

INFRARED SPECTROSCOPIC EXAMINATION OF HUMIC ACIDS, II

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Both the M. W. data, as measured by gelchromatographic fractionation, and the infrared spectra of the fractions reveal considerable structural differences between the samples studied.

Unlike the brown coal humic acid sample, which also contains considerable amounts of high M. W. aliphatic compounds, the soil and synthetic samples contain almost exclusively fractions of aromatic character.

Soil samples from moor-layers of different depths showed large deviations as to their M. W. distributions and IR characteristics.

Chelates to which biological activity has been attributed were identified in low M. W. fractions.

Introduction

In a previous paper [1] we reported on the infrared spectra of humic acids extracted from Oroszlány Eocene brown coal by gel-chromatographic fractionation. Unlike the unfractionated parent sample, the spectrum of which could not be assigned unambiguously due to its heterodispersity, the fractions showed characteristic, well-resolved spectra thereby providing information on the structure, bonding, and functional groups of humic acid samples of different origins. These data were compared with those of the fractions of compost soil.

Here we extend our previous studies to other sedimentary brown coal samples and soil samples, and compare the results with those on a synthetically prepared and fractionated humic acid sample. For the sake of comparison, the molecular weight distributions of the different fractions were also calculated.

Methods

Humic acid samples extracted from Oroszlány brown coal were investigated. Details of the extraction procedure were published elsewhere [2]. The crude extract was purified according to ref. [3]. Soil samples were obtained from the Keszthely area and represented the lower and surface moor-layers, respectively. The synthetically prepared humic acid sample was purified by the same procedure as the brown coal samples. Gel-fractionation and infrared studies were done as previously described [4].

Results and Discussion

1. *Brown coal samples.* The Oroszlány brown coal humic acid was divided into 7 fractions. Molecular weight (M. W.) data are given in Table I; the infrared spectrum of each fraction and that of the parent material are shown in Fig. 1. Apparently this humic acid sample is very heterodisperse, and preliminary studies suggested that incomplete fractionation would hardly result in unambiguously interpretable infrared spectra.

As shown in Table I, this sample consists mainly of 3 different fractions with closely similar percentage compositions. Fractions Nos. 1—3, with a M. W. range of 23—38 kdalton, represent 31.2% of the total weight. It should be noted that fraction No. 1 also contains very high M. W. components ($\cong 38$ kdalton); however this fraction could not be further subdivided with the gel applied. Fractions Nos. 1—3 contribute equally to this 31.2% of the total weight. It is interesting to note that there is a "hole" in the 14—23 kdalton M. W. range, *i.e.* a significant amount of components was not found in this mid-range. 2—14 kdalton components contribute to an extent of 33.7% to the total weight; these components include brown coal humic acid molecules and their aggregates. The low M. W. fractions (Nos. 6 and 7) give the remaining one-third of the total weight. Of these, the 1—2 kdalton fraction makes the higher contribution (24.6%) and, by definition, consists of humic acid components. The light fraction No. 7 consists mainly of himatomelan and fulvic acids, which are disproportion products due to the alkaline extraction.

Infrared spectral analysis provides further useful information concerning the molecular structures and characteristic bonds (Fig. 1). The spectrum of the parent sample, S_0 , consists of diffuse bands due to overlapping peaks of slightly different frequencies.

In contrast, the spectrum of fraction No. 1, S_1 , is rich in well-resolved bands. The main peak at 2850 cm^{-1} indicates aliphatic -CH and -CH₂ groups. This peak gra-

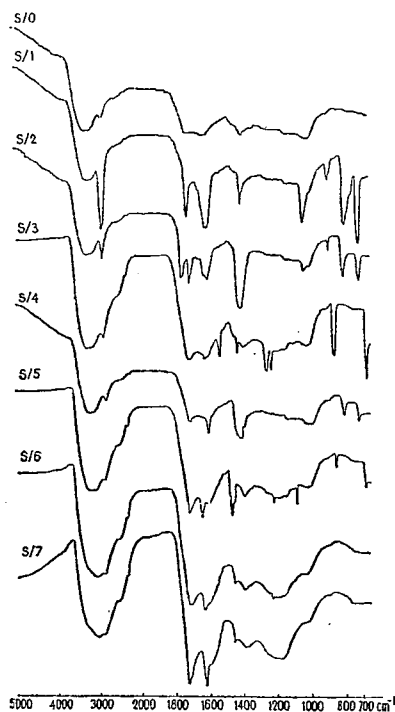


Fig. 1.

