrolysis method as well as the acid hydrolysis have been described elsewhere (ref. 2, 4).

RESULTS AND DISCUSSION

The pyrolysis behaviour of unhydrolysed humic acids have been extensively reported (ref. 2, 4). Briefly, humic acid fractions yield upon flash pyrolysis/evaporation products derived from polysaccharides, proteins, lignins, lipids and pollutants.

Figure 1 shows the pyrograms of the humic acids after hydrolysis with 6 N HCl and extraction with hexane (sonication, 2 x 10 min.). The main difference with comparison to the unhydrolysed samples is the dissapearance of characteristic pyrolysis products derived from polysaccharides, proteins and, to some extent, lignins. Also fatty acid concentrations decreased drastically, probably due to the extraction process.

The pyrolysis mixture of the humic acids shows a homologous series of straight chain alkanes, alk-1-enes and α , ω -alkadienes ranging from C_5 to C_{31}. In addition, aromatic hydrocarbons such as homologous series of alkylbenzenes and alkylnaphthalenes and some polycyclic aromatic hydrocarbons are present. Phenol and alkylphenols are also major pyrolysis products. Similar pyrograms have been obtained for hydrolysed fulvic acids, although in that case the homologous series of the aliphatic components were less prominent (unpublished data).

Recently, the occurrence of a new, non saponifiable, highly aliphatic, biopolymer in modern and fossil plant cuticles has been reported (ref. 8). The pyrograms of this cuticle biopolymers consist of series of n-alkanes, n-alkenes and α , ω-alkadienes and the pattern is similar to the aliphatic hydrocarbon pattern encountered in the soil humic acids after hydrolysis. Since unhydrolysed soil humic acids yield upon pyrolysis compounds derived from plant components, such as lignins, tocopherols, chlorophylls, terpenoids, etc. (ref. 4) it is speculated that the new biopolymer present in cuticles make up a part of the so-called core of humic acids.

The short and long chain aliphatic mono- and dicarboxylic acids obtained upon alkaline permanganate oxidation of humic acids (ref. 3) might be derived from this biopolymer. The phenolic acids and benzenecarboxylic acids identified upon permanganate oxidation of humic acids might originate from substances in the humic acid core which on flash pyrolysis yield the aromatic hydrocarbons and phenols. However, the origin and nature of these substances is as yet unclear.

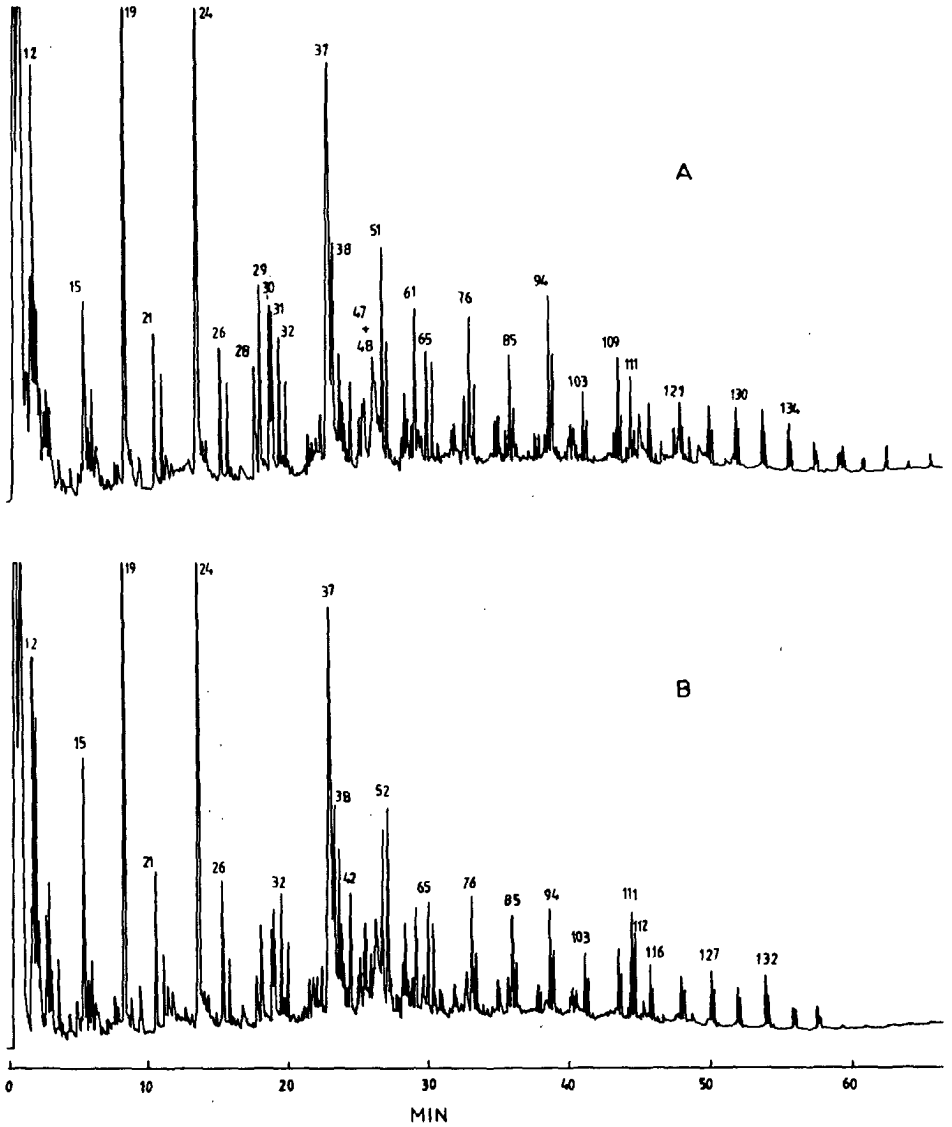

Figure 1. Flash pyrolysis-gas chromatography-mass spectrometric analysis of 6 N HCl hydrolysed and hexane extracted humic acids. A: Typic Rendoll humic acid, B: Typic Rhodoxeralft humic acid. Curie-point temperature 770°C. Peak identifications are given in Table 1. Owing to the vast number of identified compounds in each pyrolysate only major peaks are indicated in the figures.
GC conditions: Fused silica column (28 m x 0.5 mm I.D.) coated with CP-sil 5 held at 0°C for 5 min. and subsequently programmed to 300°C at a rate of 5°C/min The chromatograph (Varian 3200) was coupled to a Varian MAT 44 quadrupole mass spectrometer operated in the EI mode at 80 eV.

TABLE 1

Pyrolysis products of 6 N HCl hydrolysed and hexane extracted soil humic acids

1 Carbon monoxide
2 Carbon dioxide
3 Methane
4 Ethene
5 Ethane
6 Propene
7 Propane
8 Sulphur dioxide
9 But-1-ene
10 Butane
11 Acetone
12 Pent-1-ene
13 Furan
14 Pentane
15 Hex-1-ene
16 2-Methylfuran
17 Hexane
18 3-Methylfuran
19 Benzene
20 Cyclohexene
21 Hept-1-ene
22 Heptane
23 Methylcyclohexane
24 Toluene
25 Acetic acid
26 Oct-1-ene
27 Octane
28 Ethylbenzene
29 m- and/or p-xylene
30 Styrene
31 o-xylene
32 Non-1-ene
33 Nonane
34 C_3-alkylbenzene
35 C_3-alkylbenzene
36 C_3-alkylbenzene
37 Phenol
38 Dec-1-ene
39 Decane
40 C_3-alkylbenzene
41 Indane
42 Indene
43 C_4-alkylbenzene
44 p-cresol
45 C_4-alkylbenzene
46 $C_{4:1}$-alkylbenzene
47 Guaiacol
48 o-cresol
49 $C_{4:1}$-alkylbenzene
50 α,ω-undecadiene
51 Undec-1-ene
52 Undecane
53 $C_{4:1}$-alkylbenzene
54 C_4-alkylbenzene
55 $C_{4:1}$-alkylbenzene
56 $C_{4:1}$-alkylbenzene
57 Methylindene
58 Methylindene
59 C_4-alkylbenzene
60 C_5-alkylbenzene
61 Naphthalene
62 $C_{5:1}$-alkylbenzene
63 Methylguaiacol
64 α,ω-dodecadiene
65 Dodec-1-ene
66 Dodecane
67 Methylnaphthalene
68 Branched tridecane
69 Vinylphenol
70 C_6-alkylbenzene
71 C_6-alkylbenzene
72 Methylnaphthalene
73 Ethylguaiacol
74 Methylnaphthalene
75 α,ω-tridecadiene
76 Tridec-1-ene
77 Methylnaphthalene
78 Tridecane
79 C_7-alkylbenzene
80 C_7-alkylbenzene
81 Acenaphthene
82 C_2-alkylnaphthalene
83 Methylbiphenyl
84 α,ω-tetradecadiene
85 Tetradec-1-ene
86 C_2-alkylnaphthalene
87 Tetradecane
88 C_2-alkylnaphthalene
89 C_2-alkylnaphthalene
90 trans-isoeugenol
91 C_2-alkylnaphthalene
92 C_8-alkylbenzene
93 α,ω-pentadecadiene
94 Pentadec-1-ene
95 Pentadecane
96 C_3-alkylnaphthalene
97 C_3-alkylnaphthalene
98 C_9-alkylbenzene
99 Fluorene
100 C_{12} fatty acid
101 C_3-alkylnaphthalene
102 α,ω -hexadecadiene
103 Hexadec-1-ene
104 Hexadecane
105 Xanthene
106 C_{10}-alkylbenzene
107 C_4-alkylnaphthalene
108 α,ω -heptadecadiene
109 Heptadec-1-ene
110 Heptadecane
111 Prist-1-ene
112 Prist-2-ene

