The persulfate oxidation of fulvic acids

F. Martin and F.J. Gonzalez-Vila

Centro de Edafologia y Biologia Aplicada del Cuarto C.S.I.C. Apartado 1052, Sevilla (Spain)

Eingegangen: 6.6.1982

Angenommen: 16.9.1982

Summary – Zusammenfassung

Persulfate oxidation of two fulvic acids from different soils was accomplished. This is a new mild chemical degradative method which differentiates between fulvic acids isolated from soils developed under widely differing environments. A fulvic acid isolated from an Humic Haplorthod gives as major oxidation products phenolic acids, whereas a fulvic acid from an Andic Hapludult yields benzenecarboxylic acids as most abundant compounds. The possibility to differentiate between fulvic acids from different soils, as chemical degradation with persulfate does, offers interesting prospects to the characterization of humic materials.

Abbau von Fulvosäuren mit Kaliumpersulfat

Zwei Fulvosäuren verschiedener Herkunft wurden mit Kaliumpersulfat oxidiert. Durch diese neue milde Abbaumethode werden strukturelle Unterschiede zwischen Fulvosäuren, isoliert aus sehr unterschiedlich entwickelten Böden, festgestellt. So wurden aus einer Fulvosäure, isoliert aus „Humic Haplorthod“ Phenolsäuren als Hauptprodukte erhalten, während eine Fulvosäure aus „Andic Hapludult“ hauptsächlich zu aromatischen Carbonsäuren führt. Die Unterscheidung bezüglich der Konstitution von Fulvosäuren aus bestimmten Bodentypen durch den Abbau mit Kaliumpersulfat bietet eine Möglichkeit zur Huminstoff-Charakterisierung.

Introduction

Fulvic acids (FA) are the most extensively studied of the three fractions in which the humic substances are classically divided. Schnitzer and his collaborators have applied in the last years a great variety of methods both non-degradative and degradative ones to a FA isolated from a poorly drained podzolic soil from Canada, being the sample upon which the “building block” model of humic substances is based.

Though non-degradative methods have provided in the last time valuable information on some aspects of the structure of humic substances, the degradative methods however have been the more commonly used to characterize their primary structure. Unfortunately most of the oxidative degradation processes are often so drastic that they could lead to serious alterations in the structure of humic substances and consequently to the formation of unwanted by-products, giving an erroneous picture on the parent structure. Therefore the use of mild oxidants that would most likely minimize the extent of undersirable modifications seems worthwhile of investigation.

Recently, *Martin et al.* (1981) have developed a new oxidation method with an oxidant, the potassium persulfate, which has been used for the determination of dissolved organic

carbon in seawater. When applied to humic acids (HA) only 40 % of the starting material degrades leaving a residue that can easily be recovered for further structural investigations. As persulfate has become a mild degradative oxidation method at acid pH, we thought it would be interesting to apply it to FA from two different soil types, in order to evaluate the potential ability of this method for FA's structural studies.

Materials and Methods

Two FA's were isolated from the B_h horizon of an Humic Haplorthod (FA-P) and from the A_{h1} horizon of an Andic Hapludult (FA-A) respectively. The profile descriptions of both soils are as follows:

Classification	Humic Haplorthod
Location	Ribadeo, Lugo (Spain)
Elevation	210 m above sea level
Topography	undulating to hilly
Orientation	S
Parent material	sandy materials from weathered schists
Vegetation	heather, reforested with pine

Horizon	Depth (cm)	Description
Of	0– 5	partly decomposed forest litter
OAh	5–15	black (5 YR 2/1); friable; raw humus with remains of ferns; acid
A _c	15–30	gray (10 YR 5/1); sand; friable; homogeneous; slightly acid
B _h	30–35	dark reddish brown (5 YR 3/2); sand; crumbly; friable; very organic; acid
B _s	35–50	light brown (7.5 YR 6/4); sand; spotted by organic matter
C ₁	50–	sandy schists

Some general characteristics of the B_h horizon are: (1) grain-size distribution: 58 % (2–0.2 mm class), 17 % (0.2–0.02 mm class), 18 % (0.02–0.002 mm class) and 6 % (<0.002 mm class); (2) pH (H₂O) = 3.3; and (3) C-content = 6.6 %.

