About the Similarity between Polymaleic Acid and Water Soluble Humic Fractions

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The content of aromatic units in polymaleic acid (PMA) prepared by standard procedures is determined by C-13 NMR and three different oxydation procedures. It is shown that all destructive oxydation procedures lead to an aromatic carbon content that is significantly higher than the aromaticity content expected from the method of preparation and derived from the C-13 NMR spectra.

It is concluded, that benzenecarboxylic acids are formed in the process of oxydation of PMA. The yield increases with the oxydative strength of the method applied.

Introduction

The aromatic carbon content of humic substances is still a matter of controversy. The discussion has even increased in the last years, after the application of nondestructive methods such as ^{13}C-NMR to the structural analysis of this class of soil components. Some groups claim the predominance of aromatic units in humic substances from their data [1, 2], while other groups have derived from ^{13}C-NMR spectra that many humic extracts are largely aliphatic [3–7]. The controversial results can only in part be explained by the diversity of the humic materials analyzed (different extraction techniques, purification methods, origins, etc.) and differences in the experimental conditions and techniques employed.

Very recently Hatcher *et al.* [8] made a comparison between oxidative degradation and solid state ^{13}C-NMR spetroscopy for the determination of the aromaticity in soil humic substances from different climatic zones, and concluded that humic substances are highly aromatic (from 35 to 92%), though there remain significant discrepancies between the aromatic contents obtained with both methods.

In the present paper a similar study using synthetic polymaleic acid (PMA) was made. PMA is a purely aliphatic, alicyclic polymer highly substituted with carboxylic acid groups [9, 10]. Evidence from elemental analysis, infrared spectroscopy and hydrolytic analysis, led to the proposal that PMA is a model for fulvic acid type material [11]. In addition, Wilson *et al.* [12] consider PMA-like materials to be an important component of freshwater humic extracts, and according to Bracewell *et al.* [13] PMA behaves in flash pyrolisis like fulvic acid fractions.

In this paper the data obtained from high resolution ^{13}C-NMR spectroscopy of aqueous PMA solution and from the application of three degradative methods of different strengths *i.e.* oxidations with potassium persulfate, alkaline cupric oxide or potassium permanganete, will be used for the determination of the degree of structural similarity between PMA and fulvic acids.

Materials and Methods

Polymaleic acid (PMA) was obtained according to Braun and Pomakis [9]. Maleic anhydride (18.5 g) was allowed to stand in pyridine (50 cc) for 24 h at 25 °C. The acetone soluble polymeric material was precipitated with chloroform, and the water soluble polymer purified by ion exchange resins (H^+) in order to remove pyridine and convert the salt into the acid form. A yield of 70% was obtained. The elemental and functional group analysis of the polymer was: C 45.9%; H 4.4%; N 0.6%; O 49.1%; total acidity 12 meq/g; COOH groups 8.8 meq/g.

The high resolution ^{13}C-NMR spectrum of the PMA solution (200 mg in 3 cm^3 deuterium oxide) was recored in a 12 mm diameter tube on a Varian

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XL-100-15 FT spectrometer operating at 25.2 MHz. Proton-carbon couplings were removed by broad band noise decoupling. A sweep width of 10 kHz and acquisition time of 0.2 s with no pulse delay was chosen. The pulse width was set to ~30°. The spectrum shown results from 300 000 transients. The resolution of the spectrum is ± 10 Hz (approx. ± 0.5 ppm). The spectrometer was locked to an external F-19 lock. Shift values are given in the TMS scale, referenced to a coaxial internal neat TMS capillary. No attempt to correct for magnetic susceptibility effects was made.

Potassium permanganate oxidation was accomplished according to Khan and Schnitzer, [14], oxidation with alkaline cupric oxide was done as previously reported by Neyroud and Schnitzer [15], and oxidation with potassium persulfate according to Martin *et al.* [16]. In all cases the oxidation products were extracted with ethyl acetate for 24 h in a liquid-liquid extractor, derivatised with an excess of diazomethane and analysed by injection into a gas chromatograph-mass spectrometer/computer system (Hewlett-Packard model 5992B). The conditions for gas chromatography were 3000 × 3 mm stainless steel column packed with 3% OV 17 on Chromosorb WHMDS 80–100 mesh, programmed from 100 to 270 °C at a rate of 4 °C per min. Helium at a flow rate of 25 ml/min was used as carrier gas and the mass spectrometer was operated at an electron energy of 70 eV.