Table I
Molecular weight distribution of brown coal humic acid from Oroszlány

No. of fraction	%	Molecular weight range kdalton
1	10.1	> 38.0
2	11.5	38.0—29.0
3	9.6	29.0—23.0
4	10.4	14.0—6.0
5	23.3	6.0—2.0
6	24.6	2.0—1.0
7	10.0	< 1.0

dually decreases in lower M. W. fractions (Nos. 2—4), and is only a weak shoulder in fraction No. 5. This 2850 cm^{-1} peak not only indicates aliphatic character; its relative intensity provides qualitative information concerning the aliphatic chain length; the M. W. data in Table I and Fig. 1 are reasonable agreement. Another strong, unusually sharp peak at 720 cm^{-1} can be assigned to the >C=C< skeletal vibration and the $-(\text{CH}_2)_n-$ vibration ($n=4$). This peak is also dominant in the spectrum of fraction No. 2, although with considerably lower intensity, whereas it is totally absent from that of fraction No. 3 (and onwards).

On the basis of these data we conclude that the high M. W. fractions Nos. 1 and 2 contain mainly coal humic acids of aliphatic character.

A peak at 820 cm^{-1} is also present in the spectra of these samples, although with decreasing intensity, indicating substituted aromatic structures. The shoulders at 760 and 780 cm^{-1} are probably due to minor quantities of related structures substituted at different positions. In the spectrum of fraction No. 3 no peaks can be found in this range; the low-frequency spectrum is dominated by sharp peaks at 680 and 860 cm^{-1} , which can be assigned to deformation vibrations of substituted aromatic structures and trisubstituted molecules, respectively. The aliphatic character of the high M. W. fractions (Nos. 1—2) is taken over by an aromatic character in the fraction No. 3. This fraction has a M. W. range of 23—29 kdalton (Table I) and, thus, a different structural skeleton as compared to the high M. W. fractions.

Minor peaks in the spectra of these fractions provide further information on other structural constituents, e.g. the 900 cm^{-1} peak can be assigned to a β 1—4 glucoside vibration, indicating the probable presence of cellulose and cellulose derivatives, and their disproportionation products; this is in line with our previous suggestion [5].

A very intense peak at 1040 cm^{-1} (fraction No. 1) and a sharp peak at 1080 cm^{-1} (fraction No. 5) can be assigned to alcoholic-OH, the elongated -CO of a heterocyclic ring, and symmetric ether bond vibrations, respectively. The 1400 cm^{-1} peak due to the methyl deformation is a weak, well-resolved peak in the spectrum of fraction No. 1, whereas it is much more intense in those of fractions Nos. 2 and 4 (partly overlapping). Carboxyl vibrations are easily identified in the 1620 cm^{-1} region (fractions Nos. 1, 2, and 7); the 1720 cm^{-1} peak is due to the C=O group vibration of aromatic carboxylic acids, aldehydes and ketones. These bands are relatively weak in the spectra of fractions Nos. 3 and 4, but gain intensity in the low M. W. fractions. In the light fraction No. 7, which probably consists of himatomelan and fulvic acids according to the M. W. data, this peak dominates the spectrum. Two sharp, weak peaks at 1220 and 1260 cm^{-1} , which are exclusively present in the spectrum of fraction No. 3, can be assigned to phenolic-OH and elongated C—N/N—H deformations, respectively.

The spectrum of fraction No. 4 indicates the presence of completely different structures, e.g. a minor contribution from aromatic carboxylates and a significant amount of ionic carboxylates. In the low M. W. fractions Nos. 5 and 6 the strong, well-resolved peak at 1640 cm^{-1} indicates chelate structures. Since the chelate peak could not be found in any other case, we conclude that chelate bonds, to which biological activity was attributed by previous workers, are present only in the 1—6 kdalton fractions.

2. *Soil samples: surface moor-layer.* The M. W. data on extracted and fractionated humic acids of surface moor-soil (from the Keszthely area) are given in Table II.

