ROOM TEMPERATURE ALKALINE PERMANGANATE OXIDATION OF REPRESENTATIVE HUMIC ACIDS

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Summary—The degradation compounds released by alkaline permanganate oxidation at different temperatures from four representative soil humic acids were separated and identified by combined gas chromatography–mass spectrometry. When the permanganate oxidation was carried out at 90°C, the dominant degradation products were benzenepolycarboxylic acids, phenolic acids and fatty acids. A practically complete degradation and similar compounds were obtained after permanganate oxidation at room temperature, but a comparatively reduced proportion of aromatic molecules appeared, the alkanolic acids being major degradation compounds. As expected, the chain lengths of the latter products were found to be highly dependent on the oxidation temperature. The above differences are considered to reflect the varying resistance of the different kinds of products released in the presence of an excess of oxidizing reagent. No definitive structural conclusions are intended from the results obtained since alkaline permanganate oxidation was considered appropriate mainly to differentiate humic samples oxidized under exactly the same conditions.

INTRODUCTION

Alkaline permanganate oxidation (APO) has been extensively used in structural studies on bio- and geopolymers and was also considered a suitable method to study the composition of humic macromolecules (Schnitzer and Khan, 1972). A variety of phenolic acids, benzenecarboxylic acids and alkanolic acids have been found to be the more characteristic degradation products of humic acids (HAs). The dominance of aromatic acids amongst the permanganate degradation products together with indirect evidence based on non-destructive techniques were accepted for a long time as important reasons suggesting that the structure of HAs consisted dominantly of aromatic “building blocks”. However, further examination of HAs by ¹³C-NMR spectroscopy has led to a reconsideration of the initial estimations of the aromaticity of the humic substances based on the results of chemical degradation methods (Hatcher *et al.*, 1981).

A possible explanation is the consideration that the degradation products of HAs are not representative “building blocks” of the original polymer since APO would lead to biased structural conclusions due to the very drastic effects of permanganate on the compounds released (Maximov *et al.*, 1977). In fact, the yield of degradation products after APO generally represents a rather reduced percentage of the original weight, and the structural conclusions depended on the assumption that the final mixture of oxidation

products was a representative ‘aliquot’ of the composition of the original heteropolymer.

On the other hand, permanganate solutions with a neutral pH are used in a standard method for lignin determination (van Soest and Wine, 1968), producing a total solubilization of this relatively resistant polymer at below room temperature. This fact suggests that the humic-type polymers might also be degraded to some extent under similar mild conditions, thus avoiding the overoxidation of labile compounds, which is thought to occur during classical APO.

Our experiments have confirmed some of these assumptions, showing a high variability in the composition of the digests obtained by modifying the physical and chemical variables of the oxidation procedure. In the present article, the composition of the mixture of degradation products obtained by APO at room temperature and by a traditional degradation procedure (2 h, 90°C APO), are described for four representative soil HAs.

MATERIALS AND METHODS

The characteristics of the soils from which the HAs were isolated have already been described: soil “I” (isohumic) was a Pachic Calcixeroll (Calcic Chernozem) under grassland (Jiménez-Ballesta *et al.*, 1983); soil “P” was an Entic Haplorthod (Podsol) under heath and beech (Velasco and Almendros, 1982); soil “C” was a Typic Haplumbrept (Cambisol) under a beech forest (Almendros and Dorado, 1984; Almendros *et al.*, 1987), and soil “A” was a Mollic Ochraqualf (Acrisol) under heath (Velasco and Almendros, 1982).

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After lipid extraction (petroleum ether), the HAs were isolated by repeated extraction with 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ and 0.1 M NaOH, then precipitated (pH 1) with HCl and purified by centrifugation (27,000 *g*) at pH 12, reprecipitation, overnight demineralization with HF-HCl (1:1)1% and dialysis.

APO was carried out in a flask on a heater magnetic stirrer. The four necks of the reaction flask were connected, respectively, to a temperature controller, a 100-ml permanganate burette, an input of CO_2 -free air (a column filled with soda lime), and a reflux condenser followed by a gas-washing bottle with H_2SO_4 , a "U"-tube with $\text{Mg}(\text{ClO}_4)_2$ and finally a connection to the gas input of a CO_2 analyser "Carmograph-12".

Conditions similar to those reported by Matsuda and Schnitzer (1972) were used for 90°C oxidation (200 mg of methylated HA, 0.5 g Na_2CO_3 , 25 ml H_2O and 70 ml 4% (w/v) KMnO_4 , which were added at a rate of 2.9 ml (5 min)⁻¹. After 2-h oxidation, the mixture was cooled and acidified at pH 3, and the residual KMnO_4 was reduced with $\text{Na}_2\text{S}_2\text{O}_5$. The same was done with APO of the HAs at 20°C. The digests were extracted with ethyl acetate for 12 h, the

organic phase was treated with anhydrous Na_2SO_4 and filtered, evaporated and finally methylated with diazomethane (Schnitzer, 1974).

