

Comparison of five humic acids*

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The ultimate analyses, u.v.-visible absorption spectra, fluorescence spectra and i.r. spectra of five humic acids have been studied. One was obtained commercially from peat, two from organic-rich soils, one from an estuary and one from a marine sediment. The u.v. spectra were used to compare average molecular weights which varied from 800 to 7000 and increased with the atomic H/C ratio. Fluorescence appeared to be due to poly-nuclear aromatic structures. All the humic acids showed maximum excitation at 360 nm and developed maximum emission in the range 430–455 nm but the humic acids from sediments showed an additional emission maximum at ~410 nm. Fluorescence from the peat humic acid was broad and secondary emission was observed with a maximum at 520 nm which was attributed to the formation of excimers. Fluorescence has been used to monitor the interaction of cations with humic acids in solutions of different ionic strengths which shows promise for distinguishing between metal-humate complex formation and the coagulation of a colloid. The structures of the humic acids are discussed in terms of the structures possessed by lignites.

(Keywords: instrumental methods of analysis; humic acids; structures)

Humic acids are bio-resistant alkali-soluble organic acids formed by the degradation of naturally occurring material under aerobic (oxic) conditions. Their formation and re-assimilation by living material or their consolidation to fossil fuels forms an important part of the carbon biogeo-cycle¹. Terrestrial humic acids (which comprise about 10% of tertiary lignites and quaternary peats and up to about 1% of top soils) have very similar i.r. spectra to lignites²⁻⁷ with the addition of marked absorption in the region of 1700 cm⁻¹ which may be ascribed to carbonyl vibrations in carboxyl groups. It is reasonable therefore to accord to terrestrial humic acids chemical structures similar to those of lignites.

That is, one expects such humic acids to be composed of aromatic (including polynuclear aromatic) structures substituted by carboxyl, phenolic hydroxyl and alkyl groups and linked together through aliphatic, alicyclic and ether linkages. A detailed account of structures present in a lignite has recently been given by Doğru *et al.*⁸. Terrestrial humic acids are transported to coastal waters by rivers and the seepage of ground water but marine humic acids proper are formed by the degradation of plankton.

The structures of both zooplankton and phytoplankton (which possess no lignin) differ from those of terrestrial vegetation and consequently one might expect marine and terrestrial humic acids to differ in structure. Indeed, Hatcher *et al.*⁹ interpreted the C¹³ n.m.r. spectrum of a marine fulvic acid (related to humic acids but soluble in neutral and acidic aqueous solutions) in terms of modified polysaccharide structures; and recently Worobey and Webster¹⁰ have used the same technique to ascribe a hemicellulose component to humic

acid structures. The core of the problem has been indicated by Hartenstein¹¹ who demonstrated that whereas plots of the atomic ratios H/C against O/C suggested that humic acids, lignites and coals were structurally related, the ultimate analyses of cellulose, lignin and protein from which humic acids may have been formed are rather different.

Here u.v., fluorescence and i.r. spectra of five humic acids are compared. The first of these, F, is available commercially from Fluka and is obtained from peat; two of the humic acids, K and T, were obtained from organic rich soil in the south of Turkey; the fourth, G, was extracted from sediment in the mouth of a river, and the fifth, D, was from sediment 200 m below the surface of the Mediterranean from a location which appears to be little influenced by river water.

EXPERIMENTAL

Large objects such as stones were removed from sediments which were then extracted with excess 0.5 M NaOH at 60°C. Undissolved material was removed by centrifugation. The solutions were acidified to pH ≈ 1 with hydrochloric acid and allowed to stand overnight. Precipitated material was removed by centrifugation. Dissolution and reprecipitation were repeated twice using cold 0.1 M NaOH and a subsequent pH of ≈ 1. The final residue was washed with distilled water and dried at 50°C.

U.v. and visible absorption spectra were recorded by a Varian Techtron Model 635. I.r. spectra were obtained from a Beckman Acculab 2 spectrophotometer using the KBr disc technique. Fluorescence spectra were excited and measured using a Turner Model 430 instrument. Experiments with alkaline hypochlorite solutions were performed in test tubes at 60°C¹⁴. Surface tension was measured by the capillary technique.