Table II
Molecular weight distribution of soil humic acid
(surface moor-layer) from Keszthely

No. of fraction	%	Molecular weight range kdalton
1	30.0	> 40.0
2	15.8	40.0—28.0
3	12.6	28.0—18.0
4	9.8	18.0—6.0
5	29.9	< 6.0

The spectrum of fraction No. 1 is rich in sharp, well-resolved peaks. A strong peak at 2500 cm^{-1} is indicative of organic sulphur compounds, whereas a medium intense peak at 2300 cm^{-1} is due to aromatic nitrogen compounds. These characteristic bands are less intense in the spectrum of fraction No. 2; the 2300 cm^{-1} peak is apparently present even in the low M. W. fractions, in the form of a weak shoulder. These bands could not be identified in any of the previous spectra of humic acid samples, and thus are specific for the moor-soil samples. The presence of sulphur compounds in moor-soil is by no means surprising.

In the spectrum of fraction No. 1 there is a broad, intense peak centered at 810 cm^{-1} , which indicates substituted aromatic structures. This broad band is replaced by three sharp peaks in the spectrum of fraction No. 2; according to the literature, these peaks can be assigned to substituted aromatic structures of different forms. This pattern in the $800\text{—}850\text{ cm}^{-1}$ range is also present in the spectrum of fraction No. 3, while it is much weaker in that of fraction No. 4, and totally absent from that of fraction No. 5.

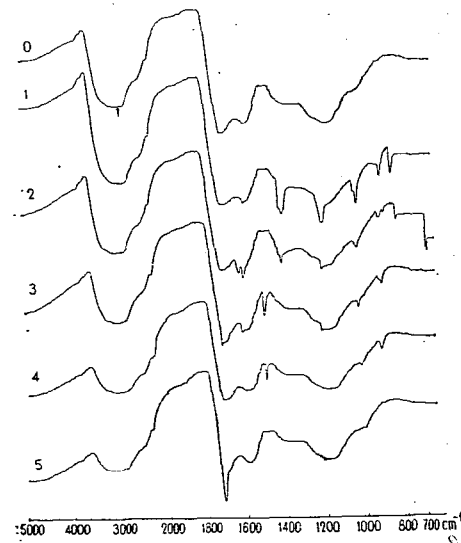


Fig. 2.

As seen in Table II, the average M. W. is shifted significantly to higher values as compared to the situation in Table I. The high M. W. fraction No. 1. (M. W. 40 kdalton) makes a contribution of 30.6%, a much higher value than in the case of brown coal samples (10%). On the other hand, the low M. W. fractions Nos. 5—7 amount to a total of 30% (compared with 60%, Table I). The medium M. W. fractions (6—40 kdalton) are also significant (40%).

The strong, sharp peaks at $910\text{—}920\text{ cm}^{-1}$, which can be assigned to the β 1—4 glucoside bond vibration, are present exclusively in the spectrum of fraction No. 2; this observation may indicate that fraction No. 2 contains the remains of plants, even macroscopically observable in the surface moor-layer. The alkaline extraction could lead to the degradation of plant tissues, resulting in 30—40 kdalton M. W. products. It is interesting to refer to our earlier result [6]: during alkaline extraction of xylites with 0.5 N NaOH cellulose products with similar degrees of polymerization were identified.

As seen in Fig. 2, there is an intense, sharp peak at 1030 cm^{-1} in the spectrum of fraction No. 1, whereas in the same region there is a doublet (at 1030 and 1010 cm^{-1}) in those of fractions Nos. 2 and 3. The

former band can be assigned to an Si—O vibration, and the latter to an elongated C—O vibration of polysaccharides. These peaks are present in the spectrum of almost every fraction. However, they are missing from the spectra of fractions Nos. 4 and 5, indicating that the inorganic silicate contamination is eluated only with higher M. W. fractions. The same holds for polysaccharides.

The most intense peak in the spectra of fractions Nos. 1—3 is at 1200 cm^{-1} , with decreasing intensity, however; this is due to the deformation vibration of the aromatic carboxylic-OH groups. An intense band at 1400 cm^{-1} in the spectra of fraction Nos. 1 and 2 can be assigned to CH_3 and CH_2 groups attached to aromatic rings.

As expected for soil humic acid, there is a band at 1520 cm^{-1} , with decreasing intensity in the spectra of fractions Nos. 1—3, which can be assigned to heterocyclic C=N vibration and is due to protein components. Since this band is missing from the spectra of low M. W. fractions, these protein components should elute with high M. W. fractions.