The yellowish colour in several digests (mainly in samples oxidized at 20°C) suggested the presence of partially-degraded oligomer fractions. These were recovered by adsorption in insoluble polyvinylpyrrolidone (Divergan P; BASF), eluted by 0.1 M NaOH and purified by cation exchange chromatography using Amberlite IR-120. After freeze-drying, these oligomer fractions were quantified gravimetrically. The methylated ethyl acetate extracts were also yellow (non-volatile material) and were purified by column chromatography in silicagel to obtain an almost colourless sample. The absorbed substances of high molecular weight were eluted with methanol and mixed with the above oligomer fraction.

The conditions for the GC and the GC-MS studies were described elsewhere (Almendros and González-Vila, 1987); the samples were injected independently on a Hewlett-Packard 5730A (FID chromatograms, for quantitative studies) and a Hewlett-Packard 5992 GC-MS system for the mass spectrometric identification of the compounds.

Fig. 1. Chromatographic separation (FID detector) of the degradation products obtained from the HA of an Umbric Cambisol (sample HA-C) upon APO at different temperatures (peak numbers refer to Table 2).

Table 1. Yield of degradation fractions from HAs after a 4-h APO period at different temperatures (weight percentages)

Sample ref.	Soil type	Degradation extent		Oligomer fractions ^a		Soluble in ethyl acetate		CO ₂ released mg (100 mg HA) ⁻¹	
		20°C	90°C	20°C	90°C	20°C	90°C	20°C	90°C
HA-I	Chernozem	96.5	100.0	3.2	0.9	11.4	14.2	2.5	5.7
HA-P	Podsol	97.3	100.0	2.7	0.1	27.9	8.0	5.0	13.1
HA-C	Cambisol	98.8	100.0	3.7	0.1	19.8	11.1	5.6	13.2
HA-A	Acrisol	100.0	100.0	2.9	0.1	13.3	5.7	6.8	18.4

^aFraction adsorbed in insoluble polyvinylpyrrolidone.

RESULTS AND DISCUSSION

The different HAs underwent extensive degradation (96–100%) at room temperature (Table 1). The yield of water-soluble oligomer fractions adsorbed in polyvinylpyrrolidone was small (about 3% at 20°C and <1% at 90°C), whereas the yields of ethyl acetate soluble products amounted to between 11–28% of the initial weight of the HAs at 20°C APO, and 6–14% at 90°C. The production of CO₂ was estimated at between 2–7 mg (100 g HA)⁻¹ at 20°C and 6–18 mg (100 g HA)⁻¹ at 90°C APO.

The organic compounds separated by GC-MS are shown in Table 2 and essentially corresponded to the degradation products described by Schnitzer and Khan (1972), but the relative amounts of the different molecules showed striking changes in terms of the temperature. Figure 1 illustrates the different chromatographic patterns in the case of the HA-C sample, and Table 3 shows the amounts of the main kinds of degradation products as percentages of the total chromatographic area. When the HAs were oxidized at room temperature, most of the products obtained were aliphatic. Except in the case of HA-I sample

(52% of aromatic products), the sum of fatty acids and alkanolic α, ω -diacids acids amounted to about 70% of the ethyl acetate soluble, volatile degradation products. After 90°C APO, however, lower percentages of alkanolic acids were found, the benzenecarboxylic acids being a major group of the degradation compounds. The low yields of aromatic products at 20°C agree with the well known fact the most aliphatics are held loosely or by low-energy bonds in the humic structure, whereas the aromatic "building blocks" require comparatively more energy for their release from the humic macromolecular systems.

The patterns of the aliphatic series also underwent important changes: the proportion of short-chain alkanolic diacids increased after heating (Table 3), whereas in the case of fatty acids (Table 3 and Fig. 2), a dominance of high molecular weight acids was found; the relative amounts of short-chain acids (<C₂₀) were more important at 20°C APO.

The dominance of short-chain fatty acids in the free lipid fractions is considered indicative of important microbial input (Simoneit and Mazurek, 1982).