TABLE 1 (continued)

113 C_{14} fatty acid
114 Anthracene
115 α,ω-octadecadiene
116 Octadec-1-ene
117 Octadecane
118 C_{15} fatty acid
119 Phytadiene
120 α,ω-nonadecadiene
121 Nonadec-1-ene
122 Nonadecane
123 Methylanthracene
124 Dialkyl phthalate
125 C_{16} fatty acid
126 α,ω-eicosadiene
127 Eicos-1-ene
128 Eicosane
129 α,ω-heneicosadiene
130 Heneicos-1-ene
131 Heneicosane
132 Docos-1-ene
133 Docosane
134 Tricos-1-ene
135 Tricosane
136 Tetracos-1-ene
137 Tetracosane
138 Pentacos-1-ene
139 Pentacosane
140 Dialkyl phthalate
141 Hexacos-1-ene
142 Hexacosane
143 Heptacos-1-ene
144 Heptacosane
145 Octacos-1-ene
146 Octacosane
147 Nonacos-1-ene
148 Nonacosane
149 Triacont-1-ene
150 Triacontane
151 Hentriacont-1-ene
152 Hentriacontane

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C H A P T E R 8

Chemical structure of a soil humic acid as revealed by analytical pyrolysis.

CHEMICAL STRUCTURE OF A SOIL HUMIC ACID AS REVEALED BY ANALYTICAL PYROLYSIS

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ABSTRACT

A humic acid isolated from a brown soil (Typic Xerochrept) was characterized by analytical pyrolysis and chemical degradation reactions. On Curie-point pyrolysis at 610 °C the humic acid yielded pyrolysis products from polysaccharides, proteins and lignins. Fatty acids and aliphatic hydrocarbons were also released. Acid hydrolysis or persulphate oxidation of the humic acid released polysaccharides, proteins and to some extent lignins and fatty acids, leaving a residue that amounted to ca. 25–30% of the initial weight. Pyrolysis of the humic acid residues indicated the abundant presence of homologous series of *n*-alkanes, *n*-alk-1-enes and α,ω-alkadienes. The data suggest that lignin is not a significant contributor to the most resistant part of the humic acid. Other plant components, mainly highly aliphatic biopolymers encountered in plant cuticles and suberin, are probably a significant and resistant part of humic acids.

Biopolymers; gas chromatography; humic acids; mass spectrometry; pyrolysis; soils.

INTRODUCTION

Humic substances, the major organic constituents of soils and recent sediments, are widely distributed over the earth's surface and occur in almost all terrestrial and aquatic environments. Soil humic substances arise from or are left over after the chemical and biological degradation of plants and animal residues by microorganisms. The products thus formed possess complex chemical structures that are more stable than the starting materials [1]. The humic substances are not well defined classes of organic compounds. Their distinction is based on differences in solubility in aqueous solutions at different pH levels. The major humic fractions are humic acid, fulvic acid and humin.

Since Archard first isolated humic acid from peat in 1786 [2], 200 years of chemical investigations of humic substances have been accomplished. In spite of this extensive research, the chemical nature of the humic substances is still unclear, and the (bio)chemistry of the formation of these substances is one of the least understood aspects of humus chemistry and one of the most intriguing [3].

Over the years, different origins have been proposed for the formation of humus in soils. However, only two of them are mainly accepted: The old and most classical theory, proposed by Waksman in 1932 [4], states that humic substances are derived from lignin. The polyphenol theory, currently accepted, states that quinones of lignin origin, together with those synthesized by microorganisms polymerize in the presence of amino compounds to form humic macromolecules [3].

Lignin building blocks have indeed been isolated from humic substances after application of degradative methods such as sodium amalgam reduction [5] and copper(II) oxide oxidation [1].

Waksman [4] assumed that humic acids are modified lignins. However, it is well known that lignins in soils or as substrates in cultures of microorganisms suffer from extensive transformations such as demethoxylation and strong oxidation of the aliphatic side-chain [6]. Investigations have been carried out on the degradation of lignin by basidiomycetes and also on the enzymes involved in the process [7], and it is clear that some fungi, for instance *Phanerochaete chrysosporium*, degrade easily 60–70% of different types of woods [8].

In spite of the processes mentioned above for the origin of humic substances, these substances can be found in environments in which lignin is absent. Thus, humic acid is encountered in a sediment of a Greenland lake, where cyanobacteria are the main organisms [9]. Also, in cold areas, humus is formed from mosses, which do not contain lignin. Stevenson [3] has reported that in the Great Plains of the U.S.A., peat-like deposits of lichen origin have been described. Accordingly, in the light of current investigations it is difficult to accept the hypothesis of a humic matrix originating from lignin.

This paper deals with the investigation of humic acids and their chemically degraded counterparts in order to gain more information about the structure of the humic acid and to reveal what role lignin plays in the process of humification.

EXPERIMENTAL

Data on the soil sample used, the chemical characteristics of the humic acid isolated and the analytical pyrolysis technique have been reported previously [10]. Briefly, the samples were suspended in methanol. One

droplet of the suspension (10–20 μg of sample) was applied to a ferromagnetic wire with a Curie temperature of 610 °C. The temperature rise time was about 0.15 s and the wire was held at the end temperature for 10 s.

The pyrolysis–gas chromatographic–mass spectrometric (Py–GC–MS) analyses were carried out using a pyrolysis unit previously described [10]. The pyrolysis products were separated on a fused silica column (28 m × 0.5 mm I.D.) coated with CP-Sil 5 (1.25 μm film thickness) held at 0 °C for 5 min and subsequently programmed to 300 °C at a rate of 5 °C/min. Helium was used as the carrier gas at a rate of 1.6 ml/min. The chromatograph (Varian Model 3200) was coupled to a Varian-MAT 44 quadrupole mass spectrometer operated in the electron impact (EI) mode at 80 eV and with a cycle time of 2 s.

Humic acid was hydrolysed for 24 h with 6 *M* HCl in sealed tubes at 105 °C. Persulphate oxidation has been described elsewhere [11]. Briefly, 200 mg of sample were mixed with 50 ml of 5% potassium persulphate and heated for 2 h at 140 °C in a bomb. The bomb was cooled and the residual humic acid was washed several times with distilled water, dried and weighed.

The extraction of the humic acid fractions with hexane was repeated ten times (sonication for 5 min). The solvent was removed using a rotary evaporator and the residue was dissolved in ethyl acetate and chromatographed with a Carlo Erba Fractovap 4160 gas chromatograph equipped with a CP-Sil 5 fused-silica column (25 m × 0.5 mm I.D.), programmed from 130 to 330 °C at a rate of 4 °C/min.

RESULTS AND DISCUSSION

The pyrolysis of humic acid at a Curie temperature of 610 °C yielded a complex chromatogram (Fig. 1). An extensive description of the pyrolysis products encountered in different humic fractions, including this humic acid, has been given elsewhere [10]. Therefore, only a few significant pyrolysis/evaporation compounds were selected in order to characterize the changes in the humic acid after chemical degradation (Table 1). Some of the compounds included in the group "Others", although not significant enough to reveal structural changes, are interesting as they have not previously been reported in humic acids.