* A preliminary account of some of these observations was presented to the UNEP Workshop on Pollution of the Mediterranean, Cagliari, 1980

Table 1 U.v.-visible absorption spectra of humic acids

Wave length (nm)	250	300	350	400	500	600	H/C	O/C	Molecular weight ^c
Humic acid: Fa	734	500	314	185	104	46	1.17	0.35	
b	1	1	1	1	1	1			800–1000 ^d
Ka	618	405	250	139	57	23	1.34	0.52	
b	0.84	0.81	0.80	0.75	0.55	0.50			980–1200
Ta	703	484	291	156	60	29	1.35	0.46	
b	0.95	0.97	0.93	0.85	0.58	0.63			840–1050
Ga	181	110	68	36	16	6	1.41	^e	
b	0.25	0.22	0.22	0.20	0.15	0.13			3500–4300
Da	105	70	42	24	13	8	1.62	0.48	
b	0.14	0.14	0.13	0.13	0.13	0.17			5700–7100

^a $\log I_0/I$ for identical concentrations in the same cell where I_0 corresponds to the solvent

^b Absorption relative to that of F for the same wave length

^c From the molecular weight of F/b value in the range 250–350 nm

^d Reference determined by Fluka by sedimentation in an ultracentrifuge

^e Not measured

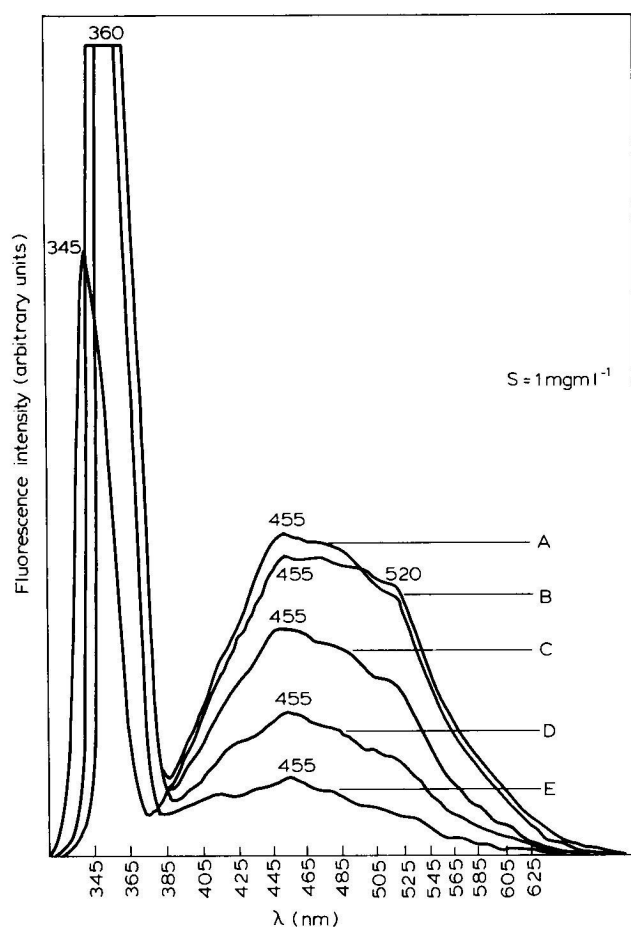


Figure 1 Effect of concentration on fluorescence of humic acid F. Excitation at 360 nm. pH 9.2, observed fluorescence intensities. A, s/20; B, s/10; C, s/40; D, s/80; E, s/160 ($s = 1 \text{ mg ml}^{-1}$)

RESULTS AND DISCUSSION

U.v.-visible absorption spectra

Table 1 compares the atomic H/C and O/C ratios and the u.v.-visible absorption spectra of the five humic acids. Solutions of humic acid in alkali absorb visible light and the absorption increases monotonously with increasing frequency. There are no peaks. The relative extinction coefficients presented in Table 1 show that the patterns of absorption of each of the five humic acids were rather similar.