Table 2. Major organic compounds identified after APO of HAs

No.	Compounds ^a	No.	Compounds ^a
1	Dimethyl glutarate	33	Methyl pentadecanoate
2	6-C Branched alkanolic α, ω -diacid dimethyl ester	34	Dimethyl dodecanedioate
3	Dimethyl adipate	35	16-C Branched fatty acid methyl ester
4	Methoxy benzenecarboxylic acid methyl ester	36	<i>n</i> -Nonadecane
5	7-C Branched alkanolic α, ω -diacid dimethyl ester	37	Methyl hexadecanoate
6	Dimethyl pimelate	38	Methoxy benzenetricarboxylic acid trimethyl ester
7	Propane tricarboxylic acid trimethyl ester	39	17-C Branched fatty acid methyl ester
8	1,2 Benzenedicarboxylic acid dimethyl ester	40	17-C Branched fatty acid methyl ester
9	8-C Branched alkanolic α, ω -diacid dimethyl ester	41	Methyl heptadecanoate
10	Dimethyl suberate	42	14-C Branched alkanolic α, ω -diacid dimethyl ester
11	1,3 Benzenedicarboxylic acid dimethyl ester	43	Dimethyl tetradecanedioate
12	1,4 Benzenedicarboxylic acid dimethyl ester	44	1,2,3,4 Benzenetetracarboxylic acid tetramethyl ester
13	Methyl dodecanoate	45	Methyl octadecenoate
14	9-C Branched alkanolic α, ω -diacid dimethyl ester (I)	46	1,2,4,5 Benzenetetracarboxylic acid tetramethyl ester
15	9-C Branched alkanolic α, ω -diacid dimethyl ester (II)	47	Methyl octadecanoate
16	Dimethyl azelate	48	1,2,3,5 Benzenetetracarboxylic acid tetramethyl ester
17	Dimethoxy benzenecarboxylic acid methyl ester	49	Methyl nonadecanoate
18	Methyl tridecanoate	50	16-C Branched alkanolic α, ω -diacid dimethyl ester
19	C-10 Branched alkanolic α, ω -diacid dimethyl ester	51	Methyl eicosanoate
20	Dimethyl decanedioate	52	Benzenepentacarboxylic acid pentamethyl ester
21	Methoxy benzenedicarboxylic acid dimethyl ester (I)	53	Methyl heneicosanoate
22	14-C Branched fatty acid methyl ester	54	Methyl docosanoate
23	Methyl tetradecanoate	55	Methyl tricosanoate
24	11-C Branched alkanolic α, ω -diacid dimethyl ester	56	Methyl tetracosanoate
25	Dimethyl undecanedioate	57	Methyl pentacosanoate
26	Methoxy benzenedicarboxylic acid dimethyl ester (II)	58	Dimethyl docosanedioate
27	Methoxy benzenedicarboxylic acid dimethyl ester (III)	59	Methyl hexacosanoate
28	1,2,3 Benzenetricarboxylic acid trimethyl ester	60	Methyl heptacosanoate
29	15-C Branched fatty acid methyl ester (I)	61	Methyl octacosanoate
30	15-C Branched fatty acid methyl ester (II)	62	Methyl nonacosanoate
31	1,2,4 Benzenetricarboxylic acid trimethyl ester	63	Methyl tricosanoate
32	1,3,5 Benzenetricarboxylic acid trimethyl ester		

^aAs methyl esters (roman numbers indicate different isomers).

Table 3. Composition of the digests obtained after APO of HAs at different temperatures

Major groups of degradation products ^a	(Chernozem) HA-I		(Podsol) HA-P		(Acrisol) HA-A		(Cambisol) HA-C	
	20°C	90°C	20°C	90°C	20°C	90°C	20°C	90°C
Fatty acids (F)	29.9	1.4	52.5	45.2	48.4	38.5	18.4	14.6
Alkanoic α , ω -diacids $> C_3$ (D)	4.8	0.3	20.5	11.2	21.3	2.6	62.3	63.2
Phenolic acids	13.7	20.0	16.2	12.0	19.4	14.5	5.9	2.2
Benzenecarboxylic acids	38.0	72.4	2.0	20.3	3.5	27.7	0.9	8.4
Total aromatics	51.7	92.4	18.2	32.3	22.9	42.2	6.8	10.6
$F \geq C_{20}$ -to- $F < C_{20}$	0.2	1.1	2.4	10.8	2.0	6.6	0.5	5.5
$D \geq C_6$ -to- $D < C_6$	6.9	2.8	13.7	2.6	28.5	1.4	19.4	0.6

^aAs methyl esters (percentages of the total chromatographic area).

However, in the case of the fatty series released upon oxidative degradation, the distribution patterns (Fig. 2) seem to reflect the effects of the experimental conditions, bearing out the experiments which demonstrate the higher resistance of the long-chain fatty acids to APO (Machihara and Ishiwatari, 1983).

The above differences may be explained in terms of selective resistance of the degradation products in the digest to an excess of permanganate: one series of compounds (Table 4) was exclusively found after 20°C APO (generally fatty acids of low molecular weight and alkanoic diacids), whereas other ones were only detected after 90°C degradation (certain benzenepolycarboxylic acids, propanetricarboxylic acid, etc.). The former group of compounds is interpreted as being composed of relatively-labile molecules, whereas the latter group would be permanganate-resistant molecules, the relative amounts

of which tend to increase as a concomitant effect of the destruction of the former.