Fig. 1 indicates that polysaccharide, protein and lignin pyrolysis products and also evaporation products (mainly lipids) are present in the humic acid. Other identified compounds were series of alkylbenzenes (C_1–C_{10}), alkylnaphthalenes (C_1–C_4), fatty acids (C_{10}–C_{27}), fatty acid methyl esters (C_{12}–C_{30}) and a few aromatic hydrocarbons such as acenaphthene and several indenes. In addition, a homologous series of *n*-alkanes and *n*-alk-1-enes from C_5 to C_{35} was identified. Most of these compounds were

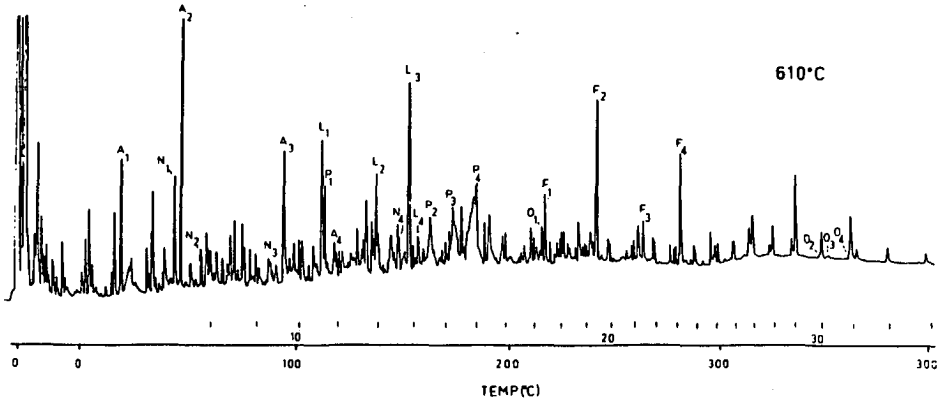

Fig. 1. Py–GC trace of humic acid. Curie temperature, 610 °C. For identification of peaks see Table 1. Lines and numbers underneath the trace represent positions of *n*-alkanes. The number corresponds to the carbon atoms.

reported previously in a pyrolysate of this humic acid obtained at a Curie temperature of 510 °C [10].

Most of the pyrolysis products obtained from the humic acid originate from well defined structures, such as polysaccharides and proteins, substances which can be removed by acid hydrolysis. To investigate further the structure of the resistant part of the humic acid, the humic acid was treated with 6 *M* HCl and subsequently extracted. About 60% of the humic acid (by weight) was removed by this acid treatment.

TABLE 1

A selection of characteristic pyrolysis products derived from humic acids

Polysaccharides	*Aromatics*
P_1 Levoglucosenone	A_1 Benzene
P_2 Levogalactosan	A_2 Toluene
P_3 Levomannosan	A_3 Phenol
P_4 Levoglucosan	A_4 *o*-Cresol
Lignins	*Fatty acids*
L_1 Guaiacol	F_1 C_{14}
L_2 Vinylphenol	F_2 C_{16}
L_3 Vinylguaiacol	F_3 C_{18}
L_4 2,6-Dimethoxyphenol	F_4 C_{20}
Proteins	*Others*
N_1 Pyrrole	O_1 Prist-1-ene
N_2 Pyridine	O_2 γ-Tocopherol
N_3 Benzonitrile	O_3 24-Ethylcholestadiene
N_4 Indole	O_4 α-Tocopherol

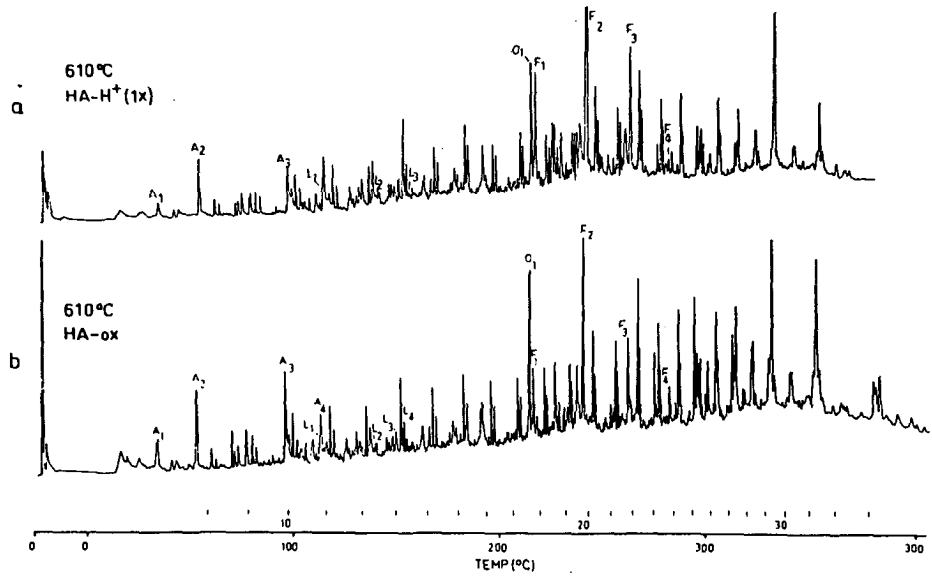

Fig. 2. (a) Py–GC trace of residual humic acid after 6 *M* HCl hydrolysis. Curie temperature, 610 °C. (b) Py–GC trace of residual humic acid after persulphate oxidation. Curie temperature, 610 °C.

The chromatogram of the pyrolysate of the residual humic acid obtained at a Curie temperature of 610 °C (Fig. 2a) displays a relatively simple picture, as the pyrolysis products from polysaccharides and proteins are no longer present and the lignin pyrolysis products are drastically reduced. As these biopolymers yielded low-molecular-weight structures on pyrolysis, their removal resulted in the absence of pyrolysis products at the beginning of the chromatogram. This chromatogram is dominated by homologous series of *n*-alkanes, *n*-alk-1-enes, α,ω-alkadienes and fatty acids. To assess the stability of the residue left after acid hydrolysis, two more acid treatments were performed. The weight loss was an additional 10% of the total weight, which means that 30% of the total humic acid is resistant to extensive acid hydrolysis. The chromatogram of the pyrolysate of the residual humic acid after the three subsequent hydrolyses is basically the same as that obtained after the first acid treatment, which suggests that the non-hydrolysable part of the humic acid is resistant and not prone to further degradation.

The untreated humic acid was also subjected to persulphate oxidation. This relatively mild oxidation appears to be less drastic than a permanganate oxidation [11]. The residue after persulphate oxidation amounted 25%. The chromatogram of the pyrolysate of the residual humic acid is shown in Fig. 2b. The trace shows basically the same compounds as those obtained for the acid-hydrolysed humic acid, indicating that the most

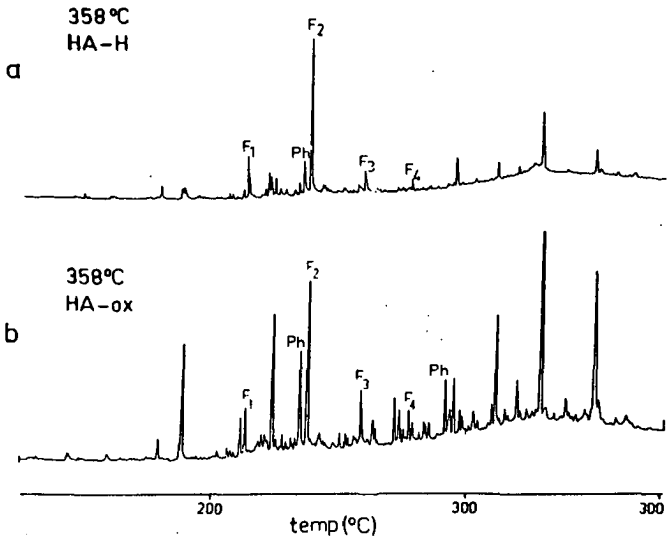

Fig. 3. (a) Py–GC trace of residual humic acid after 6 *M* HCl hydrolysis. Curie temperature, 358° C. (b) Py–GC trace of residual humic acid after persulphate oxidation. Curie temperature, 358° C. Ph denote dialkyl phthalates.

resistant parts of the humic acid after either acid hydrolysis or mild oxidation are similar. This stable part of the humic acid structure on pyrolysis yields mainly homologous series of aliphatic hydrocarbons. Similar results were obtained for two other types of humic acid from entirely different soils [12], indicating that these alkane/alkene series are common in pyrolysates of soil humic acids.

The relatively large amounts of C_{16} and C_{18} fatty acid and the C_{29} and C_{31} *n*-alkanes were thought to reflect adsorbed matter. To demonstrate this, the residual humic acid after acid hydrolysis and the residue after persulphate oxidation were analysed at a Curie temperature of 358° C. It has recently been reported that "pyrolysis" at this temperature may be an alternative to solvent extraction of rock samples [13]. Here, we selected this Curie temperature mainly to evaporate the volatile components present in the humic acid.

Fig. 3a and b show the chromatograms of both residual humic acids. It is apparent that the two traces are similar qualitatively. The evaporate is composed of fatty acids, *n*-alkanes and a few dialkylphthalates, compounds obviously adsorbed to the humic acid. Therefore, the residual humic acids were exhaustively extracted with hexane. The chromatogram of the hexane extract of the persulphate-oxidized humic acid is shown in Fig. 4. The chromatogram obtained from the acid-hydrolysed humic acid was similar. Fatty acids up to C_{32}, *n*-alkanes up to C_{35} and three dialkyl phthalates were identified.

