

# DIVISION S-3—SOIL MICROBIOLOGY AND BIOCHEMISTRY

## Pyrolysis-Gas Chromatography-Mass Spectrometry of Soil Humic Fractions: II. The High Boiling Point Compounds<sup>1</sup>

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### ABSTRACT

Pyrolysis-gas chromatography-mass spectrometry studies of soil fulvic and humic acids were made. The high boiling point compounds produced by pyrolysis were separated in a column packed with Chromosorb AW DMCS 80-100 mesh coated with 10% FFAP. Humic acids with a high nitrogen content yielded a complex variety of protein derivatives, such as alkylpyridines, alkylpyrroles, alkylbenzotriazoles, indoles, piperidines, pyrazines, and pyrrolidones. Humic acids with low N content yielded a smaller number of heterocyclic nitrogen compounds. Lignin derivatives were also identified, but the amount varied in different samples. Furanes were not as prominent as protein and lignin fragments. Acid hydrolysis released proteins, polysaccharides, and lignins, which could be considered as companion materials of a humic "core". The majority of the pyrolysis compounds from the residue after hydrolysis were identified as alkylbenzenes, alkylnaphthalenes, phenols, benzofuranes, indenes, and fluorenes. Alkanes and alkenes were noted in trace amounts. Pyrolysis behavior of fulvic acids differed from that of humic acids. They produced poorly resolved pyrograms with smaller number and a lower intensity of peaks, from which only furfurals, phenols, benzofuranes, and naphthalenes could be identified.

*Additional Index Words:* lignins, proteins, soil polysaccharides, aliphatic hydrocarbons, aromatic hydrocarbons, heterocyclic nitrogen compounds, pyrolysis products.

Martin, F., C. Saiz-Jimenez, and A. Cert. 1979. Pyrolysis-gas chromatography-mass spectrometry of soil humic fractions: II. The high boiling point compounds. *Soil Sci. Soc. Am. J.* 43: 309-312.

IN THE LAST TWO YEARS, pyrolysis-mass spectrometry studies of total soils, soil humic fractions, fungal melanins, model phenolic polymers, and plant polymers have been published (3, 10, 11, 12). As compared to the pyrolysis-gas chromatography, this technique gives immediate information about any of the thermal degradation compounds of the pyrolysate. However, the method has certain limitations because it cannot discriminate between ions of the same mass to charge ratio but with different chemical constitution, which can contribute to a single mass peak. Also, it is restricted to fragments of a mass number of about  $m/e$  150 due to the decrease of the transmissibility of the used quadrupole for larger ions.

<sup>1</sup>Contribution from the Centro de Edafología y Biología Aplicada del Cuarto, C.S.I.C., Apartado 1052, Sevilla, Spain, and the Instituto Territorial del Servicio Social de Higiene y Seguridad del Trabajo, Apartado 615, Sevilla, Spain. The authors are indebted to Dr. J. P. Martin, Riverside, California, for valuable and constructive criticism on the manuscript. Received 14 June 1978. Approved 13 Oct. 1978.

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Pyrolysis-gas chromatography-mass spectrometry-computer, more complex and time-consuming method than pyrolysis-mass spectrometry, permits the separation and safe identification of pyrolysis fragments, even for large ions. Therefore, a considerable chemical information on the pyrolyzed materials can be obtained. This method is currently used in organic geochemistry studies to elucidate the structure of complex, high molecular weight, polymer-like framework of geopolymers and carbonaceous chondrites (1, 4, 7, 8, 14, 15).

In a previous paper (6) pyrolysis-gas chromatography-mass spectrometry studies of fulvic and humic acids from different soils were reported. Samples were pyrolyzed at 700°C for 10 sec and low boiling point compounds were separated in a Chromosorb 102 column. Major components from soil humic acids were related to proteins and polysaccharides. Soil fulvic acids gave pyrograms with major peaks characteristic of soil polysaccharides.