The important differences and contrasting results obtained by the two sets of experiments at different temperatures ought to be evaluated carefully: the possibility of partial degradation of the HAs at 20°C APO was examined (i.e. a selective removal of the less-firmly associated aliphatic "building blocks" with simultaneous formation of condensed (ABHA-like) residue enriched in non-degraded aromatic structures). However, the small proportion of the non-degraded humic residue and of the oligomer fraction was not considered sufficient to explain the differences observed in the composition of the digests (Table 1). In addition, the examination of their IR spectra and atomic H/C ratios suggested that they were materials rather similar to the initial HAs with a higher degree of oxidation.

Fig. 2. Distribution diagrams (C number vs relative abundance) of fatty acids obtained upon APO of HAs at different temperatures.

Table 4

Sample	Organic compounds ^a identified after 20°C permanganate degradation; not found after 90°C degradation
HA-I	C ₆ -D; C ₁₂ -F; C ₁₃ -F; C ₁₁ -D; C ₂₀ -F; C ₂₁ -F; C ₂₅ -F
HA-P	C _{6b} -D; C _{9b} -D; C ₉ -D; C _{10b} -D; C ₁₀ -D; C ₁₄ -D
HA-A	C ₈ -D; C ₁₂ -F; C ₉ -D; 2M BC; C ₁₃ -F; C ₁₄ -F; C ₁₅ -F; C _{16b} -D; C ₂₂ -D
HA-C	C ₁₂ -F; C ₁₃ -F; C _{10b} -D; C ₁₀ -D; C ₁₄ -F; C _{11b} -D; 1M B3C; C _{14b} -D; C ₁₄ -D; C _{16b} -D
Sample	Organic compounds ^a identified after 90°C permanganate degradation; not found after 20°C degradation
HA-I	1,4 B2C; 1,3,5 B3C; 1,2,3,5 B4C
HA-P	P3C; 1,2 B2C; 1,2,3 B3C; 1,2,4 B3C; 1,3,5 B3C; 1,2,3,4 B4C; 1,2,3,5 B4C
HA-A	P3C; 1,2 B2C; 1,2,3 B3C; 1,2,4 B3C; 1,2,3,4 B4C; 1,2,3,5 B4C
HA-C	1,2,3 B3C; 1,2,4 B3C; 1,2,3,4 B4C; 1,2,4,5 B4C; 1,2,3,5 B4C; C ₂₇ -F

^aAs methyl esters (peaks representing <0.2% of the total chromatographic area were not taken into consideration).

Abbreviations used: D, alkanolic α , ω -diacid dimethyl ester; F, fatty acid methyl ester; BnC, benzene (n = di, tri, tetra) carboxylic acid n -methyl ester; M, methoxy; P3C, propanetricarboxylic acid trimethyl ester.

The horizontal sequence (left to right) follows the chromatographic retention time.

Origin of the soil HAs: HA-I = Chernozem, HA-P = Podsol, HA-C = Cambisol, HA-A = Acrisol.

The neoformation of aromatic compounds during the 90°C APO was also checked for it possibly being responsible for the large increase in the amount of aromatic compounds (Popoff and Theander, 1976). Cellulose and chitin were oxidized under the above experimental conditions at both temperatures, but no aromatic acids were found in the ethyl acetate extract of the digests. This coincided with the study on model compounds by Randall *et al.* (1938).

As indicated above, the differences found have their most likely explanation in the differential resistance of the oxidation products in the course of APO. According to the results from standard compounds (Machihara and Ishiwatari, 1983), benzenecarboxylic acids are considered to be permanganate-resistant molecules, whereas alkanolic acids and phenolic acids are progressively broken down into short dicarboxylic molecules. Our results also suggest a tendency to selective destruction of phenolic acids after permanganate attack (Maximov *et al.*, 1977).

The low yields of oxidation products obtained in both experimental conditions tested—in spite of the practically complete degradation of the original sample—suggested that APO presented an ability to depolymerize the original macromolecule similar to that of destroying the degradation products of low molecular weight. From the results obtained, the use of APO seem appropriate to obtain fingerprint chromatograms from different humic samples oxidized under exactly the same conditions, but structural studies necessarily require that the degradation products include—at least qualitatively—a representative portion of the whole polymer. In fact, both the results of the APO at 20 or 90°C suggests that the aromatic content may differ highly within the HA studied, in agreement with the NMR studies which has shown that the aromaticity of HAs may vary widely in terms of their origins. Judging from the effects of the 20°C APO, permanganate is considered a drastic oxidant for humic-type substances; the yields are not large, even in the most favourable conditions (Reuter *et al.*, 1983), whereas the composition of the digests are highly dependent on the temperature.

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