A doublet at $1600\text{--}1620\text{ cm}^{-1}$ in the spectrum of fraction No. 1 indicates aromatic C=C and ionic bound carboxylate ions. Interestingly, the 1720 cm^{-1} peak, which is characteristic for aromatic carboxylic acids, is completely missing from this spectrum. The same holds for fraction No. 2, except that there is a well-defined peak at 1600 cm^{-1} . In the spectrum of fraction No. 3 these bands are weak, whereas in that of fraction No. 4 the "chelate" peak at 1640 cm^{-1} is clearly visible. Fraction No. 4 has a M. W. range of 6—18 kdalton (Table II). From a comparison of these results with those of Table I and Fig. 1, we can conclude that in the case of soil humic acid samples higher M. W. components should play a role in chelate formation. However, the chelate formation is coupled to relatively low M. W. components, just as in the case of brown coal samples; the observed differences are due to an upward shift in M. W. distribution (*cf.* Tables I and II).

In the light fraction (No. 5) there is only one well-resolved peak, at 1720 cm^{-1} , indicating aromatic carboxylic acids. This fraction, similiary to that of brown coal samples, may consist of disproportionation products, himatomelan and fulvic acids. According to the spectrum, aromatic carboxylic acids from the dominant structure.

3. *Soil sample: lower-lying layer.* The M. W. data on extracted and fractionated humic acids of a lower moor-layer (from the Keszthely area) are given in the Table III.

This humic acid clearly differs significantly from those of both brown coal and, the surface moor-layer. The highest M. W. fraction (No. 1) has a very low percentage; this 3.8% may be compared with the 30% in Table II. The mid-fractions make a similar contribution, whereas the low M. W. fractions have a very high contribution, 57.6%. The light fraction (<1 kdalton) is particularly dominant.

From these data we can conclude that at a depth of 70 cm the humic acid material undergoes extensive degradation in moor-soil, so that the amount of the high M. W. components drops to

Table III
Molecular weight distribution of soil humic acid
(lower-lying layer) from Keszthely

No. of fraction	%	Molecular weight range kdalton
1	3.8	>40.0
2	20.5	40.0—25.0
3	17.4	25.0—10.0
4	13.6	10.0—1.0
5	44.0	<1.0

Table IV
Molecular weight distribution of synthetic humic acid

No. of fraction	%	Molecular weight range kdalton
1	13.6	>23.0
2	12.6	23.0—6.0
3	44.8	6.0—1.0
4	28.0	<1.0

one-eighth, and consequently the light fractions are considerably accumulated.

No information can be obtained from the IR spectrum of the unfractionated sample (Fig. 3), whereas those of the fractions are rich in intense, well-resolved peaks. In the light M. W. fraction (No. 5) there is only one peak, at 1720 cm^{-1} , which can be assigned to aromatic carboxylic acids, presumably fulvic acid.

In the high M. W. fraction No. 1 there is a sharp peak at 860 cm^{-1} , indicating aromatic structures. This peak loses intensity in the spectrum of fraction No. 2, but another peak at 680 cm^{-1} is prominent, indicating substituted aromatic structures (deformation vibration). These bands are missing from the spectra of lower M. W. fractions.

The weak, sharp bands at 920 and 1030 cm^{-1} indicate the presence of polysaccharides and plant fossils in fraction No. 1; these peaks decrease rapidly in the spectra of fractions Nos. 2 and 3. The 1200 cm^{-1} peak, assigned to phenolic-OH, is well-resolved in the spectrum of fraction No. 1, but loses intensity in those of the lower M. W. fractions. Furthermore, the 1420 cm^{-1} peak indicates $-\text{CH}_2$ and $-\text{CH}_3$ groups connected to aromatic rings. The 1500 cm^{-1} band, assigned to heterocyclic $\text{C}=\text{N}$, is present exclusively in the spectra of fractions Nos. 3 and 4.

Obviously, the aromatic carboxyl and carboxylate bands are missing or very weak in the spectrum of fraction No. 1. The 1620 — 1640 cm^{-1} doublet characteristic

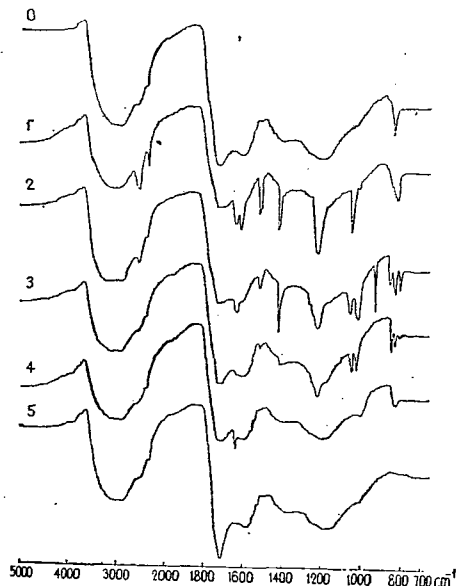


Fig. 3.