The identification of the low boiling point compounds gave only partial information about the whole humic components because the easily degraded substances, such as polysaccharides and proteins, produced most of the compounds identified. Furthermore, the column used was not able to resolve compounds with higher boiling points than toluene and phenol. Therefore, it appeared important to study the high boiling point compounds, which should shed more light on the structure of humic substances.

### METHODS

The classification, geographical origin of the soil samples, and the analytical data of the humic fractions have been reported previously (6). The pyrolysis procedure was also described. Briefly, 2-mg samples were pyrolyzed at 700°C for 10 sec directly into the injection port of a gas chromatograph. The chromatographic effluent was passed via a silicone membrane molecular separator into the mass spectrometer operated at an ionizing voltage of 70 eV. Carbowax 20 M, SE-30, OV-17, and FFAP were employed as liquid phases of the columns, the latter giving the best resolution. Therefore, the separation of the pyrolysis compounds was achieved by use of a 2,000 by 3 mm stainless steel column packed with Chromosorb AW DMCS-80-100 mesh coated with 10% FFAP, programmed from 55°C to 270°C at a rate of 4°C/min. Helium at a flow rate of 30 ml/min was used as the carrier gas.

The individual pyrolysis products were identified by comparison of their mass spectra with library reference spectra and many of them were also identified by comparison with the gas chromatographic retention time of pure compounds. The presence of two different compounds in the same area usually did not present a problem because differences were such that their individual spectra could be easily recognized. The figures presented represent pyrograms obtained with a dual flame ionization detector chromatograph under the same operating conditions as noted above.

## RESULTS

Figure 1 gives an example of a typical pyrogram of a humic and fulvic acid. The humic acid is from a Typic Chromoxerert and the fulvic acid from an Orthic Humo-Ferric Podzol. In view of the extreme complexity of the pyrograms, the present study was focused on the Typic Chromoxerert and Humic Haplorthod humic acids, as representative of the products producing the higher and lower amounts of low boiling point compounds and also with the higher and lower N contents (6). The hydrolyzed humic acid from Typic Chromoxerert and two representative fulvic acid samples were also pyrolyzed. The compounds identified in these samples are shown in Table 1.

Typic Chromoxerert humic acid (Fig. 1a) gave major peaks for C<sub>2</sub>-alkylbenzene (2)<sup>3</sup>, pyrrole (20), methylpyrrole (21), methylfurfural (22), guaiacol (35), phenol and *o*-cresol (41), *m*- and *p*-cresol (43), C<sub>2</sub>-alkylphenol (45), vinylguaiacol (46), isoeugenol (50), and vinylphenol (51). Other prominent peaks were identified as xylene (3), C<sub>3</sub>-alkylbenzene and pyridine (6), trimethylbenzene (8), acetic acid and furfural (18), benzonitrile (24), C<sub>2</sub>-alkylpyrrole (25), methylbenzoni-

<sup>3</sup> Numbers in parentheses refer to peak numbers on pyrograms.

