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Permanganate Oxidation of the Ethanol Extract of Raw Humus

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Methylated and unmethylated ethanol extracts of raw humus were oxidized by potassium permanganate at pH 9 – 10. The methylated extract gave four benzenecarboxylic acid methyl esters (3 % yield), seven methoxy-benzenecarboxylic acid methyl esters (9 % yield), 3,4-dimethoxy-benzoic acid methyl ester (22 % yield) and five dicarboxylic acid dimethyl esters (57 % yield). The unmethylated extract gave four benzenecarboxylic acid methyl esters (5 % yield), 2-methoxy-1,4-benzenedicarboxylic acid dimethyl ester (1 % yield) and eight dicarboxylic acid dimethyl esters (94 % yield). The compounds identified have also been found after oxidation of other humic fractions. Compared to these fractions, methylated and unmethylated ethanol extracts yielded a higher amount of aliphatic structures and the number of aromatic methoxy compounds obtained from methylated extract was considerable reduced. It is concluded that carboxyl and/or alkyl polysubstituted humic phenols or condensed structures evidently contribute less to the structure of the ethanol extract of raw humus than to other humic fractions of the same soil material.

The chemistry of the ethanol extractable soil organic matter is not well known. The material is believed to consist of resins, fats and waxes.[1] The purpose of the present work was to investigate the ethanol extractable material from raw humus by potassium permanganate oxidation of methylated and unmethylated samples. This method has proved useful for other humic fractions like raw humus, humin, humic acids and fulvic acids [2–4] and acid boiled raw humus.[5]

Gel filtration of the ethanol extract confirmed that it, like all other humic fractions, is a mixture of components of different chemical and physical properties with relatively high molecular weights.

The oxidation products identified are represented in Table 1. The same compounds have previously been found among the oxidation products of a number of humic fractions together with other aromatic and aliphatic

Table 1. Yields of compounds isolated from methylated and non-methylated ethanol extractable humic material after oxidation. Yields refer to 100 g extract.

Component No.	Compound	Methylated extract mg	Unmethylated extract mg
1	1,2-Benzenedicarboxylic acid dimethyl ester	11	
2	1,2,4-Benzenetricarboxylic acid trimethyl ester	24	11
3	1,2,3,4-Benzenetetracarboxylic acid tetramethyl ester	14	40
4	1,2,4,5-Benzenetetracarboxylic acid tetramethyl ester		26
5	Benzenepentacarboxylic acid pentamethyl ester	9	20
6	4-Methoxy-benzoic acid methyl ester	73	
7	3-Methoxy-1,2-benzenedicarboxylic acid dimethyl ester	37	
8	4-Methoxy-1,3-benzenedicarboxylic acid dimethyl ester	67	
9	2-Methoxy-1,4-benzenedicarboxylic acid dimethyl ester		17
10	4-Methoxy-1,2,3-benzenetricarboxylic acid trimethyl ester	14	
11	6-Methoxy-1,2,4-benzenetricarboxylic acid trimethyl ester	12	
12	2-Methoxy-1,3,5-benzenetricarboxylic acid trimethyl ester	3	
13	5-Methoxy-1,2,3,4-benzenetetracarboxylic acid tetramethyl ester	7	
14	3,4-Dimethoxy-benzoic acid methyl ester	500	
15	Adipic acid dimethyl ester	37	34
16	Pimelic acid dimethyl ester	200	270
17	Suberic acid dimethyl ester	480	480
18	Azelaic acid dimethyl ester	520	660
19	Sebacic acid dimethyl ester	100	250
20	Hendecanedioic acid dimethyl ester		180
21	Dodecanedioic acid dimethyl ester		57
22	Tridecanedioic acid dimethyl ester		10
23	Dimethoxy-carbomethoxy-diazine I	180	
24	Dimethoxy-carbomethoxy-diazine II	27	
25	Benzoyl-benzenetricarboxylic acid trimethyl ester		32
	Not identified	650	430

carboxylic acids.[2–5] A number of not identified compounds constitute a significant part of the oxidation products.

In general, the differences between the yields of oxidation products from methylated and unmethylated ethanol extract correspond to the differences found for methylated and unmethylated raw humus, humin, humic acid and fulvic acid [4] and acid boiled raw humus [5] although the number of compounds identified from the ethanol extractable material is smaller, and their yields differ from that of the above mentioned humic fractions. The discussion will therefore be limited to a comparison of the main differences found between the ethanol extractable material and other humic fractions of the same soil material.[4,5]

The chromatographic behavior (GLC and TLC) of the extracted material and the similarity to other humic fractions regarding the composition of oxidation products, indicate that the ethanol extractable matter is related to humic materials. No significant amounts of waxes or fats have been found.

