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Note

Separation of humic acid oxidation products on Sephadex LH-20

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Destructive oxidation of coals and humic acids resulted in a complex mixture of water-soluble polycarboxylic acids, and this mixture, together with aliphatic dicarboxylic and benzenecarboxylic acids, invariably included more complex compounds, the methyl esters of which were not distilled even by molecular distillation under high vacuum[1].

Numerous attempts to elucidate the nature of these acids[2–6] did not yield satisfactory results. By using inflexible methods (thermal decarboxylation and hydrogenolysis), which left room for doubt as to the character of the substances isolated, the high-molecular oxidation products were shown to include compounds with a condensed carbon skeleton (naphthalene, phenanthrene, pyrene, perylene derivatives, etc.). An increased amount of oxygen that did not correspond to the carboxyl group content indicated the possible presence of heterocyclic systems and quinones.

We have tried to elucidate the nature of these oligomeric degradation products by converting them into the corresponding hydrocarbons by mild reduction methods (using lithium aluminium hydride etc.)*. When collecting the starting high-molecular material, it was desirable to avoid the use of high-temperature vacuum distillation that could cause denaturation of non-volatile components. It appeared possible, by using gel chromatography, to separate the oxidation products into a low-molecular fraction, containing mainly benzenecarboxylic acids**, and a high-molecular fraction, composed of different polycyclic acids. For this purpose, we decided to use lipophilic Sephadex LH-20, even though the conditions required for chromatography on this gel are not simple.

In an attempt to separate a mixture of equal amounts of the methyl esters of *p*-methoxybenzoic and 2-methoxynaphthalene-1-carboxylic acids in methanol–chloroform (40:60), the separation achieved was incomplete. Moreover, contrary to the basic principles of gel chromatography, *p*-methoxybenzoic acid ester eluted first.

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* The results will be reported elsewhere.

** Blocking of phenolic hydroxyl groups of the starting material by methylation would also yield methoxybenzenecarboxylic acids.

EXPERIMENTAL AND RESULTS

In the fractionation experiments described below, we used water-soluble products obtained by oxidizing pre-methylated brown coal humic acids with alkaline permanganate solution. The preparative technique and oxidation conditions were described in a previous paper by Maximov *et al.*[7,*]. The mixture obtained was methylated first with methanol containing 10% of hydrochloric acid, and then with diazomethane.

Bearing in mind the very complex composition of the esters studied and the impossibility of determining the molecular weights of numerous fractions obtained by chromatography, we have developed a very simple test that would permit the approximate assay of the content of non-volatile components distilled under vacuum.

Portions of ester (10 mg) in a quartz weighing boat were placed in a test-tube connected to a diffusion pump. The tube was flushed with argon, evacuated to $2 \cdot 10^{-3}$ mm and inserted in a tubular oven (bronze block) heated to 175°. After heating for 40 min, the test-tube was extracted from the oven and the non-volatile esters remaining in the boat were weighed. The time and temperature of heating involved were chosen following special experiments with hexamethyl mellitate, which evaporated within the required time period. Hence, the non-volatile residue could contain only esters of acids with more complex structures than benzenecarboxylic acids. The starting mixture contained 49.2% of such esters.

The initial separation tests were run on a Sephadex LH-20 column (10 mm I.D., the height of the inflated gel layer being 530–540 mm). The weighed portion (*ca.* 100 mg) was introduced with a minimum amount of the solvent that was subsequently used for elution. The elution rate was 1.6 ml/10 min.

TABLE I

SEPARATION OF HUMIC ACID OXIDATION PRODUCT ESTERS ON SEPHADEX LH-20

No.	*Benzene*			*Chloroform–methanol (60:40)*			*DMSO*		
	Eluate volume (ml)	*Fraction yield (%)*	*Non-volatile esters (%)*	*Eluate volume (ml)*	*Fraction yield (%)*	*Non-volatile esters (%)*	*Eluate volume (ml)*	*Fraction yield (%)*	*Non-volatile esters (%)*
1	3.2	8.3	52.5	2.5	8.2	72.2	6.3	5.5	91.1
2	0 8	11.3	59.8	7.7	9.9	79.7	2.1	7.4	79.9
3	0.8	20.7	56.8	2.3	12.2	76.0	2.8	17.5	85.1
4	0.8	20.0	49.2	2.3	20.6	51.3	3.5	37.0	48.3
5	1.6	11.8	36.7	2.8	25.4	23.2	2.1	18.3	7.3
6	2.2	17.7	16.0	5.9	15.3	18.9	21.2	11.0	8.0
7	26.2	9.2	18.7	19.1	5.7	69.2	—	—	—

