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Abstract

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ON THE COMPOSITION AND CHEMICAL NATURE OF SAPROPELIC ACIDS

The sapropel of the Olera deposit lies beneath a layer of peat and is a semiliquid mass containing remnants of roots and other parts of higher plants. By means of wet sieving these fragments were removed from the sample so that their specific composition would not distort the results of analysis of the main, decomposed sapropelic mass. Microscopic examination confirmed that the amount of shaped elements in it after sieving did not exceed 0.5–1%. Thus, the composition of this mass, apart from remnants and products of transformation of microorganisms, could include only products of deep decomposition of higher plants that had already lost their morphological structure, and humic acids, if such are formed under the anaerobic conditions of a sapropelic deposit. The sapropelic mass contained 9.3% A^c; 0.54% S^c; 58.5% C^r; 7.4% H^r; 4.1% N^r. Hydrochloric acid (3%) extracted, upon heating, 38.5% of substances (RV 22%) containing, besides uronic acids and sugars ([1]), a small amount of amino acids. Bitumens were extracted with benzene (7.6%) and alcohol (2.1%). The residue was extracted with 0.1 N KOH solution. The extracts obtained were treated with KU-2 cation exchanger; the sapropelic acids were precipitated by heating to 70–80° ([2]).

Table 1

Nos. of fractions	Fraction	Solubility in alcohol*	Yield, %	Ash on dry substance, %	C	H	N (total)	N (by difference)	Before hydrolysis	After hydrolysis	– COOH	– OH
					Content on combustible mass, %		Amine N, %	Mg-eq/g				
	Sapropelic acids											

Nos. of fractions	Fraction	Solubility in alcohol*	Yield, %	Ash on dry substance, %	C	H	N (total)	N (by difference)	Before hydrolysis	After hydrolysis	– COOH	– OH
	Initial product	—	100	0.9	62.34	6.93	6.27	24.46	—	—	4.3	
1	Fraction soluble in $NaHCO_3$	NR	42	0.5	57.88	5.99	6.86	29.27	0.3	3.1	1.7	3.6
2	Same	R	3	0.1	63.66	7.39	3.15	25.80	—	2.2	1.8	3.5
3	Fraction soluble in cold NaOH	NR	22	0.7	60.98	7.03	6.63	25.36	0.2	3.5	1.3	2.6
4	Same	R	1	0.6	64.99	9.41	2.48	23.12	—	—	1.2	2.9
5	Fraction soluble in hot NaOH	NR	19	1.7	63.04	7.56	6.84	22.56	0.1	2.7	1.2	1.3
6	Same	R	2	0.0	71.21	10.82	1.37	16.60	—	—	0.9	2.6
7	Fraction insoluble in NaOH	—	11	7.3	68.62	9.00	3.58	18.80	0	1.2	1.1	1.8
	Fulvic acids											
	Initial product	—	100	2.3	47.86	6.58	6.83	38.73	—	—	6.9**	

Nos. of fractions	Fraction	Solubility in alcohol*	Yield, %	Ash on dry substance, %	C	H	N (total)	N (by difference)	Before hydrolysis	After hydrolysis	– COOH	– OH
8	Anion-ad part	NR	13	0.3	47.41	6.82	6.49	39.28	2.0	3.0	3.4	
9	Same	R	48	trace	49.12	6.17	8.52	36.19	1.7	5.5	4.1	
10	Eluted part, 1% $NaHCO_3$	—	25	1.4	40.81	4.72	1.43	53.04	1.1	0.8	12.4	
11	Eluted part, 1% NaOH	—	8	5.1	44.12	4.53	2.08	49.27	0.7	0.8	—	
12	Eluted part, 4% NaOH	—	3	52.2	—	—	1.23	—	0	0.4	—	

* R –soluble in alcohol, NR –insoluble.
** Determined in alcoholic solution by Kharitonov' s method.

Separated from the yellow solution of fulvic acids (we conditionally retain this name), they were dried in vacuo and successively extracted with 0.1 N solutions: $NaHCO_3$ and NaOH in the cold, and NaOH at 100°. However, part of the sapropelic acids remained undissolved in this process (fraction 7, Table 1). The fractions were then divided into portions soluble and insoluble in alcohol. The fulvic-acid solution was passed through a column with the anion exchanger AN-2f. The non-adsorbed portion was evaporated in vacuo and, according to its solubility in alcohol, was divided into 2 fractions. The acids retained by the anion exchanger were eluted with 1% solutions of $NaHCO_3$ and NaOH, and then with 4% NaOH.

The yield of sapropelic acids was 18.6%, and of fulvic acids 9.8%.