In particular the ratio of absorption at 350 nm to that at 250 nm was nearly the same for each humic acid. Assuming, as seems likely, that absorption in this region was due to substituted aromatic structures this suggests that the proportions of three, two and single ring aromatic structures in each humic acid were more or less the same and that the differences in absorption in the region 250–350 nm could be accounted for simply by variations in the total concentration of aromatic structures from one humic acid to the next. Since there is apparently no way of separating aromatic material from the remainder of a humic acid this is a way of stating that the differences in the absorption in the region 250–350 nm imply that the humic acids had different molecular weights.

The molecular weight of humic acid F being given, the other molecular weights in Table 1 were calculated from the relative extinction coefficients. It will be seen that the molecular weights vary by a factor of about 10, the largest molecular weight – about 7000 – being observed in humic acid D from the marine sediment. It seems likely that humic acids which have been formed directly from phytoplankton would have smaller molecular weights than this.

Table 1 shows that there were small differences amongst the humic acids in the structures that absorbed light in the visible region of the spectrum. The estimated molecular weights roughly parallel the atomic H/C values which is indeed consistent with increase in molecular weight being caused by 'dilution' of the aromatic moieties (having low H/C ratios) by increasing quantities of aliphatic or carbohydrate material (having larger H/C ratios). A lower O/C ratio was observed for humic acid F than for the other humic acids, which had similar O/C ratios.

Fluorescence spectra

Whereas u.v.-visible absorption spectra show no maxima, Ghassemi and Christman¹² observed that when excited with 360–370 nm light humic acids gave broad fluorescence from 450 nm to 460 nm. Figure 1 shows the emission spectra given by different concentrations of humic acid F when excited by 360 nm light. It will be seen that the most dilute solutions showed emission maxima at 455 nm, but as the concentration of the solution was increased a secondary maximum emission was observed at 520 nm.

Table 2 shows the variation in fluorescence intensity caused by changes in pH; neutral solutions fluoresced the

most strongly, the decline of fluorescence intensity in acid solutions being more marked than in basic solutions. Acidity had little effect on the ratio of the intensities at 455 and 520 nm.

Figure 1a and 2 and Table 3 show how the intensity of fluorescence at 455 nm varies with the concentration of humic acid present in the solution. If one starts with a very dilute humic acid solution the intensity of fluorescence increases linearly with concentration. Subsequently the intensity of fluorescence falls below the anticipated linear value because a significant fraction of the light emitted at 455 nm is reabsorbed by other humic acid molecules. The fraction of the light absorbed by the solution at 455 nm can be measured in a separate experiment and the effect of self absorption on the fluorescence intensity can be calculated. This has been done in Table 3 where constancy of the f value indicates that fluorescence intensity is being diminished solely by self absorption. At higher concentrations of humic acid f falls dramatically and this corresponds to the increase in intensity at 520 nm. This dramatic decrease in fluorescence is caused by the destruction of excited humic acid molecules by collision with other humic acid molecules, the light energy being converted into kinetic energy or being transformed by excimer formation rather than being radiated as fluorescence at 455 nm.

It is supposed that the light emitted at 520 nm corresponds to the fluorescence of the excimers. This description of the variation of fluorescence with the concentration of the humic acid corresponds closely to the description of the fluorescence of aromatic hydrocarbons¹³. The inhibition of fluorescence by collision in solutions which by most standards are dilute suggests that, as in hydrocarbon solutions, the humic acid molecules are not randomly distributed as individuals throughout the solution but are associated in small groups or clusters. Analogy with the colloidal electrolyte nature of humic acid solutions¹¹ suggests that the humic

acid clusters may be regarded as micelles. Some confirmation of this has been found in studies of the surface tension of humic acid solutions. The addition of 0.5% of humic acid F to either water or to NaOH (1 M) reduces the surface tension by about 25%. This is comparable to the effect of soaps and is consistent with humic acids being colloidal electrolytes.

Figure 3 compares the emission spectra given by very dilute solutions of the five humic acids when excited by 360 nm light. It is difficult to compare their relative intensities quantitatively. Many organic compounds fluoresce because they possess polynuclear aromatic nuclei and the similarity between the i.r. spectra of terrestrial humic acids and tertiary lignites suggests that such nuclei may be the source of humic acid fluorescence. The humic acid F from peat showed broader fluorescence than the other humic acids. In part this was due to the secondary maximum at 520 nm discussed above but it may also have been due to the possession of a greater variety of polynuclear aromatic structures produced by the greater diagenesis of this acid.

