ESR investigations of humic acids

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Investigations of humic acids (HA) extracted from fossil Danish peat and soil samples have confirmed that by the ESR method it is possible to distinguish between HA formed under acid conditions (raised bog peats, mor) and HA originating from more alkaline environments (fen peat, mull), as suggested by Atherton *et al.* (1967). An analogous classification for recent soils (mor and mull) does not seem to be possible in our case, however, presumably because the samples contain relatively simple organic molecules (originating from partly humified material) in addition to the HA fraction. – With the ESR technique no differences can be observed between different mor types (coprogenous mor, mycogenous mor), and variation in the degree of humification of raised bog peats seems only to influence the intensity of the ESR signal.

The term "humic acid" has been used with various meanings in the geological literature. The difficulty of precisely defining a humic acid (HA) has been the main reason for this confusion. By a humic acid we mean in the present context that portion of soil organic matter (humus) which is soluble in sodium hydroxide solution and which is precipitated by acidification of the alkaline extract with hydrochloric acid.

HA's are formed in nature in connection with the microbiological degradation (humification) of organic matter. Since the humification is most intense under aerobic conditions, the largest HA production generally takes place in the upper drained layers of peat bogs or on land. Under anaerobic conditions the HA production is quantitatively minor (Teichmüller & Teichmüller 1967). This explains why one often finds strongly humified fossil peat layers alternating with weakly humified ones (see f.ex. Bahnson 1968).

The chemical constitution of HA's is more or less unknown (Degens 1967) although new light has been shed on their composition from recent degradation studies (Schnitzer and de Serra 1973). Such studies have emphasized once more the complexity of HA's and made it obvious that a single HA structure scarcely exists.

One of the spectroscopic methods which in recent years has received increasing interest for characterization of HA's is the electron spin resonance (ESR) method.

Humic acids exhibit paramagnetism in the solid state, as was first observed by Rex (1960) using ESR spectroscopy. Steelink and Tollin (1962) noted ESR spectra of HA in solution. It was early accepted that the ESR signals originated from organic free radicals present in the HA core, and it was demonstrated that many solution spectra, in contrast to solid state spectra, contained resolvable hyperfine structures.

The ESR spectra of various HA's in solution appear to be almost identical or to consist of a very few slightly different types. Atherton *et al.* (1967) suggested a classification of the HA's based on resolution differences in the ESR spectra. The spectra obtained from acid boiled HA's (ABHA) originating in acid soils and peats (pH 2.8–4.3) showed a resolved four line structure (class I), whereas those obtained from ABHA produced in more basic soils (pH 4.0–7.2) showed almost structureless spectra (class II). It was later demonstrated that a pH-value of about 4.6 in the environment where HA is formed separates class I spectra from class II spectra (Cheshire & Cranwell 1972).

We have investigated a number of HA's by means of the ESR-technique with the following objectives:

1. To test the Atherton (1967) classification.
2. To examine whether it is possible to distinguish between humus from different mor types by means of the ESR technique.
3. To investigate whether the ESR signals from HA's extracted from raised bog peats are influenced by the degree of humification.

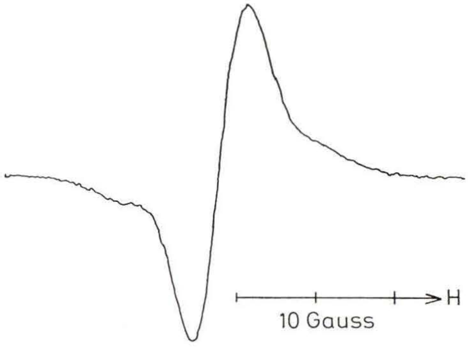
After a brief introduction of the ESR method, a discussion of the results obtained is given.

The ESR method

ESR spectroscopy detects unpaired electrons characteristic of paramagnetic species, such as organic free radicals, semiconductors, and transition metal ions as typical examples. The basic phenomenon underlying the ESR experiment is the Zeeman effect. By placing the paramagnetic sample in a static magnetic field the spin magnetic moments of the unpaired electrons are forced to align with or against the external field, the energy between the two spin states being

$$E = g\beta H_0 \tag{1}$$

Fig. 1: Solid state ESR spectrum of a humic acid.



H_0 is the applied magnetic field, β is the Bohr magneton and g the spectroscopic splitting factor or g-value. Irradiating the sample with electromagnetic energy causes electrons in the lower state to absorb energy and jump to the upper state if the frequency (υ) of the radiation fulfils the condition

$$h\upsilon = g\beta H_0 \qquad (2)$$

where h is Planck's constant. Equation (2) is called the resonance equation and is basic to all magnetic resonance experiments. For ESR spectroscopy one uses in practice a microwave radiation of about 9.5 GHz directed perpendicular to the magnetic field and sweeps the field through resonance (eqn. 2). For a "free" electron, resonance occurs at about 3400 Gauss and a single absorption line appears in the ESR spectrum. Additional lines are usually observed at the moment the unpaired electron is inherently a free radical. An interaction then arises between the magnetic moment of the electron and the magnetic moment of those nuclei having a moment different from zero. As an example, such nuclei are ^{1}H, ^{14}N, ^{17}O and ^{13}C, whereas ^{12}C and ^{16}O have magnetic moments of zero. Interaction with, say, a proton, causes the single line to split into a doublet, two chemically inequivalently positioned protons giving rise to a four line spectrum. The line pattern, or the hyperfine structure, as the pattern is called, is of immense value in elucidating structures of free radicals or processes in which free radicals take part. For more details of the hyperfine interaction see Pedersen 1969.