The combined fractions were evaporated, and the residues were dried in a vacuum at 50° to determine the content of non-volatile esters. Table I shows the results for three of the solvents tested. The first fractions yielded by the column were considerably enriched by non-volatile components, the extent of enrichment for the

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[*] See sample No. 4.

different solvents increasing in the following order: water* < benzene < methanol < chloroform–methanol (60:40) < dimethyl sulphoxide (DMSO). The fractionation of free acids in an aqueous solution was completely unsatisfactory. The separation of esters in organic solvents also proved to be incomplete, and non-volatile substances were present in all of the fractions obtained. Furthermore, the main mass of non-volatile components was in the intermediate fractions, although the latter contained them in lower concentrations than the initial fractions.

When using DMSO, the elution volumes were minimal, and the non-volatile components were obtained in the most concentrated form. Therefore, the separation in this solvent was repeated three times with a large ester portion (8.90 g) on a gel column (40 mm I.D., height 840 mm). The results shown in Fig. 1 can be regarded as satisfactory if one takes account of the fact that the method used in the procedure was rather conventional.

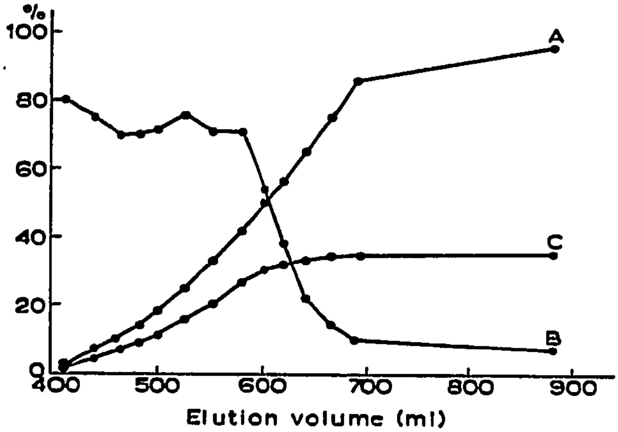

Fig. 1. Separation of methyl esters of humic acid oxidation products on Sephadex LH-20 in DMSO medium. A, cumulative yield of ester fractions; B, non-volatile ester content in fractions; C, cumulative content of non-volatile esters in eluate.

The next stage involved combining of fractions containing (a) over 70%, (b) 50–20% and (c) less than 20% of non-volatile esters. Each of the fractions obtained was re-separated on the same column for subsequent enrichment with high-molecular material (first fraction group), on the one hand, and with low-molecular substances (third fraction group), on the other. Gas–liquid chromatography was used to show that the resultant eluates, apart from the non-volatile residue, also contained benzene-carboxylic acid esters. For low-molecular fractions, in which these acids were dominant, the experiment also involved the identification of individual components with a semi-quantitative estimate of their content (see Table III and Fig. 2).

In accordance with the evidence thus obtained, the eluate groups were finally combined; the first (high-molecular) combined fraction did not contain benzene-carboxylic acid esters, while the second (intermediate) combined fraction contained trace amounts. The fraction compositions are shown in Table II. The overall fraction

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[*] Unlike with other solvents, initial water-soluble oxidation products were separated in water.

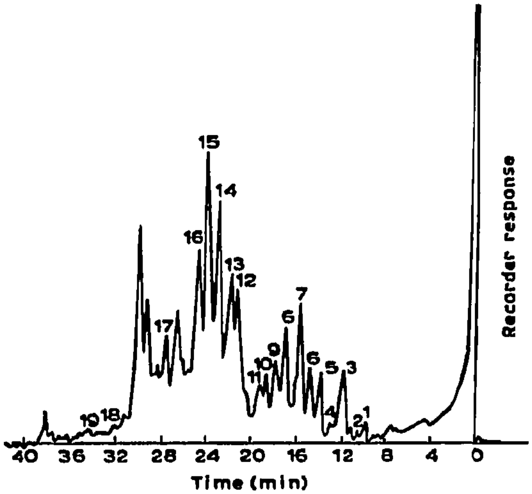

Fig. 2. Chromatogram of low-molecular ester portion. Gas chromatograph: Pye Unicam, Model 104, flame ionization detector, and dual glass columns (1500 × 6 mm I.D.) containing 3% OV-1 on Gas-Chrom Q (100–120 mesh). Injection port temperature, 350°; column temperatures were programmed from 100° to 300° at 5°/min. Argon and hydrogen flow-rate, 60 ml/min. For peak numbers, see Table III.