The fluorescence spectra from the soil humic acids K and T were very similar. All the humic acids gave an emission maximum in the range 430–450 nm but G and D, obtained respectively from sediments in a river mouth and the Mediterranean sea bed, showed a further sharp pronounced maximum close to 410 nm suggesting, perhaps, that they possessed two distinct types of fluorescing structure.

In an attempt to confirm the hypothesis that humic acids fluoresce because they contain polynuclear aromatic structures, acids were treated with an alkaline solution of hypochlorite anions. Hypochlorite solutions react with organic compounds in many ways but if the solution is sufficiently alkaline the major reaction appears to be the degradative oxidation of polynuclear aromatic structures to give benzene carboxylic acids¹⁴.

When an $\approx 1.0 \text{ g dm}^{-3}$ solution of humic acid F was treated with alkaline hypochlorite its colour changed from dark coffee brown, to redder cocoa brown, to orange, to yellow, to milk white. The reaction could be stopped at each stage by the addition of acid which produced a coloured precipitate of somewhat lighter hue. Figure 4 shows i.r. spectra of the products at successive stages of the reaction. Carbonyl absorption by carboxyl groups around 1700 cm^{-1} increased with the severity of the treatment whilst aromatic absorption at 1600 cm^{-1}

Table 2 Effect of pH on observed fluorescence intensity of humic acid F

Observed fluorescence intensity (arbitrary units)	8	13	22	20	19	18
pH	3	5	7	9	11	13
Excitation = 360 nm; emission = 455 nm; band width = 15 nm; concentration = $0.004 \text{ mgm dm}^{-3}$						

Table 3 Variation of humic acid fluorescence with concentration. Typical results, excitation at 360 nm, emission maximum 520 nm (high concentrations), 455 nm (low concentrations)

Concentration ^a	A/2	A/4	A/8	A/16	A/32	A/64	A/128	A/256	A/512	A/1024	A/2048
Fluorescence intensity											
455 nm	1.8	31	76	104	95	67.5	43.9	24.9	13.5	9	6.9
520 nm	8.4	38	75	85.5	75	51	30	15.9	9	5	3.5
f^c	1	2.55	2.48	2.65	2.67	2.63	2.62	2.41	3.04	d	d
Concentration ^b	B/2	B/4	B/8	B/16	B/32	B/64	B/128	B/256			
Fluorescence intensity											
455 nm	9	48.5	85.5	93	71.5	43.5	20	10.5			
520 nm	19	53	78	75	52.5	29.9	13	6.5			
f	1.55	2.65	2.43	2.47	2.80	d	d	d			

^a A = 0.4 mg humic acid per ml of solution buffered at pH 9.2

^b B = 0.45 mg humic acid per ml of solution buffered at pH 7.0

^c f = fluorescence intensity at 455 nm in arbitrary units $(T_{455})^{-1} (1 - T_{360})^{-1}$; where T_x = optical transmittance at x nm