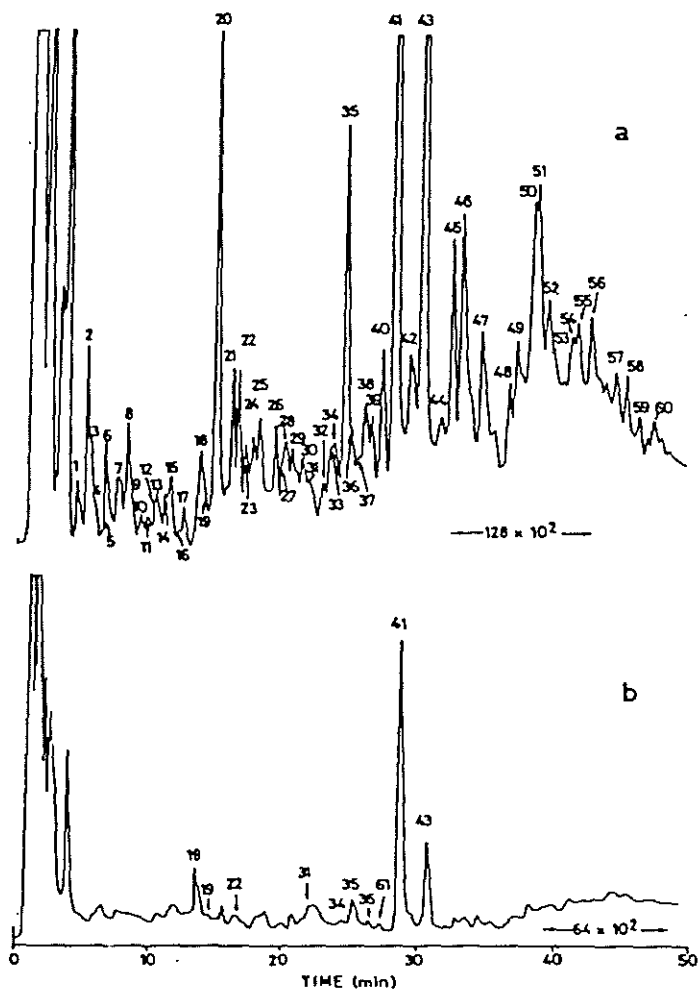


Fig. 1—(a) Pyrogram of Typic Chromoxerert humic acid; and (b) Pyrogram of Orthic Humo-Ferric Podzol fulvic acid.

trile (26), methylguaiacol (40), C<sub>2</sub>-alkylguaiacol (42), dimethoxyphenol (47), C<sub>2</sub>-alkylpiperidine (48), methylmethoxyphenol (49), C<sub>2</sub>-alkyldimethoxyphenol (52), methylpyrrolidone (55), and C<sub>2</sub>-alkyldihydroxybenzene (56). As minor or trace fragments, series of n-alkanes ranging from C<sub>10</sub> to C<sub>13</sub>, series of alkylbenzenes from C<sub>2</sub> to C<sub>7</sub>, and a few components of heterocyclic nitrogen compounds were present.

Hydrolyzed Typic Chromoxerert humic acid showed as major fragments acetic acid, methyl-naphthalene, phenol and *o*-cresol, and *m*- and *p*-cresol. Other prominent peaks were related to C<sub>2</sub>-alkylbenzene, styrene, naphthalene, C<sub>2</sub>-alkylphenol, and C<sub>4</sub>-alkyl-naphthalene. Minor components identified were series of alkylbenzenes from C<sub>2</sub> to C<sub>11</sub>, n-alkanes from C<sub>10</sub> to C<sub>17</sub>, and n-alkenes from C<sub>12</sub> to C<sub>17</sub>. Also in this pyrogram, peaks for polycyclic aromatic hydrocarbons such as fluorene, methylfluorene, and C<sub>2</sub>-alkylfluorene were present.

The Humic Haplorthod humic acid pyrogram was similar to that of the hydrolyzed Typic Chromoxerert humic acid. Major peaks represented styrene, furfural, pyrrole, phenol and *o*-cresol, *m*- and *p*-cresol, and C<sub>2</sub>-alkylphenol. Other important components were methylstyrene, methylfurfural, C<sub>2</sub>-alkylnaphthalene, and xanthene. Peaks related to lignin and N-containing compounds were present as well, but were not as prominent as those for Typic Chromoxerert humic acid. As minor fragments series of alkylbenzenes from C<sub>2</sub> to C<sub>5</sub>, series of alkanes from C<sub>10</sub> to C<sub>20</sub>, and series of alkenes from C<sub>11</sub> to C<sub>20</sub> were present.