Results and Discussion

Fig. 1 gives the ^{13}C-NMR spectrum of PMA. It exhibits a very pronounced signal in the aliphatic region with significant intensity between 10 and 80 ppm and a distinct maximum of intensity at 50 ppm. The only other resonance that can be observed with certainty is the signal centered at 175 ppm which must be assigned to the carboxyl groups of PMA. In the aromatic range from 110 to 150 ppm some very weak broad peaks can be seen, which hardly extend above the noise level of the spectrum. Aromatic carbons thus contribute at most very little to the total peak area of this spectrum. An identical result was reached by Braun and Pomakis, [9], in their ^{1}H-NMR investigation of PMA. Wilson and Goh, [4], which to our knowledge published the only PMA-^{13}C-NMR data, find according to their own statements for their sample, which was prepared by procedures similar to the ones described above, a poor ^{13}C-NMR spectrum with a small signal at 175–182 ppm. Comparing the PMA ^{13}C-spectrum of Fig. 1 with ^{13}C-spectra of fulvic acids from various sources [3, 7, 8, 17] reveals significant differences, which may only in part be explained by the presence of non humic materials coextracted with humic substances. In the region from 60–100 ppm only a broad shoulder of the main peak is seen in PMA. ^{13}C-spectra of fulvic acids from sludge [17], marine sediments [7] and soil [1, 8, 18] do normally possess pronounced and resolved signals in this range, which are ascribed to polyhydroxyl compounds and certain carbons of amino acid residues. The most pronounced difference is seen between 110 and 160 ppm where the ^{13}C-NMR-spectra of most humic and fulvic acids [3, 7, 8, 17] do show distinct signals ascribable to the aromatic carbons of these compounds, while in the case of PMA only a marginal envelope is observed, which could result from sp^2 carbons and/or included pyridine residues.

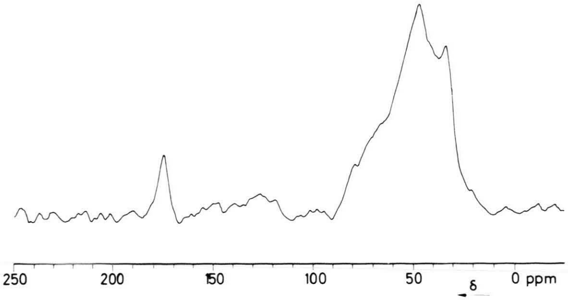

Fig. 1. ^{13}C-NMR spectrum of polymaleic acid (PMA).

Although care must be taken in comparing intensities of ^{13}C-NMR solution spectra of different substances [7, 12, 18, 19] and the results of the various authors do yield fairly different estimates of the aromatic carbon content, it can be concluded, that this spectral region accounts in the fulvic acid spectra for more than 20% of the total intensity while it is ≦5% in the PMA spectrum of Fig. 1. The difference observed in this range appears to be of structural significance. The broad resonance centered at 50 ppm carries approximately 90% of the intensity of the whole spectrum. This signal stems mainly from unsubstituted aliphatic carbons in a variety of paraffinic structures but could on the low field side also contain minor contributions from O- and N-substituted aliphatic carbons.