Classification	Andic Hapludult
Location	Tenerife Island, Spain
Elevation	400 m
Topography	highly undulating
Orientation	NW
Parent material	basalt
Vegetation	laurisilva

Horizon	Depth (cm)	Description
Ah1	2– 50	very friable; thixotropic porous; high biological activity
Ah2	50– 90	black (10 YR 2/2); silty-clay texture friable; light porous; thixotropic; abundant roots
B	90–120	dark reddish brown (5 YR 3/4); porous; very developed structure; without cutans; crumbly; friable

Some general characteristics of the A_{h1} horizon are: (1) grain-size distribution: 0.7 % (2–0.2 mm class), 3.4 % (0.2–0.02 mm class), 23 % (0.02–0.002 mm class), 41.9 % (<0.002 mm class); (2) pH (H_2O) = 5.4; and (3) C-content = 11.3 %.

The extraction and purification procedures in both cases were as follows: two hundred grams of soil were suspended in water to eliminate roots and plant debris and then were shaken with two liters of 0.5 N NaOH solution under N_2 . After centrifugation, the supernatant was shaken with cationic resin (Dowex 50, H^+ form) and the precipitate removed by filtration, the FA solution was concentrated under vacuum, and freeze-dried.

Table 1 shows the ultimate and functional groups analyses of both FA's.

Table 1: Elementary analysis (%) and major oxygen-containing groups (meq/g)

Tabelle 1: Elementaranalyse (in %) und sauerstoffhaltige Gruppen (mval/g) zweier Fulvosäuren aus Böden

Type of fulvic acid	C	H	N	O	Total acidity	Carboxyl	Hydroxyl
Humic Haplorthod (FA P)	50.2	4.6	1.8	13.4	12.1	7.9	4.2
Andic Hapludult (FA-A)	40.6	4.1	1.4	53.9	10.2	8.8	1.4

The oxidation procedure was as follows: two hundred milligrams of each FA were dissolved in 20 ml of distilled water, mixed with 50 ml of a saturated potassium persulfate solution and heated for 2 hours at 140°C in a Teflon digestion bomb under stirring. The digest was cooled and transferred to a liquid-liquid extractor and extracted with ethyl acetate for 24 hours. After solvent evaporation, the residue was suspended in methanol and methylated with diazomethane. The methylated oxidation products were analysed by direct injection into a gas chromatographic-mass spectrometric computer system (Hewlett Packard model 5992 B). The gas chromatographic conditions were: 3000 x 3 mm stainless steel column packed with 3 % OV-17 on Chromosorb W HMDS 80–100 mesh, programmed from 90 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 at an ionizing voltage of 70 eV.

The identity of the compounds in each peak was confirmed by running its mass spectrum, searching in the computer library, and matching the mass spectrum with the known compound to which it corresponds and by co-chromatography of known compounds. No quantitative data are reported because accuracy cannot be ensured by the method employed, as discussed by *Maximov et al.* (1977).

For methylation FA was suspended in methanol and methylated with an excess of diazomethane until a constant methoxyl value content had been obtained.

Results and Discussion

Chemical structures of the identified oxidation products are listed in Table 2. In addition, the material removed consists of amino acids and carbohydrates not studied here. Because the oxidation products were methylated prior to the gas chromatographic separations, and in view to the low methoxyl content of the original FA's, it is likely that most of the carboxyls and hydroxyls occurred as such rather than as esters and ethers.

Table 2: Compounds isolated from fulvic acids after persulfate oxidation**Tabelle 2:** Bruchstücke, die sich aus Boden-Fulvosäuren nach Persulfat-Oxidation isolieren ließen (FA-P: Humic Haplorthod, FA-A: Andic Hapludult, M: methyliert)