The pyrolysis of fulvic acids and soil polysaccharides produced poorly resolved peaks with some unusual mass fragmentations, which made difficult the identification. Figure 1b shows the pyrogram of an Orthic Humo-Ferric Podzol fulvic acid. The low number and intensity of peaks in comparison with those humic acid are obvious although the pyrogram is double size (half of attenuation). Major peaks represent acetic acid and furfural (18), phenol and *o*-cresol (41), and *m*- and *p*-cresol (43). Other components were indene (19), methylfurfural (22), naphthalene (31), methyl-naphthalenes (34 and 36), guaiacol (35), and furfurylalcohol (61). Typic Chromoxerert fulvic acid showed similar peaks, but benzofuranes were present and guaiacol and naphthalenes were absent. For comparison the compounds produced in the pyrolysis of a protein (peptone) and a soil polysaccharide are shown in Table 1.

## DISCUSSION

A study of the high boiling point compounds produced by pyrolysis of soil fulvic and humic acids has been reported by Wershaw and Bohner (16). These authors identified 18 compounds: benzene, toluene ethylbenzene, styrene, cumene, phenol, cresol, indane indene, naphthalene, and methyl-naphthalene as aromatics; methylfuran, methylfuraldehyde, furfurylalcohol, dimethylfuran, and methylformylfuran as carbonyl derivatives; and methylindol. All these, except methylfuraldehyde and methylformylfuran were identified in this study.

In our previous paper (6) it was shown that most of the low boiling point compounds produced upo

Table 1—Pyrolysis products from humic fractions, soil polysaccharide, and peptone.