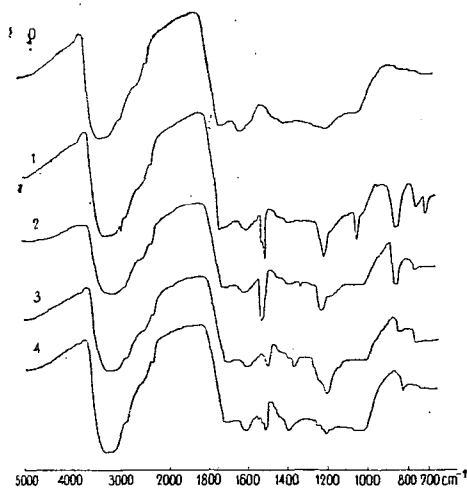


Fig. 4.

for carboxylate ions and chelates is only present in that of fraction No. 2. In the light fractions the ionic character is less marked, and the spectrum is dominated by the 1720 cm^{-1} peak.

4. *Synthetic humic acids.* The M. W. data on a synthetically prepared and fractionated humic acid sample are given in Table IV.

From a comparison of Table IV with Tables I—III, it is obvious that the average M. W. is much lower in the case of the natural humic acids. About 75% of the total weight is due to the light M. W. fractions (<6 kdalton). This marked difference is also reflected in their IR spectra.

No bands characteristic for aromatic carboxylic acids (at 1720 cm^{-1}) or chelates (at $1620\text{--}1640\text{ cm}^{-1}$) can be seen in the spectra of these fractions. There are very strong bands in the $1500\text{--}1520\text{ cm}^{-1}$ range, which can be assigned to the heterocyclic C=N group vibration. Each fraction shows a band at 1200 cm^{-1} with different intensities, indicating aromatic carboxylic -OH groups. There is a sharp peak at 1040 cm^{-1} in the spectrum of fraction No. 1, which is probably due to the presence of alcoholic -OH groups. Broad bands at 830 and 860 cm^{-1} , and minor bands at 740 and 690 cm^{-1} in this spectrum, indicate aromatic structures substituted at different positions. The former two peaks are also present in the spectrum of fraction No. 2 and in those of fractions Nos. 3 and 4, although with low intensity. These data indicate that this synthetic humic acid sample is of a much simpler structure, with a strong aromatic character.

Conclusions

1. Both the M. W. data, as measured by gelchromatographic fractionation, and the infrared spectra of the fractions reveal considerable structural differences between the samples studied. The data of these two methods, however, can be brought into parallel.

2. Unlike the brown coal humic acid sample, which also contains considerable amounts of high M. W. aliphatic compounds, the soil and synthetic samples contain almost exclusively fractions of aromatic character. There is a significant difference between the structure of the brown coal sample described here and that previously reported [1].

3. Soil samples from moor-layers of different depths showed large deviations as to their M. W. distributions and IR characteristics.

4. Chelates to which biological activity has been attributed were identified in low M. W. fractions.

In order to clarify the structures of this group of biologically-active compounds, the M. W. measurements should be complemented by other methods, and the main components should be quantitatively identified on the basis of their IR spectra.

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ИССЛЕДОВАНИЕ ГУМИНОВЫХ КИСЛОТ МЕТОДОМ ИНФРАКРАСНОЙ СПЕКТРОСКОПИИ, II

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В испытанных образцах гуминовых кислот наблюдаются значительные различия не только в молекулярно-весовом распределении, определенной методом гель-фракционирования, но и в инфракрасных спектрах разных фракций. В том случае, пока образец угольной гуминовой кислоты содержит кроме ароматических значительное количество и алкильных компонентов, то образцы почвенных и синтетических гуминовых кислот состоят только из ароматических составляющих.

Наблюдаются также значительные различия между структурой гуминовых кислот взятых с одного и того же места, но с различной глубины почвы, как по их молекулярно-весовому распределению, так и по их инфракрасным спектрам.

В исследованных образцах, хелаты имеющие значение с точки зрения их биологической активности содержатся в меньших фракциях.