^d = optical transmittance inaccurate

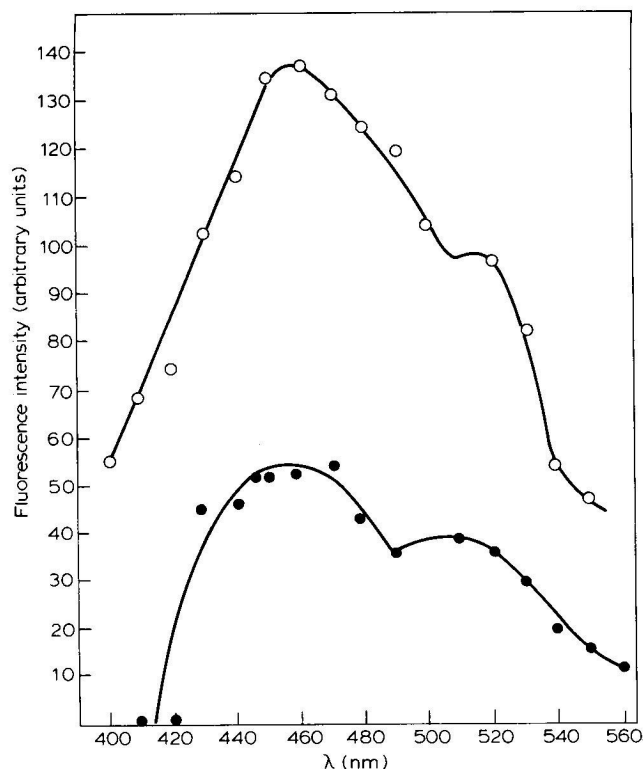


Figure 2 Effect of concentration on fluorescence of humic acid F. Excitation at 360 nm. pH 11.4, fluorescence intensities corrected for self absorption. Fluorescence intensity as in Table 3. ○, s/80; ●, s/4 ($s = 0.5 \text{ mg ml}^{-1}$)

decreased relative to absorption by aliphatic groups in the region of 1450 cm^{-1} . Milk white solutions gave u.v. absorption spectra having a maxima at $\approx 260 \text{ nm}$ indicating the presence of benzenoid structures.

In fact, relatively increased concentrations of benzenoid structures must have been present since the maxima are not distinguished in u.v. spectra of solutions of the parent humic acid. Thus the i.r. and u.v. spectra suggest that alkaline hypochlorite degrades polynuclear aromatic material in the humic acid to benzene carboxylic acids. As the severity of the hypochlorite treatment increased fluorescence diminished. The milk white solutions resulting from the treatment of humic acid F did not fluoresce and the fluorescence of the solutions of all the other humic acids was inhibited by warm hypochlorite.

This study of fluorescence may have relevance beyond the field of humic acids. It is known that spores and pollens, both fresh and fossil, excited by light of 365 nm wave length give broad fluorescence with a peak in intensity situated between 460 and 600 nm¹⁵. With increasing diagenesis spores and pollen grains change in colour from transparent yellow to orange, to red, reddish brown and finally to black¹⁶. Similar colour changes accompanied by increase in the wavelength of maximum emission of fluorescence was caused by heating pollen grains¹⁵.

Such sequences of colour are more or less the reverse of the sequence produced by the action of alkaline hypochlorite on humic acid. This suggests that increasing diagenesis or heat increases concentrations of polynuclear aromatic material and that increase in the wavelength of maximum emission may be due, at least in part, to excimer formation made possible by the formation of local clusters

of ordered polynuclear aromatics; more work is needed to test such an interpretation. Similar arguments should apply to the fluorescence of huminite-vitrinite which, when immature, may contain considerable proportions of humic acids.

Intensities of fluorescence provide a ready means of monitoring interactions between metals and humic acids. Figure 5 shows the change of intensity of emission when humic acid F was titrated with ferric ions. The 'end point' indicated by minimum fluorescence was seen, after a period of standing, to correspond to coagulation of the humic acid. Table 4 shows the concentration of some cations needed to cause coagulation of this humic acid. Much work has been reported on the interaction of cations and humic acids¹⁷. The subject is complicated and it has not been clear whether coagulation of a colloid or complex formation was being studied.

The distinction may perhaps be made by investigating the effects of varying the ionic strength of the electrolyte. The Debye-Hückel theory of dilute electrolytes shows that a complex increases in solubility with the square root of the ionic strength¹⁷ whilst the Verwey-Overbeek-Dejaguin description of a lyophobic colloid solution suggests that increase in the ionic strength of the solution will usually decrease the amount of a coagulating cation needed to cause precipitation¹⁸.

Table 5 shows that the concentration of precipitant varies in a complicated manner with ionic strength and