Permanganate oxidation of methylated samples, recently demonstrated that the total amount of dicarboxylic acid dimethyl esters increased (from

17 to 44 %) and the amount of dimethoxy-benzenecarboxylic acid methyl esters decreased (from 54 to 30 %) in the order humin, humic acid, "high molecular weight" fulvic acid and "low molecular weight" fulvic acid.[5] Thus, the more soluble humic fractions had a higher contribution of aliphatic structures. As seen from Table 2, summarizing the compounds isolated, this trend is even more pronounced for the ethanol extractable material.

Table 2. Groups of compounds isolated from methylated and non-methylated ethanol extractable humic material after oxidation. Yields refer to 100 g samples. The contribution of each group relative to the total amount of identified compounds is given in per cent.

	Methylated extract		Unmethylated extract	
	g	% [a]	g	% [a]
Benzenepolycarboxylic acid polymethyl esters	0.06	3	0.10	5
Methoxy-benzenepolycarboxylic acid polymethyl esters	0.21	9	0.02	1
Dimethoxy-benzenecarboxylic acid methyl ester	0.50	22		
Dicarboxylic acid dimethyl esters	1.33	57	1.93	94
Dimethoxy-carbomethoxy-diazines	0.21	9		
Total	2.31	100	2.05	100

[a] Represented as % of total yield.

Between methylated and unmethylated ethanol extractable material, the difference in yield of benzenecarboxylic acids found by oxidation is small (Table 2). This is in contrast to results found from other humic materials, and might indicate a smaller contribution of the condensed structures expected to be present in soil organic matter.[3-6]

The decrease in amount of condensed structures might be connected to the smaller number of methoxy-benzenecarboxylic acid methyl esters detected. The usually relatively important 3-methoxy-1,2,4-benzenetricarboxylic acid trimethyl ester and 4-methoxy-1,2,3,5-benzenetetracarboxylic acid tetramethyl ester are not at all found among the products of the methylated ethanol extractable material.

The most striking difference between the ethanol extractable material and other humic fractions of the same raw humus material was found for the dimethoxy derivatives isolated. The methylated ethanol extract yields exclusively 3,4-dimethoxy-benzoic acid methyl ester (compound 14) by oxidation and methylation, whereas also six other dimethoxy-benzenepolycarboxylic acid polymethyl esters are usually found from other humic fractions.[4,5] The yields and number of aromatic methoxy and dimethoxy compounds clearly indicate that alkyl and/or carboxyl polysubstituted phenols contribute relatively little, and carboxyl substituted or alkyl polysubstituted *o*-diphenols nothing, to the composition of the ethanol extractable material. This will also apply to the condensed structures.

The relative contribution of the different dicarboxylic acid dimethyl esters identified, compound 15 – 22, Table 1, corresponds well with results found earlier [4,5] and will not be discussed here.

The significance of the dimethoxy-carbomethoxy-diazines (compounds 23 and 24) has been described elsewhere.[5] The fact that they are isolated in relatively high yield from the methylated sample, shows that the structures yielding these compounds are of significant importance also for the ethanol extractable material.

Compound 25 is most probably a benzoyl-benzenetricarboxylic acid trimethyl ester. The mass spectrum shows peaks at m/e 105, 77, and 51 due to the benzoyl cation and its decomposition products. The base peak is at m/e 279 representing a tricarbomethoxyl-benzoyl cation. Peaks at m/e 356 and 325 represent the molecular ion and $M-31$, respectively. The infra-red spectrum gives a strong ester carbonyl absorption (1725 cm^{-1}) and a weaker carbonyl absorption (1675 cm^{-1}).

Further investigations showed that compound 25 is an oxidation product of a number of unmethylated humic fractions, the same as earlier investigated.[4,5] Compound 25 was not detected in humic materials methylated prior to oxidation. Thus, the original humic structure, probably phenolic, yielding compound 25 by oxidation, is apparently stabilized toward permanganate oxidation by methylation to give other oxidation products. It might therefore well constitute a part of the condensed phenolic structures of humic materials previously reported.[5]

The results obtained here indicate that the ethanol extractable material is similar to other humic fractions in composition, although more aliphatic. Its occurrence in soil, therefore, probably originates from biochemical reactions in humus formation and/or degradation.

EXPERIMENTAL

Material. Details of the raw humus and the extraction are given elsewhere.[6] The dry raw humus was extracted with ether and thereafter ethanol. The ethanol extract is a dark waxy substance extracted in 1.8 % yield. (C 63.6; H 8.98; OCH_3 6.2). After methylation with diazomethane in methanol the methoxyl content increased to 14.0 %.

Oxidation. The sample (5 g extract or methylated extract) was oxidized by potassium permanganate at pH 9 – 10 according to the method outlined elsewhere.[5] Yields: 5 g ethanol extract gave 0.70 g acids, after methylation the yield was 0.50 g of esters; 5 g methylated extract gave 0.84 g acids which were methylated to give 0.68 g esters. The loss of matter by methylation is due to volatile esters; these have not been analyzed.

The ester mixture obtained by methylation of the extracted oxidation products was analyzed by preparative thin-layer and gas chromatography, followed by micro infrared and mass spectroscopy. Details of the procedure are given elsewhere.[5]

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