HA's in the solid state exhibit single line spectra (figure 1) with g-values around 2.0033. The four line spectra from HA's in solution (see figure 2) are composed of two splittings of 1.3 and 0.4 Gauss, respectively, from two inequivalent protons. The g-values and the hyperfine splittings as well as the

Table 1

Sample No.	Locality		Nature of soil	Age	ESR spectrum
Dr. 1	Draved Skov, Sønderjylland. The samples are taken in a vertical mor-profile, located close to Draved 381:8 (Iversen 1969, p. 38)		mycogenous mor	fossil	class I
Dr. 2			coprogenous mor	–	–
Dr. 3			yellowish pollen layer (mor)	–	–
Dr. 4			yellowish pollen layer (mor)	–	–
Dr. 5			humic sand	–	–
Dr. 6			humic sand	–	–
	Elevation (m)				
2218	31.56–31.54	Fuglsø Mose Djursland The samples are taken from profile 420 (Bahnson 1968)	raised bog peat	fossil	class I
2224	31.44–31.42		–	–	–
2228	31.36–31.34		–	–	–
2238	31.16–31.14		–	–	–
2243	31.07–31.05		–	–	–
4507	30.81–30.79		–	–	–
4511	30.73–30.71		–	–	–
4524	30.48–30.46		–	–	–
4528	30.40–30.38		–	–	–
4537	30.22–30.20		–	–	–
4546	30.03–30.01		–	–	–
4555	29.85–29.83		–	–	–
4559	29.77–29.75		–	–	–
El 1	Eldrup Skov, Djursland		mor	fossil	class I
El 3	Eldrup Skov, Djursland		humic sand below El 1	–	–
Ra 21	Randers Fjord area (Villumsen 1973)		fen peat	fossil	class II
Ra 23			– –	–	–
14703	Draved Skov, Sønderjylland		coprogenous (zoogenous) mor	recent	see text
14705			insectmull (initial stage of mor formation)	–	–
14706			insectmull	–	class II or I
14707			hydromull	–	class II or I
14708			mycogenous mor	–	see text
14713			mull	–	class II or I
14714			mull	–	class II or I

peculiar redox behaviour of the HA's in alkaline solutions (Atherton 1967) are strong evidence that the HA core contains radicals of the semiquinone type. No definite explanation for the unusual stability of the radicals or their actual structure has as yet been given, however.

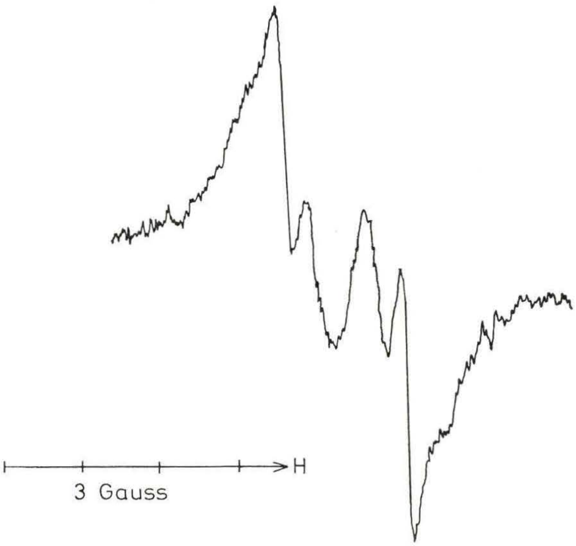


Fig. 2: Four line ESR spectrum of a humic acid in alkaline solution. (Class I spectrum).

Experimental details

The extraction of HA's and preparation of ABHA's were carried out according to the procedure described by Atherton *et al.* (1967). ESR spectra were obtained from solid samples of ABHA either using an E–3 or an E–15 Varian Associates ESR spectrometer. Solution spectra were obtained from 1 per cent solutions of ABHA's in 0.1 N NaOH. Careful air oxidation of the alkaline solution was performed by a circulation method (Pedersen 1973) in order to get reliable and fully resolved spectra. The best resolution was found to occur in the temperature range 30–60°C.