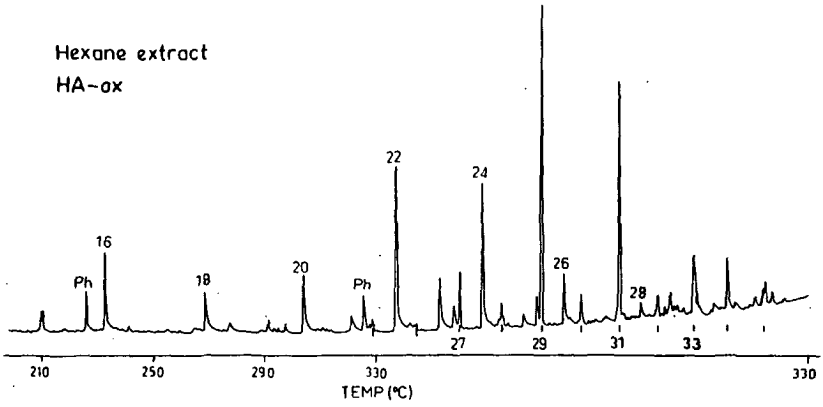

Fig. 4. GC trace of the hexane extract of residual humic acid after persulphate oxidation.

Fig. 5a and b show chromatograms of pyrolysates of the hexane-extracted residual humic acids. They were subjected to pyrolysis at a Curie temperature of 770 °C, so that they could be compared with chromatograms reported in the literature for a cuticle biopolymer [14].

The chromatograms of these extracted residual humic acids are greatly dominated by the homologous series of aliphatic hydrocarbons. The fatty

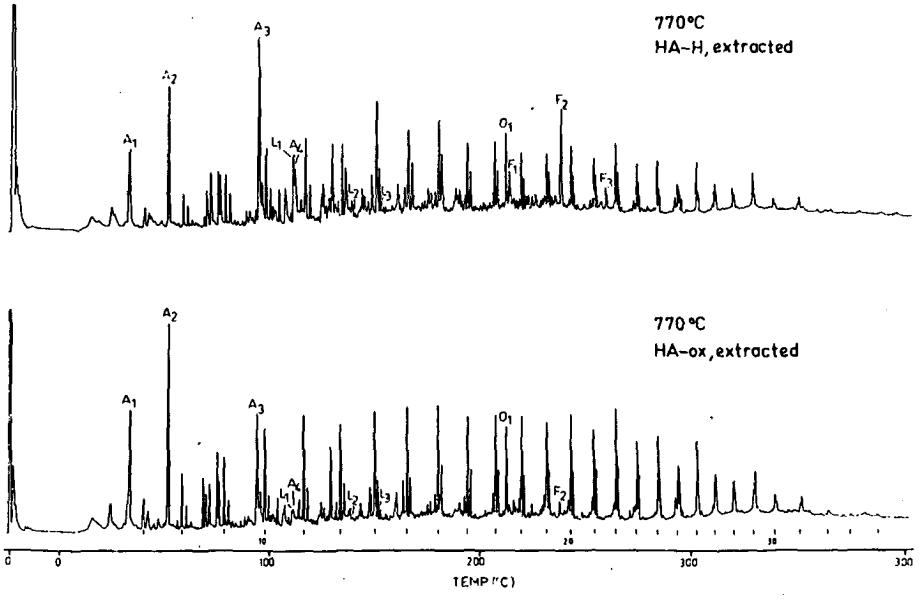

Fig. 5. (a) Py–GC trace of residual humic acid after 6 *M* HCl hydrolysis and hexane extraction (ten times). Curie temperature, 770 °C. (b) Py–GC trace of residual humic acid after persulphate oxidation and hexane extraction (ten times). Curie temperature, 770 °C.

TABLE 2

Main classes of pyrolysis or evaporation products identified from acid-hydrolysed or persulphate-oxidized humic acids

Classes of compounds	Range/compound	Abundance
Alkanes	C_1-C_{35}	+ + +
Alk-1-enes	C_2-C_{35}	+ + +
α,ω-Alkadienes	$C_{14}-C_{28}$	+
Acyclic isoprenoids	Prist-1-ene	+ + +
	Prist-2-ene	+ +
	Phytadiene	+
Phenols	Phenol	+ + +
	Cresols	+ +
	Vinylphenol	+
Alkylbenzenes	C_1-C_{10}	+
	Benzene and toluene are abundant compounds (+ + +)	
Alkylnaphthalenes	C_1-C_4	+
PAHs	(Methyl)indenes	+
	Acenaphthene	+
	(Methyl)fluorenes	+
	(Methyl)anthracenes	+
	Fluoranthene	+
Lignins	(Alkyl)guaiacols	+
	2,6-Dimethoxyphenol	+
Fatty acids	$C_{10}-C_{28}$	+
Dialkyl phthalates	C_1-C_4	+

TABLE 3

Plant components identified in soil humic substances [10,12,15]

Components	Origin	Presence
α- and γ-Tocopherol	Plants, algae	Pyrolysate of humic acid
Pristenes	Pyrolysis products of tocopherols	Pyrolysates of humic acid, humin and hymatomelanic acid
Phytadienes	Pyrolysis products of chlorophylls	Pyrolysates of humic acid
24-Ethylcholestadiene	Plants	Pyrolysate of humic acid
Terpenoids	Plants	Pyrolysates of humin and hymatomelanic acids
Lignin	Plants	Pyrolysates of all humic fractions
New biopolymer from plant cuticles and suberin	Plants	Pyrolysates of humic acid, fulvic acid and humin

acids and the C_{29} and C_{31} *n*-alkanes are reduced in comparison with the unextracted samples. The lignin pyrolysis products are very minor compounds. Table 2 indicates the nature of the pyrolysis products obtained for the extracted residual humic acids and their relative abundances.

Because of the striking resemblance between the extracted residual humic acids and the biopolymer encountered in plant cuticles, it is concluded that a large part of the most resistant (non-hydrolysed by acid, non-oxidized by persulphate) humic acid is made up of aliphatic components, probably representing the "polyethylene" moiety of a biopolymer, which has been isolated from several recent plant cuticles [14].

This highly aliphatic biopolymer has also been noted in suberin fractions of plants. In this and other studies [10] many plant components other than lignin have been identified by analytical pyrolysis in humic acids (Table 3), thus emphasizing the importance of these other plant components in the formation of humic acid. A possible origin of the most resistant part of humic acids from plant cuticles and suberin seems likely.

CONCLUSIONS

(1) Humic acids may be formed by different chemical and biochemical processes, in which many high- and low-molecular-weight plant components are involved.

(2) The role of the lignin in the formation of humic substances seems to be overestimated. Lignin and degraded lignins may be coextracted with humic acids or may be linked to humic acid substances, but they do not contribute significantly to the resistant part of the humic acids.

(3) Other more resistant plant components, such as the highly aliphatic biopolymer present in plant cuticles and suberin, could represent important moieties of the humic acid structure.

ACKNOWLEDGEMENTS

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C H A P T E R 9

Sludge from waste water of the olive processing industry: A potential soil fertilizer?

SLUDGE FROM THE WASTE WATER OF THE OLIVE PROCESSING INDUSTRY: A POTENTIAL SOIL FERTILIZER?

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SUMMARY

The humic acid fraction of sludge obtained from waste water of olive mills after disposal in isolated lagoons was chemically characterized by amino acid and sugar analyses and by flash pyrolysis-GC-MS. The sludge humic acid fraction consists of polysaccharides, proteins, lignins and relatively high amounts of C_{16} and C_{18} fatty acids. Although the composition of this material is different from soil humic acids it is concluded that the sludge has good soil fertilizer properties.

INTRODUCTION

The large amount of waste water (1-2 million cubic meters per season) generated during the production of olive oil is a major environmental problem in Spain. This waste water is mainly composed of particulate matter from olive pulp, sugars, pectins, tannins, mucilages, organic acids and polyphenols (ref. 1). Because of the extremely high BOD these waste water pollute the surface water seriously when spilled. Over the last five years, ponds and lagoons have been built for disposal of the waste water from which the water evaporates during the dry spring-summer period. The residual sludges are recovered in fall, before a new olive processing season starts. Some farmer used to irrigate olive tree orchards with this type of waste water to fertilize the soil. Therefore, it is of interest to study in detail the chemical composition of the sludge to evaluate its potential value as soil fertilizer. This preliminary study was focussed on the chemical characterization of the base-extractable and acid-precipitable humic acid fraction, because it is well-known that, at least in soils, humic acids are the most important substances related to fertility properties.

METHODS

The sludge sample was obtained from a lagoon located in Villanueva del Trabuco (province of Malaga, Spain). The humic acid fraction was prepared in the

usual way by solution in 0.5 N NaOH and subsequent precipitation with HCl (ref. 2). To investigate the possible changes in the humic acid fraction during the period of disposal as a consequence of microbial fermentations and transformations, a "fresh" waste water sample was also investigated. This sample was obtained from an experimental olive mill (Instituto de la Grasa, C.S.I.C., Sevilla). The waste water sample was taken immediately after formation and stored at -15°C until analysis. The humic acid fraction was obtained after centrifugation at 15.000 g, addition of 0.5 N NaOH to the solution and precipitation with HCl. The results of the sludge and the waste water humic acids can be compared since the olive oil production processes at both locations are very similar.