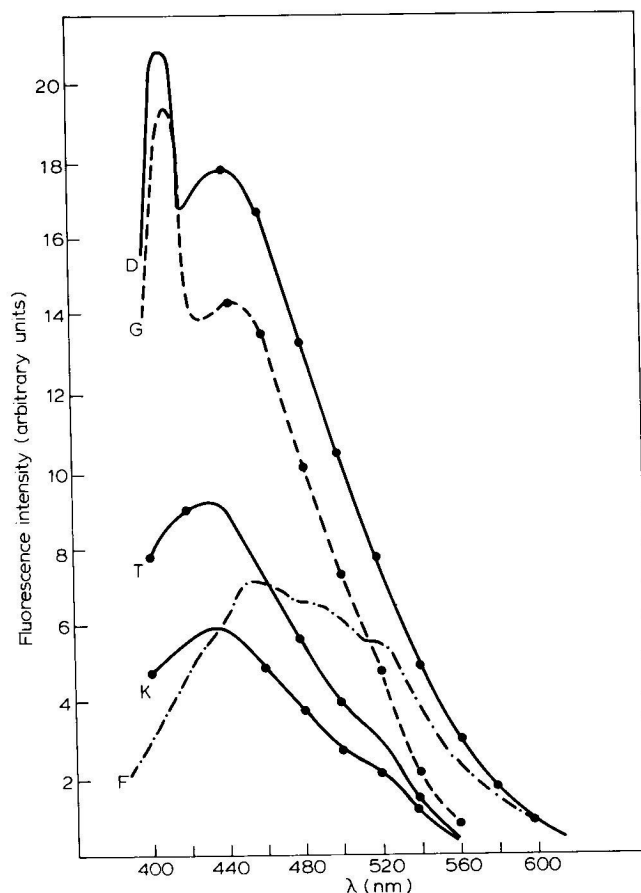


Figure 3 Fluorescence emission by humic acids (excitation 360 nm). The spectra have been staggered in the vertical (fluorescence) direction so as to display them clearly. Letters indicate humic acid types

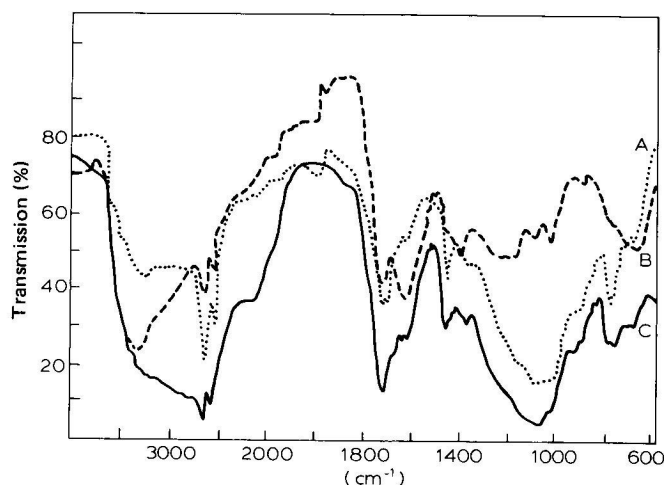


Figure 4 I.r. spectra of humic acid F after reaction with alkaline hypochlorite. ... A, after drastic treatment (yellow residue); --- B, untreated humic acid F; — C, after mild treatment (brown residue)

Table 4 Amount of metals needed for coagulation of 1 g of humic acid F^c

Metal	Amount needed (g)	
	A ^a	B ^b
Fe ⁺³	0.23	0.1
Al ⁺³	0.51	0.2
Mg ⁺²	0.39	
Ca ⁺²	1.23	0.4–0.5

^a A = visual coagulation end point

^b B = fluorescence end point

^c Initial humic acid concentration 0.05 g dm⁻³

probably both colloid coagulation and complex formation are involved. With the trivalent ferric and aluminium ions complex formation was clearly the predominant effect but in contrast ferrous ions caused the coagulation of a colloid. Magnesium ions gave an increase in concentration with ionic strength suggesting that complex formation occurred but the variation with ionic strength was much less marked than with ferric and aluminium ions and it seems likely that complex formation and colloid coagulation happened simultaneously. Table 4 suggests that generally the maximum humic acid content of a natural water will be limited by the concentrations of calcium and magnesium ions present; on the other hand, the many studies of metal ion–humic acid interactions by previous workers^{19–21} indicate that the concentrations of trace metals may well be determined by the humic acid content of the water.

I.r. spectra

The i.r. spectrum of humic acid F has been given in Figure 3 and has been partly interpreted – in discussing the hypochlorite experiment – in terms of substituted (polynuclear) aromatic structures. The spectrum shows broad absorption by H-bonded OH groups, absorption near 2930 cm⁻¹ and in the region 1400–1450 cm⁻¹ by

alkyl groups, by carbonyl groups in carboxyl groups at 1700 cm⁻¹, by C–O and O–H vibrations around 1200 cm⁻¹ and by aromatic rings and carboxylate anions near 1600 cm⁻¹²³. All these features were present in the spectra of the other four humic acids but were less well resolved, especially in the 1600–1700 cm⁻¹ region, possibly as a result of their higher molecular weights.