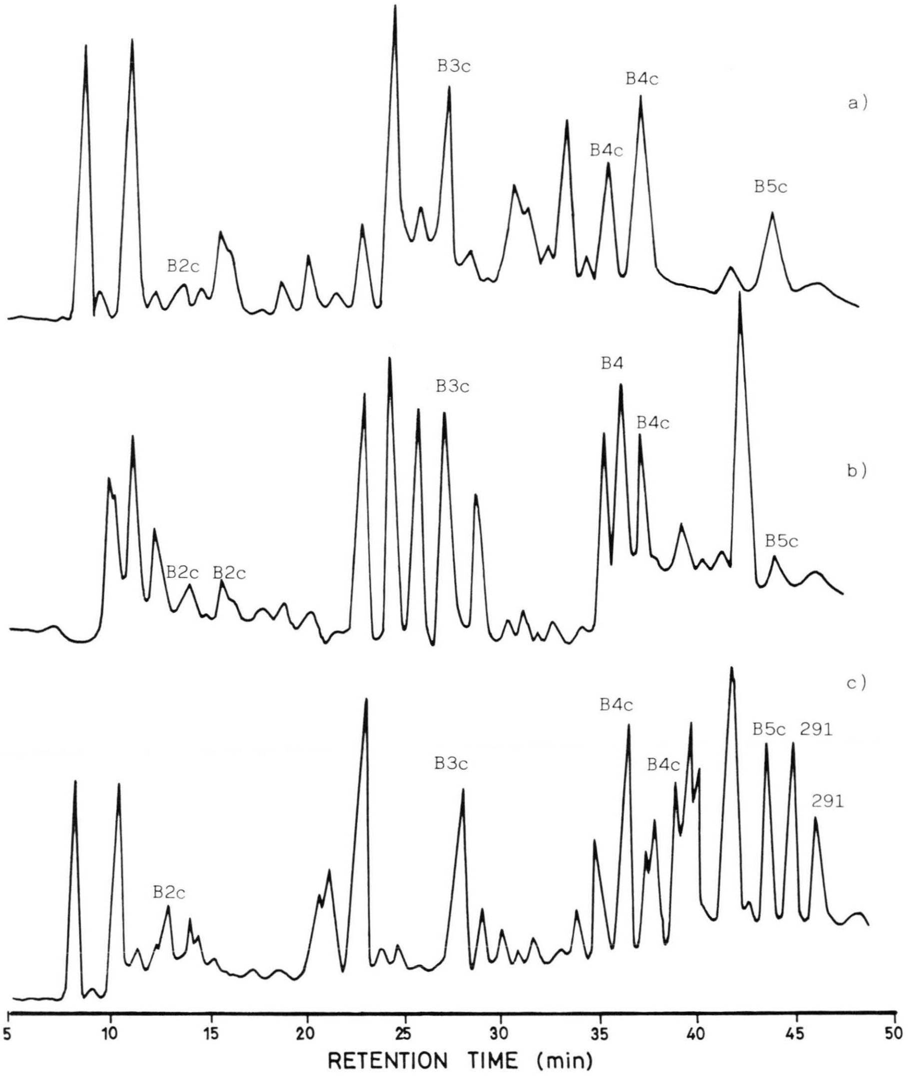

Fig. 2. Total ion chromatograms of the oxidation products of PMA isolated after a) oxidation with potassium persulfate, b) oxidation with alkaline cupric oxide, and c) oxidation with potassium permanganate.

The relatively strong carboxyl resonance at 175 ppm is a prominent feature of the PMA spectrum. It contains ~ 5% of the carbons. The carboxyl content is thus lower than calculated from chemical methods *i.e.* the classical Ca acetate procedure (8.8 meq/g) [20]. Apart from methodological limitations of this chemical method [21] one has to keep in mind, that quaternary carbons do possess relatively long spin lattice relaxation times and might, because of partial saturation, yield low intensity signals at the high pulse repetition rate employed in the spectrum of Fig. 1. Potentiometric titrations [10] demonstrate, that in PMA COOH groups are distributed randomly throughout the polymer skeleton, while they occur in local clusters in fulvic acids.

In order to have an independent control for the spectroscopic data, the behaviour of PMA against three degradative methods of different oxidative strength was studied.

Fig. 2 shows the total ion chromatograms of the oxidation products isolated after chemical degradation of PMA with a) potassium persulfate, b) alkaline cupric oxide, and c) potassium permanganate.

No quantitative relationship exists between the relative concentration of the decomposition products that appear in these chromatograms and their actual concentration in the original material [7, 22]. However it appears relevant that one observes, compared to humic acids, a much higher percentage of aliphatic constituents in the PMA chromatogram.

The aliphatic products isolated are mainly di-, tri-, and tetracarboxylic acids, alicyclic ethers and some unidentified products. The details will be presented in a subsequent paper in a discussion of the structure of PMA.

Quite unexpectedly, one observes, the presence of benzenecarboxylic acids among all three oxidation products. The peaks of these compounds are marked with B2C, B3C, B4C, and B5C in the chromatograms. In addition, other polysubstituted aromatic units with a base peak at m/e 291, also found by Oritz de Serra and Schnitzer [23], as permanganate oxidation products of humic materials, are indicated in the permanganate chromatogram.

The relative concentrations of aromatic acids increase with the strength of the oxidation methods in the order: persulfate < alkaline CuO < permanganate. However, in all these cases the aromatic unit content calculated from the yields of benzene carboxylic acids is much higher than the low contribution expected for PMA from its method of preparation and also from the ^{13}C-NMR spectrum of Fig. 1.

This conclusion is corroborated by Hatcher *et al.* [8] which find, that the ^{13}C-NMR spectrum of a soil fulvic acid yields an aromaticity value of $\sim 35\%$ while chemical oxidations lead to a calculated concentration of 80–100%; also in this case the highest aromaticity is found for permanganate oxidation.

Three plausible explanations have been advanced for the appearance of benzenecarboxylic acids among the products of oxidative degradation of humic substances [24]: they are a) the results of the oxidation of straight- and branched-chain substituents on a single benzene ring, b) the residues of cyclic aliphatic structures that were linked to an aromatic unit, c) the remnants of a fused aromatic structure. Because of the basically aliphatic nature of the starting material, PMA, neither of these possibilities can really explain the appearance of the large amounts of polysubstituted aromatic acids among the oxidation products of this compound.