Compounds†	A‡	B	C	D	E	F	G	Compounds	A	B	C	D	E	F	G
(1) C <sub>10</sub> -alkane dimethylfuran	+	+	+					(31) naphthalene	+	+	+		+		
(2) C <sub>7</sub> -alkylbenzene	+		+					n-C <sub>7</sub> -alkylbenzene		+					
(3) xylene	+	+	+					(32) C <sub>7</sub> -alkylpyrrol	+						+
(4) C <sub>11</sub> -alkane n-C <sub>7</sub> -alkylbenzene C <sub>7</sub> -alkylbenzene	+	+	+					C <sub>11</sub> -alkene		+	+				
(5) C <sub>11</sub> -alkane	+	+	+					(33) C <sub>7</sub> -alkylbenzofuran	+						
(6) pyridine	+		+					C <sub>11</sub> -alkane			+				
(6) C <sub>7</sub> -alkylbenzene	+		+					(34) methylnaphthalene	+	+	+		+		
(7) methylpyridine	+		+					(35) guaiacol	+		+		+		
(7) styrene	+	+	+					(36) methylnaphthalene	+	+	+		+		
(8) trimethylbenzene	+	+	+					(37) piperidine	+						+
(9) C <sub>11</sub> -alkane	+	+	+					(38) methylpyrazine	+						+
(10) C <sub>7</sub> -alkylpyridine	+		+					C <sub>11</sub> -alkene			+				
(11) C <sub>7</sub> -alkylbenzene	+	+	+					n-C <sub>11</sub> -alkylbenzene		+					
(12) C <sub>7</sub> -alkylpyridine	+		+					(39) C <sub>7</sub> -alkylnaphthalene	+	+	+				
(13) C <sub>7</sub> -alkylbenzene	+	+	+					(61) furfurylalcohol				+	+	+	
(13) C <sub>11</sub> -alkene	+	+	+					(40) methylguaiacol	+						
(14) methylstyrene	+	+	+					(41) phenol	+	+	+	+	+	+	+
(15) C <sub>7</sub> -alkylbenzene	+	+	+					o-cresol	+	+	+	+	+	+	+
(15) C <sub>7</sub> -alkylpyridine	+		+					n-C <sub>11</sub> -alkylbenzene		+					
(16) methylstyrene	+	+	+					(42) C <sub>7</sub> -alkylguaiacol	+						
indane	+	+	+					C <sub>11</sub> -alkane			+				
(17) C <sub>7</sub> -alkylbenzene	+	+	+					C <sub>11</sub> -alkylbenzene		+					
(18) acetic acid	+	+		+	+	+		(43) m-cresol	+	+	+	+	+	+	+
C <sub>11</sub> -alkene	+	+	+					(43) p-cresol	+	+	+	+	+	+	+
(18) furfural	+	+	+	+	+	+		(44) C <sub>7</sub> -alkylphenol	+	+	+				
(19) indene	+		+		+			methylbiphenyl		+					
(19) C <sub>7</sub> -alkylpyridine	+		+		+			C <sub>11</sub> -alkane			+				
C <sub>11</sub> -alkane	+	+	+					(45) C <sub>7</sub> -alkylphenol	+	+	+				
C <sub>7</sub> -alkylbenzene	+	+	+					C <sub>7</sub> -alkylnaphthalene	+	+	+				
furylmethylketone	+	+	+			+		methylformylpyrrol		+					+
(20) pyrrol	+	+	+					methylpiperidine							+
benzofuran	+	+		+				C <sub>7</sub> -alkylnaphthalene	+	+					
C <sub>7</sub> -alkylbenzene	+	+		+				(46) vinylguaiacol	+						
(21) methylpyrrol	+	+	+					C <sub>7</sub> -alkylnaphthalene		+					
C <sub>11</sub> -alkene	+	+	+					C <sub>7</sub> -alkylnaphthalene		+	+				
(22) methylfurfural	+	+	+	+	+	+		(47) dimethoxyphenol	+						
(23) methylbenzofuran	+	+	+	+				C <sub>11</sub> -alkene			+				
(23) methylindene	+	+	+					(48) dimethylpiperidine	+						
C <sub>11</sub> -alkane	+	+	+					(49) methyl dimethoxyphenol	+						
(24) benzonitrile	+	+	+					fluorene		+	+				
(25) C <sub>7</sub> -alkylpyrrol	+		+					(50) isoeugenol	+						
C <sub>7</sub> -alkylpyridine	+		+					(51) vinylphenol	+						
(26) methylbenzonitrile	+	+	+					xanthene			+				
(27) n-C <sub>7</sub> -alkylbenzene	+	+	+					(52) C <sub>7</sub> -alkyldimethoxyphenol	+						
(28) C <sub>7</sub> -alkylpyrrol	+		+					(53) indol	+	+					+
C <sub>11</sub> -alkene	+	+	+					(54) methylindol	+	+	+				+
(29) C <sub>7</sub> -alkylbenzofuran	+	+	+	+				(55) methylpyrrolidone	+		+				
(30) C <sub>7</sub> -alkylindene	+		+					methylfluorene		+	+				
C <sub>11</sub> -alkane	+	+	+					(56) C <sub>7</sub> -alkyldihydroxybenzene	+						
								(57) C <sub>7</sub> -alkylindol	+		+				+
								(58) acetoguaiacone	+						
								C <sub>7</sub> -alkylfluorene		+					
								(59) 4,4'-dihydroxybiphenyl	+		+				+
								(60) methyl 4,4'-dihydroxybiphenyl	+		+				+

† Numbers in parentheses refer to peak numbers on pyrograms.

‡ A: Typic Chromoxerert humic acid; B: Typic Chromoceret hydrolyzed humic acid; C: Humic Haplorthod humic acid; D: Typic Chromoxerert fulvic acid; E: Orthic Humo-Ferric Podzol fulvic acid; F: Typic Chromoxerert soil polysaccharide; G: peptone.

pyrolysis of fulvic and humic acids could have come from polysaccharides and proteins. This study of the high boiling point compounds shows as well that protein derivatives such as alkylpyridines, alkylpyrroles, alkylbenzonitriles, indoles, piperidines, pyrazines, and pyrrolidones are common to humic acids with a high N content. Humic acids with lower N content yield fewer heterocyclic N compounds, such as noted for Humic Haplorthod humic acid.