Elemental composition, sugar and amino acid analyses and pyrolysis technique have been described in detail elsewhere (ref. 2, 3).

RESULTS AND DISCUSSION

The sludge contained 40 % of humic acid and 28 % of acid soluble materials on a dry weight basis. Table 1 shows the elemental composition of the sludge (S-HA) and the waste water (W-HA) humic acids, before and after 6 N HCl hydrolysis.

TABLE 1

Elemental composition of sludge and waste water humic acid fractions (on a moisture- and ash-free basis)

%	W-HA	W-HA-H[1]	S-HA	S-HA-H[1]
C	62.5	66.4	61.3	61.2
H	8.5	7.9	7.0	7.0
N	3.4	1.2	2.6	1.9
S	0.3	0.3	0.3	n.d.
O	25.3	24.2	28.8	29.9
ash	10.8	0	8.0	0
weight loss after hydrolysis	46.9	-	27.8	-
N loss after hydrolysis	64.7	-	27.0	-
atomic H/C ratio	1.6	1.4	1.4	1.4

[1]Acid hydrolysed samples

Hydrolysis was carried out to release polysaccharides, proteins, phenols and metals. In this way it was thought that the so-called core of the humic acid would remain, making a specific core investigation possible (ref. 4).

The elemental compositions of the W-HA and S-HA fractions were slightly different from those of soil humic acids (C and H are higher and O is lower) (ref. 5). As indicated by the weight loss and N loss the S-HA sample was much more resistant to hydrolysis than the W-HA sample, indicating that easily hydro-

lysable substances are probably metabolized for the greater part by microbial attack during the disposal in the lagoon. The lower N-content of the S-HA sample compared to that of the W-HA sample also indicates biodegradation processes.

TABLE 2

Sugar distribution in 2 N H_2SO_4 hydrolysates of sludge and waste water humic acid fractions (mg/100 mg of sample)

sugar	W-HA	S-HA
glyceraldehyde	-	6.8
treose	-	0.3
rhamnose	1.6	1.3
ribose	0.2	0.3
arabinose	0.4	0.3
xylose	0.2	0.1
mannose	0.4	0.4
galactose	0.1	trace
glucose	2.8	2.0

The composition of the polysaccharides was investigated after hydrolysis with 2 N H_2SO_4. Comparison of the S-HA and W-HA monosaccharides indicates that the pentose and hexose contents were slightly higher in W-HA. In the S-HA hydrolysate glyceraldehyde and treose were also present. The relatively high amount of rhamnose was not surprising in view of the abundant occurrence of this sugar as glucoside in olive anthocyanins (ref. 1).

TABLE 3

Amino acids distribution in hydrolysates of sludge and waste water humic acid fractions (mg/100 mg sample)

amino acid	W-HA	S-HA
aspartic acid	1.43	0.80
threonine	0.65	0.26
serine	0.76	0.39
glutamic acid	1.81	0.98
proline	0.97	0.77
glycine	0.70	0.43
alanine	0.68	0.39
valine	0.79	0.36
methionine	0.10	0.03
isoleucine	0.46	0.24
leucine	0.96	0.46
tyrosine	0.56	0.29
phenylalanine	0.72	0.27
glucosamine	0.15	0.08
histidine	0.32	0.13
lysine	0.12	0.04
arginine	0.74	0.27
NH_4^+	0.26	0.13

The amino acid distribution analysed after 6 N HCl hydrolysis was similar to that reported for pulp olives as far as the most abundant components are concerned (ref. 2). The W-HA fraction yielded about twice as much amino acids compared to S-HA fraction. The compositions of W-HA and S-HA were somewhat different: proline was relatively more abundant in W-HA and phenylalanine and arginine were relatively increased in S-HA.

Apart from the amino acids, three phenols were released after 6 N HCl hydrolysis. They were identified by HPLC and TLC as protocatechuic acid, caffeic acid and p-coumaric acid, protocatechuic acid being the most abundant. These compounds have previously been found as free phenols in the waste water (ref.1).

Curie point evaporation/pyrolysis-gas chromatography-mass spectrometric analysis of the humic acid fractions was applied to characterize other components than proteins and sugars. A complex mixture of evaporated components and pyrolysis products from high molecular weight substances resulted. The identifications of the major products are listed in Table 4. The peak numbers in Figure 1 correspond to the numbers indicated in Table 4.

TABLE 4

Pyrolysis/evaporation products of sludge and waste water humic acid fractions

1 Carbon monoxide	20 Pyrrol
Carbon dioxide	21 Toluene
Methane	22 Dihydropyran
Ethene	23 Oct-1-ene
Ethane	Ethylpyrrol
Propene	24 Maleic anhydride
Propane	25 Octane
But-1-ene	3-Furaldehyde
Butane	26 2-Furaldehyde
2 Acetone	Benzyl alcohol
3 Pent-1-ene	27 Ethylbenzene
4 Ethanethiol	28 Methylpyridine
5 2-Methylpropanal	3-Methylcyclopent-2-en-1-one
6 Butan-2,3-dione	29 m- and/or p-Xylene
7 2-Methylpentane	30 Styrene
8 2-Methylfuran	31 o-Xylene
9 3-Methylfuran	32 C_3-alkylfuran
10 Crotonaldehyde	33 α-Angelicalactone
11 Hexadiene	34 Non-1-ene
Hexatriene	35 Nonane
12 Hexatriene	36 Ethylpyridine
3-Methylbutanal	37 Benzaldehyde
13 2-Methylbutanal	38 Dimethylpyridine
Benzene	39 Vinylpyridine
Acetic acid	40 4-hydroxy-5,6-dihydro-2H-pyran-2-one
14 Hept-1-ene	41 Dimethylpyridine
15 Heptane	42 Phenol
16 2,5-Dimethylfuran	43 Methylethylpyridine
17 2,4-Dimethylfuran	44 2-Hydroxy-3-methyl-2-cyclopenten-1-one
18 N-Methylpyrrol	45 4-Hydroxy-6-methyl-5,6-dihydro-2H-pyran-2-one
19 Pyridine	

46 Indene
Limonene
47 Methylvinylpyridine
48 p-Cresol
Tolualdehyde
49 C_4-alkylbenzene
50 3-Hydroxy-6-methyl-3,4-dihydro-2H-pyran-2-one
51 Methylvinylpyridine
52 Guaiacol
Methylethylpyridine
53 o-Cresol
54 Benzyl cyanide
55 C_3-alkylhydroxypyridine
56 Undec-1-ene
57 Undecane
58 Ethylphenol
59 Xylenol
60 Methylguaiacol
61 1,4:3,6-Dianhydro-α-D-glucopyranose
62 Catechol
63 Vinylphenol
64 Dodec-1-ene
65 C_6-Alkylbenzene
66 Ethylguaiacol
67 Indole
68 Vinylguaiacol
69 Methylcatechol
70 Levogalactosane
71 2,6-Dimethoxyphenol
72 Eugenol
73 Methylindole
74 Methylquinoline
75 Ethylcatechol
76 Levomannosane
77 Methyl-2,6-dimethoxyphenol
78 Levogluconase
79 trans-Isoeugenol
80 Vanillic acid
81 Ethylquinoline
82 Pentadec-1-ene
83 Pentadecane
84 Vinyl-2,6-dimethoxyphenol
85 $C_{14:0}$ fatty acid
86 Dialkyl phthalate
87 trans-Propenyl-2,6-dimethoxyphenol
88 Heptadecadiene
89 Heptadec-2-ene
90 Heptadec-1-ene
91 Heptadecane
92 Octadec-1-ene
93 $C_{16:1}$ fatty acid methyl ester
94 Dialkyl phthalate
95 $C_{16:0}$ fatty acid methyl ester
96 Dialkyl phthalate
97 $C_{16:0}$ fatty acid
98 $C_{16:0}$ fatty acid ethyl ester
99 Eicosane
100 $C_{18:2}$ fatty acid methyl ester
101 $C_{18:1}$ fatty acid methyl ester
102 $C_{18:0}$ fatty acid methyl ester
103 $C_{18:2}$ fatty acid ethyl ester
104 $C_{18:1}$ fatty acid
$C_{18:1}$ fatty acid ethyl ester
$C_{18:0}$ fatty acid
105 $C_{18:0}$ fatty acid ethyl ester
106 $C_{18:1}$ fatty acid propyl ester
107 $C_{18:1}$ fatty acid butyl ester
108 Dialkyl phthalate

The chromatograms of both humic acids were relatively similar, although distinct qualitative and quantitative differences were observed. The major compounds were free and esterified n-C_{16} and n-C_{18} fatty acids. It can be assumed that these acids are present as such in the samples and just evaporate since they are the major lipids present in olive oil. These fatty acids and their esters probably adsorb to particulate matter due to incomplete removal of the oil during the production process. The occurrence of fatty acid methyl esters is in agreement with the findings of such esters in the oil extracted from microbial degraded olives (ref. 6) and indicates a biological activity of microorganisms during the olive storage and/or disposal in the lagoon. The presence of ethyl-, propyl-, and butyl-C_{18} esters is noteworthy since these esters have not yet been reported in microbially altered olives.