		FA-P	FA-PM*	FA-A
1	Dimethyl succinate	+	+	+
2	Dimethyl glutarate			+
3	1,2-benzenedicarboxylic acid methyl ester	+	+	+
4	1,3-benzenedicarboxylic acid methyl ester	+	+	+
5	Dimethyl pimelate		+	+
6	Dimethyl adipate		+	
7	n-C ₁₄ fatty acid methyl ester		+	+
8	3,4-dimethoxybenzoic acid methyl ester			+
9	n-C ₁₆ fatty acid methyl ester	++	+	++
10	3 methoxy-1,2-benzenedicarboxylic acid methyl ester	+	+	
11	2 methoxy-1,5-benzenedicarboxylic acid methyl ester	+		
12	4 methoxy-1,2-benzenedicarboxylic acid methyl ester	+		
13	Isobutyl phthalate		+	+
14	1,2,3-benzenetricarboxylic acid methyl ester	++	+++	++
15	Dibutyl phthalate	+	+	+
16	n-C ₁₈ fatty acid methyl ester	++		++
17	1,3,5-benzenetricarboxylic acid methyl ester		+++	
18	3,4-dimethoxy-1,2-benzenedicarboxylic acid methyl ester	+		
19	3 methoxy-1,2,4-benzenetricarboxylic acid methyl ester	+++		++
20	2 methoxy-1,3,5-benzenetricarboxylic acid methyl ester	+++	+	++
21	4,5-dimethoxy-1,2-benzenedicarboxylic acid methyl ester		+	
22	5 methoxy-1,2,4-benzenetricarboxylic acid methyl ester	+++	++	
23	4 methoxy-1,2,3-benzenetricarboxylic acid methyl ester		++	++
24	4,5-dimethoxy-1,2,3-benzenetricarboxylic acid methyl ester	+++	+	
25	1,2,3,4-benzenetetracarboxylic acid methyl ester	++	+++	+++
26	1,2,4,5-benzenetetracarboxylic acid methyl ester	++	+++	+++
27	1,2,3,5-benzenetetracarboxylic acid methyl ester	+	+	+
28	5 methoxy-1,2,3,4-benzenetetracarboxylic acid methyl ester	+++	++	++
29	2 methoxy-1,3,4,5-benzenetetracarboxylic acid methyl ester		+	
30	2,4-dimethoxy-1,3,5-benzenetetracarboxylic acid methyl ester		+	
31	Dimethoxybenzenetetracarboxylic acid methyl ester	+		
32	Methyl benzenetetracarboxylic acid methyl ester	+		+
33	Dimethyl benzenetricarboxylic acid methyl ester	+		+
34	Dimethyl benzenetetracarboxylic acid methyl ester	+		+
35	Di-(2-ethyl hexyl) phthalate	+	+	
36	Benzenepentacarboxylic acid methyl ester	+	+	+++
37	Methoxybenzenepentacarboxylic acid methyl ester	+	+	+
38	Benzenehexacarboxylic acid methyl ester	+	+	+

* Methylated ++ medium content
 + low content +++ high content

The most abundant compounds isolated from the oxidation of FA-P, according to the peak height and in decreasing order were the phenolic acids 28, 20, 19, 22 and 24, followed by the benzenecarboxylic acids 25, 26 and 14 and the fatty acids 9 and 16. In FA-A and with the same criteria as above, the most abundant compounds were the benzenecarboxylic acids 36, 25, 26 and 14, followed by the fatty acids 9 and 16 and the phenolic acids 28, 20, 19 and 23. In the methylated FA-P major compounds were the benzenecarboxylic acids 17, 14, 25 and 26 followed by the phenolic acids 22, 23 and 28.

No alkanes were detected and methylation prior to oxidation failed to increase the variety and yield of the oxidation products, as shown for HA (*Martin et al.*, 1981). In addition to the above cited compounds some dialkyl phthalates were also identified, and although *Matsuda* and *Schnitzer* (1972) have pointed out that humic substances could adsorb large amounts of hydrophobic organic compounds, we assume that in this case they are most likely contaminants introduced in the work up procedure.

The identification of compounds 32, 33 and 34 is tentative though methyl derivatives have been found by *Schnitzer* (1974) after CuO-NaOH oxidation of a FA. The presence of methyl groups has been explained by *Hayes* and *Swift* (1978) by the generation of a carbonyl group on a β -carbon, when the reaction elapse at basic pH. As the persulfate oxidation is at acid pH the above mechanism may not be valid.

Reaction of persulfate with FA is complex and we suggest that the mechanism, as occurs for peracetic acid and potassium permanganate may be an electrophilic attack. Coupling products as reported by *Baumgartner* (1973) for oxidation of single phenolic compounds were not observed.