Table 6 compares the features of the spectra showing the (minor) differences between them. It will be seen that humic acids K, T, G and D had very broad absorptions by OH groups and this prevented accurate quantitative measurement of the absorbance around 2930 cm⁻¹ since, as Table 6 shows, such absorbance did not appear to parallel the H/C ratios. Bending absorption by CH₂ (and CH₃) groups was generally near to 1400 cm⁻¹ suggesting that most alkyl groups were adjacent to aromatic rings or carboxyl groups.

The ratio of the absorbance at 1400 cm⁻¹ to that of 1600 cm⁻¹ was lower for humic acid F than for the other acids but the ratio did not vary regularly with the atomic H/C ratios of the humic acids and it may be that the extinction coefficients varied from one acid to another. The soil humic acids, K and T, showed no resolved absorption near 1370 cm⁻¹ by CH₃ groups. Some humic acids, notably D, showed weak absorption in the 1500–1550 cm⁻¹ region, presumably due to aromatic ring vibrations, though other explanations are possible. Buravas *et al.*⁴ suggested that absorption near 1500 cm⁻¹ was a sign of immaturity in lignites and further work on its origin might be worthwhile. It is not possible to determine the patterns of substitution around the aromatic rings from the i.r. spectra; presumably a variety of patterns was present. These spectra were similar to those obtained by previous workers^{2–7} and, as noted in the introduction, akin to those of lignites.

It is not too difficult to postulate molecules having atomic H/C and O/C ratios, molecular weights and i.r. and u.v. spectra similar to those of the humic acids investigated here. Figure 6 shows a hypothetical component of humic acid F. Such a molecule does not have the range of polynuclear aromatic structures necessary to explain the observed u.v. spectra; plainly a

Table 5 Dependence of coagulation of metal–humic acid F complexes on the ionic strength of solution

Ionic strength ^b	Fluorescence intensity ^a			
	Fe ⁺³	Al ⁺³	Mg ⁺²	Fe ⁺²
0.00	2.5	2.5	46.0	46.0
0.09	11.7	6.5	46.5	6.5
0.20	14.5	11.0	48.0	7.0
0.30	17.5	23.5	56.5	—
0.40	26.5	—	—	5.5
0.51	39.5	27.5	—	—
0.72	43.5	49.0	61.0	—

^a A known amount of humic acid solution was titrated with metal ions. The precipitate was filtered through a 0.2 millipore membrane filter and the resulting precipitate was then washed to bring it into equilibrium with sodium chloride solutions of various ionic strengths at 26°C. The fluorescence intensity of each solution was measured

^b Ionic strength = $\frac{19.935 S}{1000 - 1.005 S}$ where S = salinity in parts per thousand

Table 6 I.r. spectra of humic acids

Humic acid	OH stretching absorption	absorbance ^a at 2930 cm ⁻¹ absorbance at 1600 cm ⁻¹	absorbance at 1400 cm ⁻¹ absorbance at 1600 cm ⁻¹	CH ₃ peak near 1370 cm ⁻¹	1700 and 1600 cm ⁻¹ absorption	Absorption in 1500–1550 cm ⁻¹ region
F	maximum at 3440 cm ⁻¹ least broad	0.34	0.64	yes	resolved	no
K	maximum 3200–3400 cm ⁻¹	0.11	0.76	no	poorly resolved	slight
T	maximum 3000–3400 cm ⁻¹	not determinable	0.84	no	poorly resolved	no
G	maximum 3050–3350 cm ⁻¹	0.14	0.80	yes	poorly resolved	slight
D	maximum 3200–3500 cm ⁻¹	0.27	0.78	yes	unresolved	yes

^a $\equiv \log I_0/I$, where I_0 is the intensity estimated in the absence of the peak being considered