TABLE II

ESTER FRACTION COMPOSITIONS AFTER REPEATED SEPARATION ON SEPHADEX LH-20

Ester fraction	*Yield (%)*	*Non-volatile esters (%)*	*C (%)*	*H (%)*	*OCH_3 (%)*	*Oxygen in neutral form (%)*	*Mol. weight*	*Content (mequiv./g)*		
								*COOH**	*$COOCH_3$***	*OCH_3****
Initial mixture	100.0	49.2	53.32	5.61	26.60	12.0	295	1.06	7.50	1.07
High-molecular	33.6	97.2	53.62	5.45	19.90	16.4	570	1.37	6.16	0.25
Intermediate	22.2	41.5	50.06	5.35	23.64	16.5	264	1.25	7.52	0.10
Low-molecular	24.0	6.3	52.66	5.60	30.96	10.4	—	1.08	7.45	2.52

* Titration with alkali.

** Saponification with alkali.

*** Difference between total methoxyl and carbomethoxyl group content.

yield was approximately 80% of the starting material; losses were caused by partial volatilization of esters during solvent removal, and also by consumption of material used to determine the non-volatile components (see above) in numerous eluate fractions.

The selective loss of volatile esters resulted in certain disproportions, *e.g.*, in neutral oxygen content.

The results in Table III show that water-soluble humic acid oxidation products apparently do not contain compounds with molecular weights considerably above 500.

An almost complete absence of phenolic hydroxyl groups (low OCH_3 content) and a high proportion of compounds containing oxidation-resistant neutral oxygen forms are characteristic of the high-molecular and intermediate fractions.

Thus, with the aid of preparative gel chromatography on Sephadex LH-20, it

TABLE III

COMPOSITION OF LOW-MOLECULAR PORTION OF HUMIC ACID OXIDATION PRODUCTS RE-SEPARATED ON SEPHADEX LH-20 COLUMN

No.	*Acid*	*Fraction No. in order of elution from column*[*]					
		1	*2*	*3*	*4*	*5*	*6*
1	3,4-Methoxybenzoic	—	—	—	—	—	++
2	4-Methoxyisophthalic	—	—	—	—	tr	+
3	3,4,5-Trimethoxybenzoic	—	—	—	++	++	+++
4	5-Methoxyisophthalic	—	—	—	+	+	++
5	4-Methoxyphthalic	—	—	—	+	+	+++
6	Hemimellitic	—	—	tr	++	++	+++
7	Trimellitic	—	—	tr	++	+++	+++
8	Isohemipinic	—	—	tr	++	+++	+++
9	Trimesic	—	—	+	++	+++	+++
10	3-Methoxytrimellitic	—	+	+	++	++	++
11	5-Methoxytrimellitic	—	+	+	++	++	tr
12	2-Methoxytrimesic	—	+	++	++	++	tr
13	6-Methoxytrimellitic	—	+	+	+++	++	tr
14	Prehnitic[**]	++	++	+++	+++	++	+
15	Pyromellitic	++	+++	+++	+++	++	+
16	Mellophanic[***]	+++	+++	+++	+++	++	+
17	5-Methoxyprehnitic	+++	+++	++	+	tr	tr
18	Benzenepentacarboxylic	tr	tr	tr	—	—	—
19	Mellitic	tr	tr	tr	—	—	—

[*] tr = trace; + = small amount; ++ = medium amount; +++ = large amount.
[**] Benzene-1,2,3,4-tetracarboxylic acid.
[***] Benzene-1,2,3,5-tetracarboxylic acid.

was possible to separate humic acid oxidation products into groups of compounds that were very different in composition and properties. This was not feasible with the techniques used previously.

In conclusion, certain peculiarities in the chromatographic behaviour of benzenecarboxylic acid esters on Sephadex LH-20 should be noted. Thus, with re-separation of the low-molecular ester portion, the elution of polybasic acid esters took place first, mono- and dicarboxylic acid esters appearing only in the last fractions. At the same time, aliphatic dicarboxylic and phthalic acids, found previously in the composition of the total oxidation products of the same material[7], proved to be absent. They were probably lost when removing DMSO from the eluates.

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