Results and Discussion

The present investigation has by and large confirmed the classification proposed by Atherton *et al.* (1967). As stated in table 1, listing all the results obtained, the series with sample Nos. Dr. 1 to Dr. 6, 2218 to 2243 and 4507 to 4559 all exhibit class I spectra with four clearly resolvable hyperfine lines (see figure 2). Identical line patterns are observed for the samples E1 1 and E1 3 although the resolution of the four lines is less pronounced for the spectra of those two samples. No hyperfine lines are

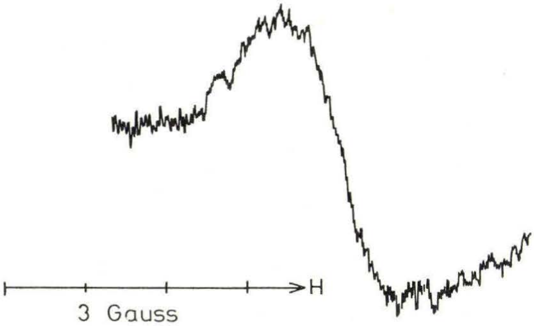
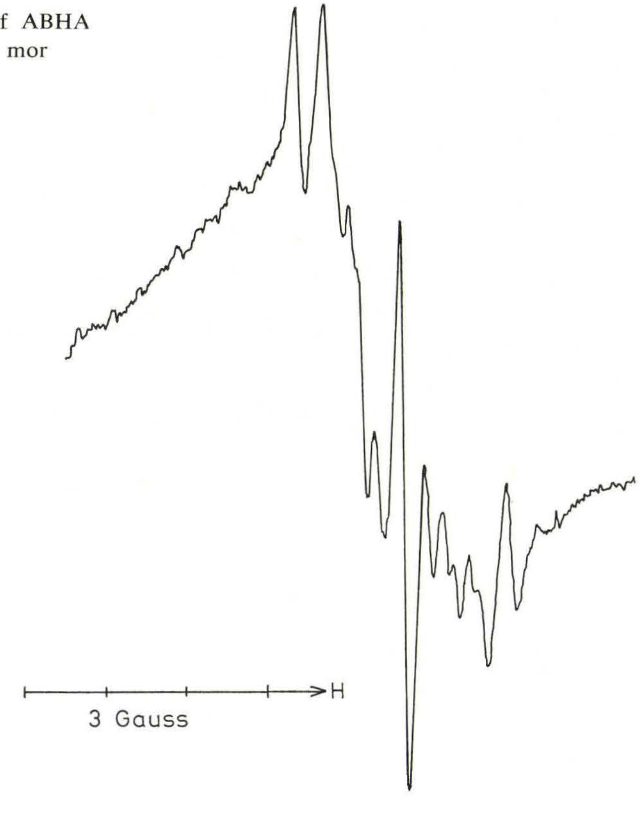


Fig. 3: Class II spectrum of a humic acid alkaline solution.

observed from the spectra of the fen peat samples Ra 21 and Ra 23 (see figure 3), the corresponding HA's thus belonging to class II as expected (mull type HA). From these results it seems possible to distinguish between mull and mor type HA's by the ESR method. For the more recent series with sample Nos. 14703 to 14714 some discrepances are observed. The

Fig. 4: ESR spectrum of ABHA extracted from a recent mor sample.



samples 14706, 14707, 14713 and 14714, all expected to give class II spectra (compare figure 3), thus exhibit a faint and distorted four line structure. The spectral resolution is quite poor, and whether the spectra are properly termed as class I or class II is an open question. Concerning the samples 14703, 14705 and 14708, expected to yield class I spectra, we obtain spectra containing 10–15 sharp lines as seen in figure 4. An explanation for this widely different result is that the samples in addition to the HA fraction contain residues of only partly humified matter consisting of relatively simple organic molecules. It is of interest to notice that no difference in the ESR spectra is observed regardless whether the HA is a result of a mycogenous mor-formation (sample No. 14708) or produced through a coprogenous process (sample No. 14703). For the fossil soil samples no differences between mycogenous and coprogenous mor could be observed by the ESR-method (samples Nos. Dr. 1 and Dr. 2).

We have investigated a series of raised bog peats in order to observe what effect a different degree of humification makes on the ESR spectrum. We observe only a change in signal intensity, i.e. samples with a humification larger than 50 % yield spectra with signals of strong intensity, whereas those less humified yield spectra with intensities of weak or medium strength. A qualitative correlation between degree of humification and ESR signal thus seems to exist.

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Dansk sammendrag

Electron spin resonans (ESR) undersøgelser har i de senere år haft stigende interesse for bl.a. undersøgelser af humussyrer (HA). Metoden har vist sig at være anvendelig til at skelne mellem HA, der er dannet under sure forhold (højmosetørv, mor) og HA, der er opstået i mere basiske miljøer (kærtørv, muld). Nærværende undersøgelse bekræfter denne klassificering af HA for fossilt materiale. For recent muld og mor er klassificeringen usikker, antagelig fordi der i den extraherede fraktion af prøvematerialet sammen med HA forekommer relativt simple organiske molekyler, stammende fra organisk stof, der endnu ikke er humuficeret. – HA stammende fra forskellige mortyper (mycogen mor, coprogen mor) giver samme ESR signal. – Undersøgelser af højmosetørv med forskellig humuficeringsgrad viser, at humuficeringsgraden kun påvirker ESR signalets intensitet.

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