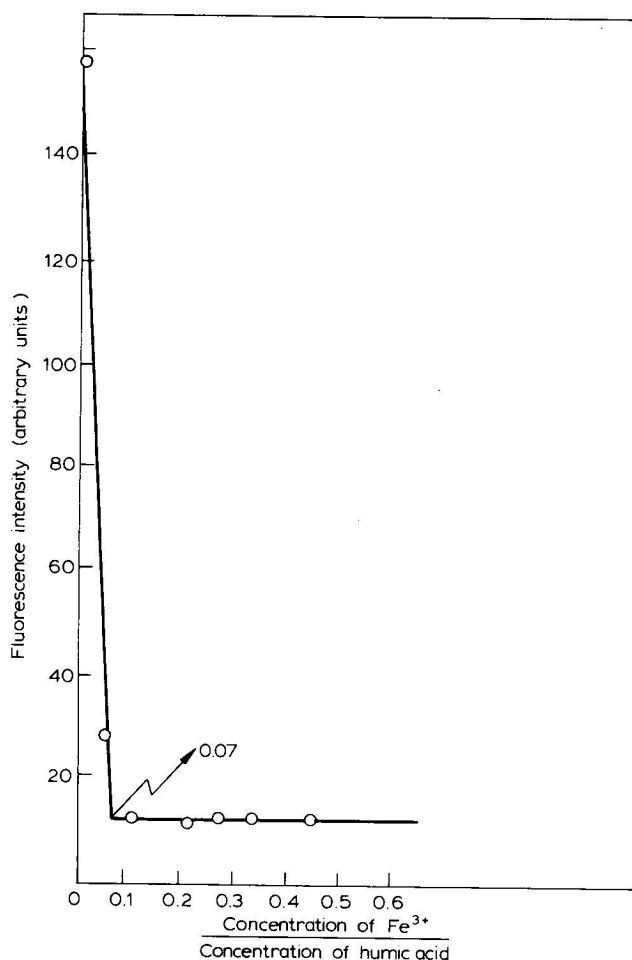


Figure 5 Change in fluorescence intensity on addition of ferric cations to humic acid F

mixture of such components is necessary to model these spectra.

The rather simpler structures for humic acids such as those proposed recently¹¹ do not appear to account for the molecular weights, the u.v. spectra and the

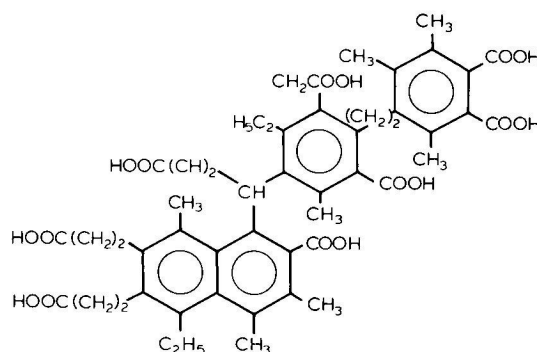


Figure 6 Structure of a hypothetical component of humic acid F

fluorescence observed in the present investigation. The suggested bonding between the aromatic nuclei shown in Figure 6 must be regarded as speculative; it is probably here that least is known about the structures of humic acids. From Figure 6 it seems that to obtain a more detailed understanding of the chemical structure of humic acids it will be necessary to fragment molecules as mildly as possible and identify the individual fragments. Such an investigation is in progress.

CONCLUSIONS

The five humic acids examined here had atomic H/C ratios in the range 1.17–1.6, the ratio increasing from peat to marine sediment humic acids. The u.v. absorption spectra of the humic acids were similar to each other and similar also to those of extracts of solid fuels²⁴ and indicate that humic acids contain a mixture of single ring and polynuclear aromatic structures. Comparison of their u.v. spectra suggested that the molecular weights of the humic acids lay in the range 800–7000 there being a parallelism between the molecular weight and the atomic H/C ratios. Investigation of their fluorescence spectra also indicated that humic acids contained polynuclear aromatic structures.

The similarity between the spectra of all five humic

acids suggested that they may all be expected to have structures similar to those found in lignites. Numerous samples of humic acids need to be examined to see whether these findings may be generalized to provide a basis for the classification of humic acids whilst investigation of the mild degradation of the humic molecules and the identification of the resulting fragments is needed to provide details of their chemical structures.

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