Interestingly, $C_{18:2}$ fatty acids were only present in the W-HA sample, which means that these acids are removed after disposal in the lagoon, either biologically or photochemically, since an oily layer was present at the water-air interface.

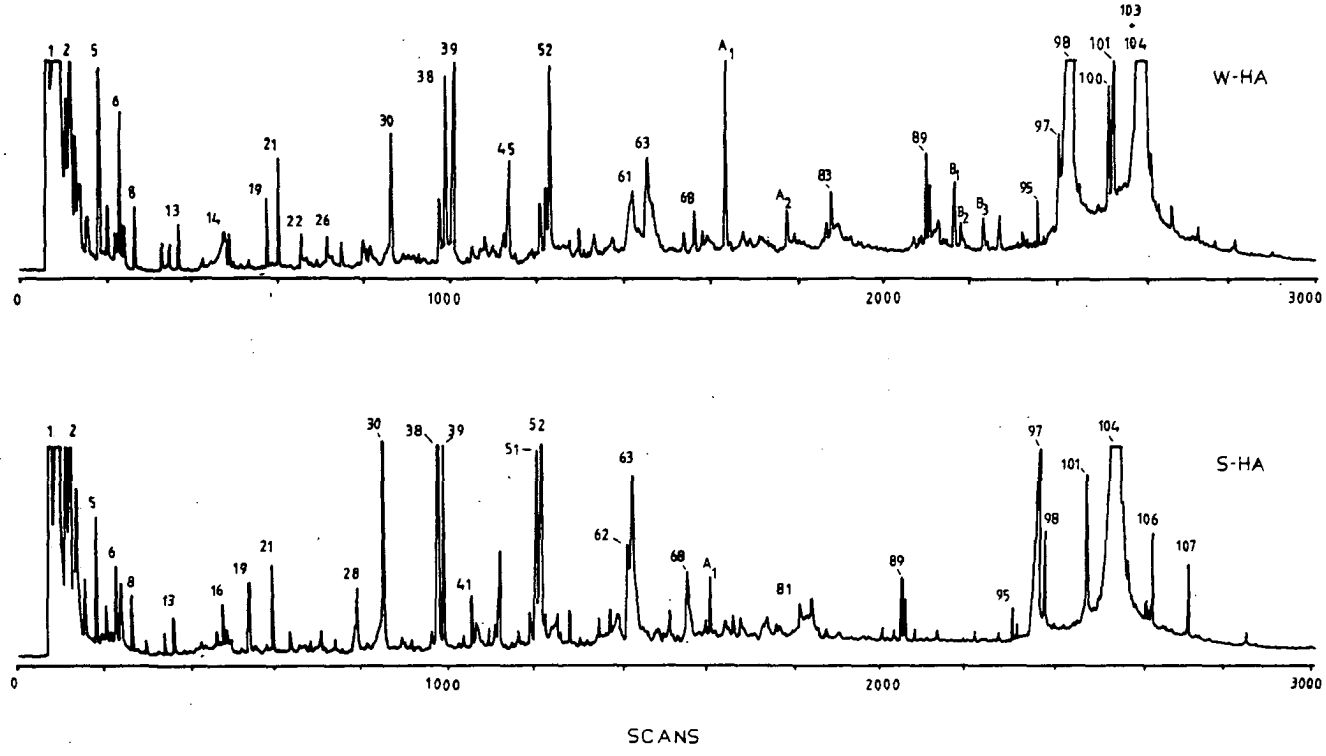

Figure 1. TIC traces of waste water (W-HA) and sludge (S-HA) humic acid fractions. Peak numbers refer to the compounds listed in Table 4. Compounds A_1 and A_2 are unidentified nitrogen-containing products. Compounds B_1-B_3 are probably cyclic sesquiterpenoids.

All three types of lignin unit pyrolysis products were present and they were relatively more abundant in the S-HA sample. This suggests that lignin, which is reported to be resistant to biodegradation in anaerobic environments (ref. 7), accumulate in the lagoon. Although typical pyrolysis products from the p-coumaryl lignin unit such as phenol and vinylphenol were present, an origin of these phenolic compounds from other substances present in the waste water can not be precluded. Catechol might originate from protocatechuic acid, which was present in the hydrolysates after HCl treatment. It is known that the carboxyl group in these type of molecules is easily removed upon pyrolysis (ref. 8).

Sugar pyrolysis products were somewhat more abundant in the pyrolysate of the W-HA sample. Relatively high amounts of pyridine and alkylpyridines were encountered in both the S-HA and W-HA samples. These compounds are only present in minor amounts in pyrolysates of soil humic acids. Although one might speculate about a protein origin of the alkylpyridine-type pyrolysis compounds they have not yet been reported in pyrolysis studies of proteins (ref. 9).

By comparing S-HA and W-HA with soil humic acids the differences are evident. Although both types of materials contain polysaccharides, proteins, lignins and lipids, their relative amounts differ. Thus, the C_{16} and C_{18} fatty acid were very high in S-HA and W-HA and the abundantly present alkylpyridines are virtually absent in pyrolysates of soil humic acids (ref. 3).

In spite of these differences the sludge can be considered as a good fertilizer on a chemical basis. Proteins and sugars are still present in the sludge and the C_{16} and C_{18} fatty acids are high quality substrates for microorganisms and plants. On the other hand, the high concentrations of the fatty acids might cause some problems since they give the sludge a hydrophobic nature.

Two additional aspects are important in the utilization of the sludge. From an environmental point of view, the disposal of waste water in isolated lagoons protects the surface waters againts pollution. The second aspect is of economical nature. The evaporation of water results in a more concentrated product which has not lost much of its nutrient value and it can be distributed much cheaper. It is further demonstrated that the olive processing industry is losing relatively high amounts of valuable oil. A more efficient removal of C_{16} and C_{18} fatty acids before waste water disposal would improve both the economy of the olive oil industry and the physico-chemical properties of the soil to which the sludge has been added.

Long-term experiments are required to further test the sludge as a soil fertilizer.

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C H A P T E R 10

Evidence of lignin residues in humic acids isolated from vermicomposts.

EVIDENCE OF LIGNIN RESIDUES IN HUMIC ACIDS ISOLATED FROM VERMICOMPOSTS

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ABSTRACT

Three vermicomposts derived from animal manures exposed to the action of the earthworms Eisenia foetida or Lumbricus rubellus were investigated to determine the nature of the humic acid fraction in relation to the potential fertility of the vermicomposts. The isolated humic acid fractions were analysed by pyrolysis-gas chromatography-mass spectrometry. The greater part of the humic acids consisted of lignin and/or lignin residues as revealed by the very prominent and characteristic methoxy- and dimethoxy-phenols pyrolysis products. The chromatograms of the pyrolysates are similar to those of grass lignin. This points to an incomplete degradation or selective preservation of grass lignin during earthworm composting. The results further indicate that neither the quality nor the quantity of the humic acids has changed considerably by vermicomposting.

INTRODUCTION

Vermicomposting is the biological transformation of organic matter present in agricultural, urban and industrial wastes mediated by earthworms feeding on these wastes. This process is supposed to generate a useful material and to ameliorate the severe problems associated with the disposal of large quantities of organic wastes.

Earthworms play an important role in processing organic matter in nature. Darwin (1881) already pointed out that earthworms are essential components of soils because they macerate litter materials thus mixing it with the soil. This phenomenon can be observed by inspection of worm casts which contain more organic matter and more of the smaller particle size fractions than the surrounding soil.

The interest in vermicomposting has increased over the last years due to a shortage of natural organic fertilizers and the

necessity of organic amendment in most of Southern Europe's soils which have a very low carbon content due to climatic conditions and extensive and milenary culturing.

Vermicomposts contain up to 17 per cent alkali-extractable, acid precipitable humic acids (Hervas et al., unpublished data). This paper deals with the chemical characterization of vermicompost humic acids applying analytical pyrolysis. This analytical approach provides data on structural moieties in macromolecular materials and avoids problems encountered in wet chemical degradation methods due to lengthy and tedious procedures.

MATERIALS AND METHODS

Three different types of vermicomposts, commercially available in Spain, were studied. Animal manures, the parent materials, were composted for at least three months with the earthworms Eisenia foetida or Lumbricus rubellus.

The vermicomposts are darkly coloured, show a well decomposed state and a fine texture and are not polluted with metals, plastics, fabrics or glass.