Bracewell *et al.* [13], found after pyrolysis of PMA some simple aromatic hydrocarbons which according to their opinion "may arise from a small proportion of aromatic rings in the polymeric structure or may be formed by elimination reactions leading to polyconjugated chains that readily cyclise".

However, in our opinion, this mechanism should not lead to the formation of large amounts of polysubstituted aromatic units in the oxidation process. Furthermore it is of significance, that phenolic acids are absent among the oxidation products of PMA while they do make a significant contribution to the oxidation products of methylated and unmethylated fulvic fractions [16, 25].

The results presented lead to the conclusion, that the chemical degradation methods produce aromatic units from purely aliphatic precursors.

The extrapolation of the results obtained here to humic substances makes it a questionable procedure to take the isolation of benzenecarboxylic acids from the products of oxidation as a proof of the aromatic nature of the parent compound. These results could alternatively be explained by the presence of PMA-like material as structural components.

This conclusion corroborates the statement by Hatcher *et al.* [1982], that a careful reevaluation of the data resulting from the application of chemical techniques to the study of humic materials is necessary and indicated.

[1] M. A. Wilson, A. J. Jones, and B. Williamson, Nature **276,** 487–489 (1978).
[2] P. Ruggiero, F. S. Interesse, and O. Sciacovelly, Geochim. Cosmochim. Acta **40,** 1109–1114 (1979).
[3] D. H. Stuermer and J. R. Payne, Geochim. Cosmochim. Acta **40,** 1109–1114 (1976).
[4] M. A. Wilson and K. M. Goh, J. Soil Sci. **28,** 645–652 (1977).
[5] G. Ogner, Soil Biol. Biochem. **11,** 105–108 (1979).
[6] R. H. Newman, K. R. Tate, P. F. Barron, and M. Wilson, J. Soil Sci. **31,** 623–638 (1980).
[7] P. G. Hatcher, R. Rowan, and M. A. Mattingly, Org. Geochem. **2,** 77–85 (1980).
[8] P. G. Hatcher, M. Schnitzer, L. W. Dennis, and G. E. Maciel, Soil Sci. Soc. Am. J. **45,** 1089–1094 (1982).
[9] D. Braun and J. Pomakis, Makromol. Chem. **175,** 1411–1425 (1974).

[10] S. D. Young, B. W. Bache, D. Welch, and H. A. Anderson, J. Soil Sci. **32,** 579–592 (1981).

[11] H. A. Anderson and J. D. Russell, Nature **260,** 597 (1976).

[12] M. A. Wilson, P. F. Barron, and A. H. Gillam, Geochim. Cosmochim. Acta **45,** 1743–1750 (1981).

[13] J. M. Bracewell, G. W. Robertson, and D. I. Welch, J. Anal. Appl. Pyrol. **2,** 239–248 (1980).

[14] S. U. Khan and M. Schnitzer, Geoderma **7,** 113–120 (1974).

[15] J. A. Neyroud and M. Schnitzer, Soil Sci. Soc. Am. J. **38,** 907–913 (1974).

[16] F. Martin, C. Saiz-Jimenez, and F. J. Gonzalez-Vila, Soil Sci. **132,** 200–203 (1981).

[17] G. Sposito, G. D. Schaumberg, T. G. Perkins, and K. M. Holtzclaw, Environ. Sci. Technol. **12,** 931–934 (1978).

[18] F. J. Gonzalez-Vila, H. Lentz, and H.-D. Lüdemann, Biochem. Bioph. Res. Comm. **72,** 1063–1070 (1976).

[19] F. J. Gonzalez-Vila, C. Saiz-Jimenez, H. Lentz, and H.-D. Lüdemann, Z. Naturforsch. **33c,** 291–293 (1978).

[20] M. Schnitzer and V. C. Gupta, Soil Sci. Soc. Am. Proc. **28,** 374–377 (1964).

[21] K. M. Holtzclaw and G. Sposito, Soil Sci. Soc. Am. J. **43,** 318–323 (1979).

[22] O. B. Maximov, T. V. Shvets, and Yu. N. Elkin, Geoderma **19,** 63–78 (1977).

[23] M. I. Ortiz de Serra and M. Schnitzer, Soil Biol. Biochem. **5,** 287–296 (1973).

[24] M. H. B. Hayes and R. S. Swift, in The chemistry of soil constituents (D. J. Greenland and M. H. B. Hayes, ed.), Chap. 3, p. 179. John Wiley and Sons, Chichester (1978).

[25] M. Schnitzer, Recent findings on the characterization of humic substances extracted from soils from widely differing climatic zones. Proc. Symp. "Soil Organic Matter Studies". IAEA. Vienna, 117–132 (1977).