To a better understanding of the degradative action of persulfate oxidation, it may be interesting to compare the types of products resulting from the oxidation of FA with different oxidants. Potassium permanganate like peracetic acid at 80 °C releases as major products benzenecarboxylic acids. Sodium hydroxide, alkaline cupric oxide and peracetic acid at 40 °C give as major compounds phenolic acid structures (*Neyround* and *Schnitzer*, 1977). Most of the compounds listed in Table 2 have been obtained by the above cited degradative methods, but as for HA's (*Martin* and *Gonzalez-Vila*, 1981), persulfate differentiate between FA's formed under widely differing environments. Thus, whereas FA-P gives as major oxidation products phenolic acids, FA-A yields as major compounds benzenecarboxylic acids i.e. while FA-P gives a ratio benzenecarboxylic acids/phenolic acids similar to that formed if alkaline cupric oxide oxidation was used, FA-A gives a value for the above ratio as if potassium permanganate was employed.

The results illustrate the variability of structure of the two FA under study, since the yield of the major types of compounds is different from sample to sample. This finding is in contrast with those reported by *Schnitzer* and collaborators who failed to observe any differences in the weight ratios of benzenecarboxylic to phenolic compounds between FA's from different origins.

Recently it has been shown by non-destructive techniques such as ^{13}C NMR that humic materials can be either highly aromatic or aliphatic according to their origin and the precursors from which they are formed (*Hatcher et al.*, 1980, *Wilson et al.*, 1981). In fact, taking into account that the humification process is a complex oxidative degradation

reaction depending on numerous factors such as climate, vegetation, microbial activity, physico-chemical properties of soil, etc. it is not surprising that the basic chemical structure of the humic substances might be different in every soil. The reason why humic materials from different soils show similar structural characteristics depends probably on the drastic extraction techniques employed, and on the lack of adequate study methods, since spectroscopic techniques have been relatively unsuccessful, while most of the degradative methods alter or destroy the original chemical structure providing a biased information about the chemical make-up of these macromolecules, as have been previously discussed (Maximov et al., 1977, Hatcher et al., 1980).

The possibility to differentiate between FA's of different soils, as persulfate does, offers very interesting prospects to the characterization of humic materials.

References

- Baumgartner, J. (1973): Beitrag zur Oxidation pflanzlicher Phenole insbesondere der Ferulasäure. Thesis E.T.H. Zürich Nr. 4923 Jurin Verlag. Zürich.
- Hatcher, P.G., Rowan, R. and Mattingly, M.A. (1980): ^1H and ^{13}C NMR of marine humic acids. *Org. Geochem.* **2**, 77–85.
- Hayes, M.H.B. and Swift, R.S. (1978): The chemistry of soil constituents: (Ed. D.J. Greenland and M.H.B. Hayes). John Wiley and Sons, London.
- Martin, F. and Gonzalez-Vila, F.J. (1981): L'Oxydation au persulfate des acides humiques du sol. In Colloque Humus-Azote vol. I INRA, Reims pp. 14–18.
- Martin, F., Saiz-Jimenez, C. and Gonzalez-Vila, F.J. (1981): The persulfate oxidation of a soil humic acids. *Soil Sci.* **123**, 200–203.
- Matsuda, K. and Schnitzer, M. (1972): Reaction between fulvic acid, a humic material and dialkyl phthalates. *Bull. Environm. Contam. Toxicol.* **6**, 200–203.
- Maximov, O.B., Shvets, T.V. and Elkin, Yu.N. (1977): On permanganate oxidation of humic acids. *Geoderma* **19**, 63–78.
- Neyroud, J.A. and Schnitzer, M. (1977): Structure chimique des acides humiques et fulviques du sol. In soil organic matter studies vol. 2, IAEA Vienna pp. 157–169.
- Schnitzer, M. (1974): Alkaline cupric oxide oxidation of a methylated fulvic acid. *Soil Biol. Biochem.* **6**, 1–6.
- Wilson, M.A., Barron, P.F. and Gillam, A.H. (1981): The structure of freshwater humic substances as revealed by ^{13}C NMR spectroscopy. *Geochim. Cosmochim. Acta* **45**, 1743–1750.

[P 4167 B]