Pyrolysis of proteins yields heterocyclic N compounds and some aromatics, such as benzene, toluene, ethylbenzene, propylbenzene, styrene, *o*-cresol, *p*-cresol, phenol, ethylphenol, and xylenols (9). These compounds are also present in the humic acid pyrograms. However, the hydrolyzed humic acid also

yield these aromatic compounds, which indicates that they also originate from aromatic units of the humic molecule. This is further substantiated by the presence of a great variety of additional aromatic compounds (alkylbenzenes, alkylphenols, lignin derivatives, polycyclic aromatic hydrocarbons) among the pyrolysis products.

Furan derivatives and other typical polysaccharides fragments are not as abundant as protein derivatives. Only a few compounds, dimethylfuran, furfural, methylfurfural, furylmethylketone, and furfurylalcohol, were present. This is probably due to the fact that pyrolysis of polysaccharides yields mainly low boiling point compounds, which are eluted during the first minutes from the column. These were iden-

tified in the previous paper (6) and no complex molecular structures were noted. The presence of protein and polysaccharides in the humic fractions was discussed in the previous paper.

A relatively high number of lignin fragments were identified in the Typic Chromoxerert humic acid pyrogram. Guaiacol, methylguaiacol, C<sub>2</sub>-alkylguaiacol, vinylguaiacol, isoeugenol, vinylphenol, acetoguaiacone, and dimethoxyphenols were found, which indicate the presence of lignins or altered lignins in the humic acids. Schnitzer and Khan (13) have summarized information on the isolation of lignin related compounds from different degradative reactions, which have been regarded by several authors as building blocks of the humic polymers. However, these lignin derivatives were not present in the pyrogram of the hydrolyzed humic acid. It has been shown (5) that during acid hydrolysis the lignin cannot be extensively condensed because a great percentage of it can be gradually dissolved. Another indication for smaller amounts of condensed structures is provided by the 1% yield of benzenepolycarboxylic acids obtained by permanganate oxidation of acid-boiled lignins. Therefore, this indicates that lignins present in the humic acids tested could be released by acid hydrolysis rather than transformed to more condensed structures.

Haider et al. (2) pointed out that under natural conditions the lignin molecules slowly decompose and that the units which are linked into the soil humus molecules will have the greater resistance to biodegradation. The high amounts of residual <sup>14</sup>C from coumaryl alcohols in the lignin recovered in the soil humic fraction indicated that before lignin is fully degraded or converted to humus a substantial portion may be extracted with NaOH solution and will be recovered with the humic acid fraction.

The results presented here indicate that a variety of heterogeneous materials including proteins, polysaccharides, and lignins are components of the humic acid molecules, which can be cleaved by relatively mild treatments such as acid hydrolysis. It appears that these classes of bio-organic matter found in living systems and associated with the humic acid "core" may be relatively young, while the "core" may be older, originating from organic materials present in the environment during the early soil forming processes.

Acid-boiled humic acid could, therefore, represent the more resistant structure or "core" and pyrolysis will release part of the structural units. The majority of the pyrolysis compounds were identified as series of alkanes, alkenes, alkylbenzenes, alkyl-naphthalenes, phenols, benzofuranes, indenes, and fluorenes. The pyrolysate is composed mainly of aromatic and substituted aromatic hydrocarbons with smaller and trace amounts of aliphatics. Inasmuch as aromatic compounds comprise the vast majority of the total pyrolysate it can be inferred that the humic acid "core" is primarily of an aromatic nature. A tentative model could be a system of loosely condensed aromatic rings

with aliphatic hydrocarbons either as bridges or side chains of undetermined length.

Fulvic acids yield compounds which suggest that the main components of the pyrolysable moiety are polysaccharides and aromatics, together to a few nitrogen derivatives.

The results of this study indicate that the pyrolysis-gas chromatography-mass spectrometry method is appropriate for the elucidation of the structure of soil humic fractions.

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