The humic acids were extracted with a cold solution of 0.1 M $Na_4P_2O_7$ and NaOH (1:1) under N_2 (Saiz-Jimenez et al., 1979). The elemental compositions and amounts of humic acids obtained are reported in Table 1.

Grass lignin (Bambusa ssp.) was kindly supplied by Dr. O. Faix, Hamburg, F.R. Germany.

One hundred mg of humic acid of sample B (Table 1) was extracted 5 times with 10 ml acetone-water (9:1 v/v) by sonication for 10 min. The extract after evaporation of the solvent weighed 38 mg. The humic acid was subsequently extracted with 10 ml dichloromethane-methanol (2:1) and another 11 mg of extract was thus obtained. The residual amount of humic acid accounted for 51 mg.

Pyrolysis-gas chromatography was performed with a Curie point pyrolysis unit described by van der Meent et al. (1980). The pyrolysis units were mounted in the injection blocks of a Packard Becker 419 gas chromatograph and in a Varian 3700 gas chromatograph of a pyrolysis-gas chromatorgraphy-mass spectrometry system. The pyrolysates were separated on a 25 m. fused silica column (i.d. 0.26 mm) coated with DB-1701 (film thickness 0.25 µm). The GC oven was programmed from 30°C to 290°C at a rate of 3°C/min. Gas chromatography-mass spectrometry was performed with a MAT 44 quadrupole mass spectrometer operated in the EI mode at 80 eV and with a cycle time of 2 sec.

RESULTS AND DISCUSSION

Table 1 shows the yields and elemental composition of the three humic acids isolated from different vermicomposts. The elemental compositions are almost identical and they are similar to those reported for humic acids extracted from soils from widely different climatic zones (Schnitzer, 1977). From these data it is, however, impossible to detect structural similarities and dissimilarities between vermicomposts and soil humic acids.

Over the last years analytical pyrolysis had been demonstrated to be a suitable method for the chemical characterization of high molecular weight substances, providing detailed information of the structural moieties present in the macromolecular assemblages (Nip et al., 1986; Saiz-Jimenez and de Leeuw, 1987 c). Since data of investigations concerning soil humic acids characterization using this method have been reported (Saiz-Jimenez and de Leeuw, 1986 a, 1987 b) a comparison with pyrolysis data of vermicompost humic acids is possible.

Figure 1 shows the chromatogram of the pyrolysis products of humic acid from vermicompost B. Peak numbers refer to the compounds identified and listed in Table 2. All three humic acids generated the same pyrolysis products with no significant quantitative differences. The major pyrolysis products produced

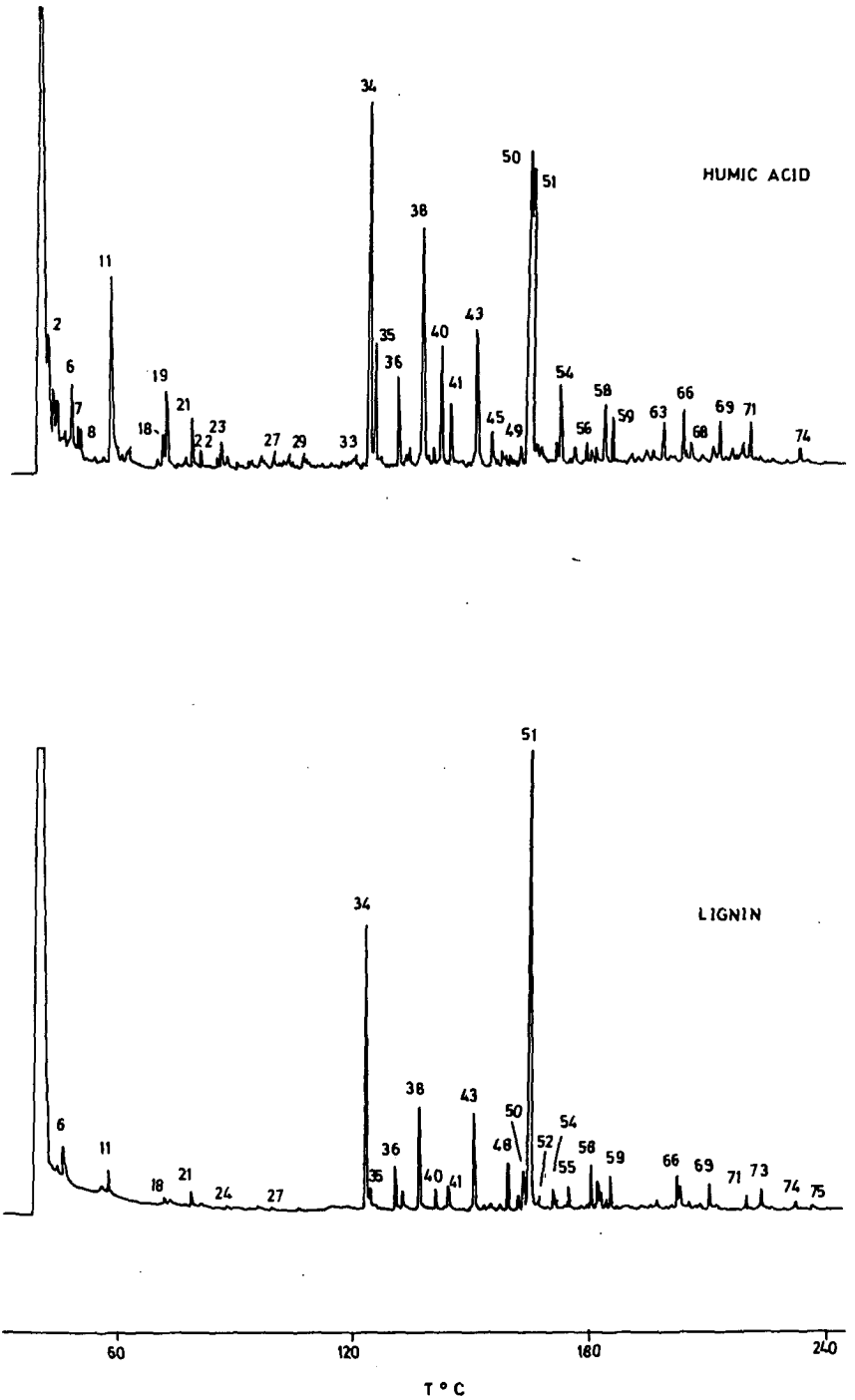

Figure 1. Pyrolysis gas chromatography trace of humic acid from vermicompost B.

Figure 2. Pyrolysis gas chromatography trace of grass lignin (Bambusa ssp.)

from these humic acids are highly characteristic of lignins, especially grass lignin (Saiz-Jimenez and de Leeuw, 1986 b). The compounds identified in the pyrolysates of this grass lignin are the same as those in the pyrolysates of the humic acids, phenol (34) and vinylphenol (51) being predominant. This was further substantiated by analysis of Bambusa grass lignin pyrolysed under exactly the same conditions (Figure 2). The major compounds identified in the pyrolysate of this lignin are indeed dominantly present in the pyrolysates of the vermicompost humic acids and the distribution patterns of the pyrolysis products show a high degree of similarity.

The somewhat more abundant presence of quaiacol (35), methylguaiacol (40), vinylguaiacol (50) and 2,6-dimethoxyphenol (54) in the pyrolysates of the humic acids when compared with the relative intensities of these compounds in the grass lignin pyrolysate can be explained easily. Grass lignin contains p-hydroxyphenyl units esterified with guaiacyl-syringyl lignin (Higuchi et al., 1967). It is expected that a significant amount of these bonds are cleaved during saponification with cold alkali (Sarkanen and Hergert, 1979) during the extraction and purification process of the humic acids. As a result the p-hydroxyphenyl units may end up in other fractions and the relative amount of p-hydroxyphenyl pyrolysis products is thus reduced in comparison with guaiacyl and syringyl pyrolysis products. Alternatively, partial hydrolysis of the ester bonds might have taken place already in the digestive tracts of the ruminants.