(Figure: Fig. 1. IR spectra of sapropelic acids)

Fig. 1. IR spectra of sapropelic acids:
1 –soluble in $NaHCO_3$;
2 –soluble in cold NaOH;
3 –soluble in hot NaOH;
4 –insoluble residue. (NaCl prism)

The data given in Table 1 show that sapropelic acids are mixtures of substances,

a small part of which, soluble in alcohol, is close in its nature to bituminous fractions. The main mass of the sapropelic acids (94%) consists of products insoluble in alcohol and rich in nitrogen, 40-50% of which is in hydrolyzable form. The infrared spectra (Fig. 1) indicate a commonality in the structure of these substances. In addition to carboxyls, hydroxyls, and a large number of aliphatic side chains, the presence of –O–bonds, as well as amino and tertiary amide groups, has been established. The presence of 5- and 6-membered heterocycles with nitrogen and oxygen is very probable. The absence of condensed aromatic structures, typical of humic acids of soils and peat, is demonstrated by oxidation of fractions 1, 3, 5, and 7 with an alkaline permanganate solution ([3]). In the reaction products, acetic, propionic, butyric, and caproic acids were found, as well as oxalic, malonic, succinic, and adipic acids; benzenecarboxylic acids were absent. In searches for lignin residues, all fractions of sapropelic acids, as well as alcohol bitumen, were subjected to nitrobenzene oxidation in an alkaline medium ([4], [5]); however, after oxidation, not even traces of syringaldehyde, vanillin, or *p*-hydroxybenzaldehyde were detected in any of them.

In the composition of sapropelic fulvic acids, 2 groups of substances are revealed: highly nitrogenous amphoteric compounds of the non-adsorbed portion (fractions 8 and 9) and nitrogen-poor acidic products of the eluted fractions. The amount of hydrolyzable nitrogen in the fulvic acids reaches 70–80% of its total content. The IR spectra of the non-adsorbed fractions indicate the presence of hydroxyls, as well as primary and secondary amide groups (1640, 1580, 1200, and 680 cm^{-1}). Nitrogenous rings are probably present. The band at 2600 cm^{-1} suggests the presence of a peptide bond characteristic of proteins. Carboxyl groups appear weakly. The IR spectrum of the fraction eluted with $NaHCO_3$ gives distinct absorption bands corresponding to carboxyls, as well as to the hydroxyls of primary, secondary, and tertiary alcohols (1035, 1112, 1178 cm^{-1}). This product, which is very hygroscopic, should be assigned to oxy acids, possibly of the uronic type.

Hydrolysis of the fulvic-acid fractions and of all fractions of sapropelic acids with 6 N HCl at 120° makes it possible to detect in the hydrolysates, by means of paper chromatography (⁀6), a number of amino acids: glycine, leucine, glutamic and aspartic acids, alanine, valine, serine, lysine, and others. It is of interest that isoleucine and β-phenylalanine were found only in hydrolysates of fulvic acids, whereas norleucine and valine were found only in the products of hydrolysis of sapropelic acids.

The alkali-insoluble residue of the original sapropelic mass (17.2%) had the following composition: C^c 22.8%; S^c 0.4%; C^r 66.3%; H^r 8.6%; and N^r 3.5%. The content of acidic groups was 0.39 mg-eq/g. By all indices, and also with respect to permanganate, nitrobenzene, and acid hydrolysis, the residue is similar to the 7th (insoluble) fraction of sapropelic acids.

Thus, in the composition of the sapropelic mass we found neither lignin residues nor humic acids similar to peat or soil humic acids, although the participation of higher plants in the formation of the Oler sapropelic deposit undoubtedly

took place. Sapropelic acids differ sharply from humic acids not only in their composition but also in their structure, which is based on aliphatic and, probably, heterocyclic nitrogen-containing structures. It should be supposed that these substances are formed from products of the anaerobic decomposition of organic residues under the influence of synthetic reactions, in which protein and also carbohydrate residues must apparently play the leading role. One may agree with the opinion ([7,8]) that this process proceeds mainly according to the well-known Maillard scheme ([9]); however, it should be taken into account that synthetic melanoidins are hydrolyzed by acids only to a very slight extent ([10]). Therefore, while retaining Maillard' s basic conception, one may arrive at the conclusion that under natural conditions the predominant process is the condensation of sugars not with free amino acids, but with larger units of protein molecules, capable also of hydrolyzing along the lines of peptide bonds. In such a case, however, the formation of a nonhydrolyzable bond between the aldehyde and the amino group of the terminal member of the peptide chain may constitute a serious obstacle to determining the "carbohydrate component" of sapropelic acids.

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Note: Figure translations are in progress. See original paper for figures.