In addition to the lignin pyrolysis products several nitrogen-containing compounds were identified in low amounts. They are typical protein/peptide pyrolysis products. Also a few furan derivatives, 3-methylpyrocatechol and prist-1-ene were present. Furans are common in pyrolysates of carbohydrates and prist-1-ene in probably a pyrolysis product of tocopherols (Goossens et al., 1984). 3-Methylpyrolcatechol has recently been identified as a major pyrolysis product of a fungal melanin (Saiz-Jimenez and de Leeuw, unpublished data). All these

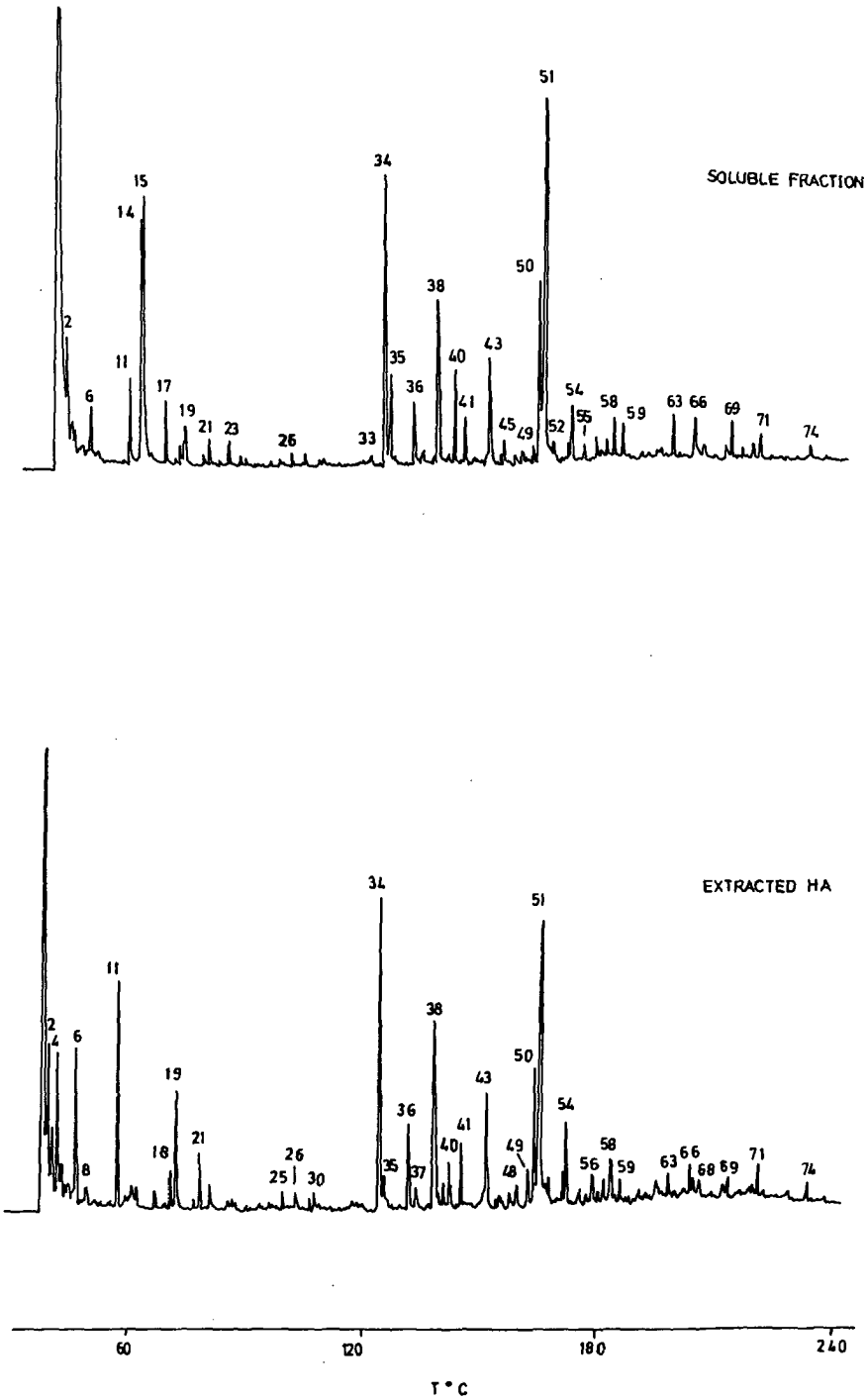

Figure 3. Pyrolysis gas chromatography trace of acetone-water soluble fraction.

Figure 4. Pyrolysis gas chromatography trace of solvent extracted humic acid.

pyrolysis products point to the presence of minor amounts of proteins, carbohydrates, tocopherols and some microbially synthesized phenols.

To further substantiate the dominant presence of lignins in these humic acid fractions, the vermicompost humic acid isolated from sample B was extracted with acetone-water, a common solvent for lignins (Freudenberg, 1968). The chromatogram of the pyrolysis products of this acetone-water extract is shown in Figure 3. Figure 4 represents the residual humic acid after acetone-water and dichloromethane-methanol extraction. The chromatogram of the pyrolysis products of the dichloromethane-methanol extract (not shown) was similar to that of the acetone-water extract. In summary, the four traces are similar to one another. This probably means that the so-called humic acids from vermicomposts mainly consist of slightly altered grass lignin with only secondary contribution of other origin.

The origin of this lignin seems to be clear. The dietary ingestion of ruminants is based on grasses and other herbaceous plants. They contain lignocellulosic complexes which are not or virtually not metabolized either by the animal or by the microbial gut flora. The predominant bacterial strains in the gut flora of earthworms are facultative anaerobes, from which 73 per cent belong to the genus Vibrio and none of the isolated strains were able to degrade lignin as demonstrated by Marialigeti (1979). Neuhauser et al. (1978) stated that neither Eisenia foetida nor its faecal bacteria are capable to depolymerize lignin and degrade its aromatic constituents. The influence of other bacteria present during the vermicomposting process seems to have been limited as well with respect to lignin degradation.

The vermicompost humic acids cannot be compared with those of soil humic acids previously studied (Saiz-Jimenez and de Leeuw, 1986 a; 1987 a, b). The differences are obvious; upon pyrolysis soil humic acids generate a wide range of components related to polysaccharides, proteins, lignins, and an aliphatic biopolymer, as well as alkylbenzenes, alkylnaphthalenes, polycyclic aromatic hydrocarbons and lipids. The main pyrolysis products generated

from vermicompost humic acids are almost exclusively derived from lignins as proved above.

From the results presented here it is clear that the potential fertilizing capacity of vermicomposts cannot be predicted by simple determinations such as weight percentages of humic acids. This is caused by the fact that the humic acids are operationally defined in terms of acid-base solubility and not by chemical structural criteria. Therefore, the prediction of the nature and properties of humic acids can only be based on more detailed structural analyses such as pyrolysis in combination with gas chromatography and gas chromatography-mass spectrometry, ^{13}C NMR, FT-IR and, in some cases, by data obtained from chemical degradations.

TABLE 1

Elemental composition of vermicompost humic acids.

sample	origin	yield	C	H	N	S	O	ash
		%						
A	mixed manures	6.0	52.6	5.3	4.7	1.5	35.9	2.7
B	cow manure	17.2	54.3	5.5	4.3	1.3	34.6	2.6
C	animal manure	5.0	55.6	5.6	4.2	1.3	33.3	5.7

TABLE 2

Pyrolysis products of vermicompost humic acids

1	Butyne
1	Butene
1	Methanethiol
2	Furan
3	Acrylonitrile
4	Methylfuran
5	Hexadiene
6	Benzene
7	Ethylfuran
8	2,5-Dimethylfuran
9	Dimethylfuran
10	C_7H_{10}
11	Toluene
12	Methylthiophene
13	Pyrrole
14	Furfurylalcohol
15	C_3-Alkylfuran
16	Pyridine
17	Benzenemethanol
18	Ethylbenzene
19	o-Xylene
20	p-Xylene
21	Styrene
22	C_3-Alkylbenzene
23	C_3-Alkylbenzene
24	C_3-Alkylbenzene
25	C_3-Alkylbenzene
26	$C_{3:1}$-Alkylbenzene
27	C_3-Alkylbenzene
28	C_3-Alkylbenzene
29	Indene
30	Benzonitrile
31	$C_{4:1}$-Alkylbenzene
32	$C_{4:1}$-Alkylbenzene
33	$C_{4:1}$-Alkylbenzene
34	Phenol
35	Guaiacol
36	o-Cresol
37	Naphthalene
38	p-Cresol
39	Benzeneacetonitrile
40	Methylguaiacol
41	o-Xylenol
42	Methylnaphthalene
43	p-Xylenol
44	Methylnaphthalene
45	Ethylguaiacol
46	C_3-Alkylphenol
47	C_3-Alkylphenol
48	C_3-Alkylphenol
49	C_3-Alkylphenol
50	Vinylguaiacol
51	Vinylphenol
52	Eugenol
53	Dimethylnaphthalene
54	2,6-Dimethoxyphenol + Indole
55	cis-Isoeugenol
56	3-Methylpyrolcatechol
57	Terephthalaldehyde
58	trans-Isoeugenol Methylindole
59	Methyl-2,6-dimethoxyphenol
60	Vanillin
61	C_3-Alkylnaphtalene
62	Ethyl-2,6-dimethoxyphenol
63	Prist-1-ene
64	Homovanillin
65	Acetovanillin
66	Vinyl-2,6-dimethoxyphenol
67	Guaiacylpropanone
68	Allyl-2,6-dimethoxyphenol
69	cis-Propene-2,6-dimethoxyphenol
70	Coniferaldehyde
71	trans-Propene-2,6-dimethoxyphenol
72	Biphenol
73	Syringaldehyde
74	Ethanone-2,6-dimetoxyphenol
75	Propanone-2,6-dimethoxyphenol

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