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HYDROCARBONS AND CAROTENOIDS OF THE MEDICINAL MUD OF LAKE KARACHI

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The composition of the hydrocarbons and carotenoids of the lipids of a typical ooze-like sulfide mud from Lake Karachi has been studied. The presence of carcinogenic 3,4-benzopyrene in the hexane fraction of the liquid has been established. A high antioxidant activity has been found in the chloroform fraction, which also possesses a pronounced hepatoprotective action.

Experimental pharmacological investigations have shown the high therapeutic efficacy of the lipids of the medicinal mud from Lake Karachi in toxic hepatitis, myocarditis, and acute and chronic arthritis [1-3]. The curative action of the lipids is connected with the presence in them of unsaturated fatty acids, phospholipids, and prostaglandins [4]. The aim of the present investigation was a detailed study of the chemical composition of the lipids of a typical ooze-like sulfide mud from Lake Karachi and the determination of the biologically active and ballast substances and possible toxic substances. The results are given of an investigation of the composition of two classes of compounds – hydrocarbons (HCs) and carotenoids. Biological activity was tested on the model of acute toxic hepatitis caused by CCl_4. In the organism, CCl_4 undergoes enzymatic homolytic breakdown with the formation of the free radicals $CCl_3^{\cdot}$ which induce the peroxide oxidation of the lipids in the membrane of the liver. Consequently, for a preliminary estimate of the hepatoprotective action we also determined the antioxidant activity of fractions of the lipids of the medicinal mud.

The medicinal mud of Lake Karachi accumulated under the conditions of pronounced hydrogen sulfide contamination from residues of planktonic organisms (Microcystis salina, Artemia salina) transformed by the bottom microflora. The mineral fraction is represented by clays, carbonates, and salts (NaCl, Na_2SO_4, $CaSO_4$, $MgSO_4$).

A lipid extract of the medicinal mud from Lake Karachi consists of a resinous substance of dark-brown color with a specific balsamic odor, soluble in chloroform and ethanol and partially soluble in hexane and petroleum ether. This property of the lipids was used for their separation in two fractions – a hexane (nonpolar) and a chloroform (polar) fraction. According to the experimental results, the yield of the polar fraction of lipids was 59% and that of the nonpolar fraction 41%.

The antioxidants in the hexane fraction consisted of one type of weakly active inhibitors and those in the chloroform fraction of two types of inhibitors possessing a pronounced antioxidant action. The characteristics of the antioxidant properties of the lipid fractions of the medicinal mud of Lake Karachi are given below:

Function	$k_{7_1} \cdot 10^{-4}$, liter/mole·sec	$k_{7_2} \cdot 10^{-4}$, liter/mole·sec	Concentration of inhibitors, mole/kg
Hexane	–	1.40	0.23
Chloroform	7.10	0.96	0.28
Ionol	2.40	–	–

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To detect possible toxic substances, the lipid fractions were investigated for the presence of polycyclic aromatic hydrocarbons (PAHs) – common components of the majority of modern marine and lacustrine deposits, including peloids [5]. Some of them possess carcinogenic properties (3,4-benzopyrene, anthracene, and others). The hexane fraction contained the only PAH – 3,4-benzopyrene, in an amount of $4.2 \cdot 10^{-8}$ g/liter; it was completely absent from the chloroform fraction.

A considerable amount of HCs (16% by weight of the fraction) consisting predominantly of unsaturated compounds was found in the hexane fraction. In a hydrogenated sample, paraffinic, bicyclic naphthenic, tricyclic naphthenic, and tetracyclic naphthenic HCs were found in amounts of 51, 20, 17, and 10%, respectively, of the total weight of the hydrogenated sample. Among the HCs the predominating components were n-heptadecane and n-heptadec-1-ene, inherited from the lipids of blue-green algae [6], and a number of C_{23}-C_{35} n-alkanes with a predominance of the odd homologs, the initial bioproducers of which may have been higher terrestial plants [7]. A number of n-olefins with a predominance of even homologs produced by anaerobic microflora [8] was present. A considerable amount of isoprenoids (norpristane, pristane, phytane, squalane) and unsaturated sterane components (isocholesterol, isoergostene – the products of the geochemical transformation of sterols – was found. There were no HCs in the chloroform fraction.

The lipid extract contained co-extracted elementary sulfur (8% on the lipids), which is included in the ballast substances. The producing agents of the elementary sulfur are thione and sulfur bacteria.

The carotenoid pigments – biologically active compounds – were represented by two classes – carotenes and xanthophylls – and were distributed in both lipid fractions in accordance with their polarities. The predominating carotene was 9-cis-β-carotene, and among the xanthophylls rhodovibrin, myxoxanthophylls, and antheraxanthin.

The amounts and chromatographic mobilities (R_f) of the carotenoids in the medicinal mud from Lake Karachi were as follows [the chromatographic mobilities of the carotenoids are given relative to β-carotene in the hexane–acetone (8:3) system on Silufol]:

Pigment	Amount, mg/g of lipids	Relative R_f
all-trans-β-Carotene	0.56	1.00
9-cis-β-Carotene	1.35	1.00
γ-Carotene	0.02	1.00
Echinenone	0.60	0.74
Spheroidenone	0.80	0.59
Rhodovibrin	1.00	0.46
Canthaxanthin	0.86	0.44
Antheraxanthin	1.12	0.22
Lutein	0.23	0.09
Zeaxanthin	0.25	0.06
Myxoxanthophylls		
rhamnoside	0.90	0.02
glucoside	0.20	0.02

The carotenoids identified in the medicinal mud from Lake Karachi can be divided into three groups according to their origin. The first, predominating, group is represented by a set of pigments specific for blue-green algae – β-carotene, echinenone, canthaxanthin, antheraxanthin, zeaxanthin, and myxoxanthophylls [9]. The second group, also present in considerable amount, consists of pigments characteristic for anaerobic photosynthesizing bacteria: purple – spheroidenone and rhodovibrin – and green – γ-carotene [10]. The third group, detected in the smallest amount, consisted of pigments typical for higher plants and blue-green algae – β-zeacarotene and lutein [11].

Thus, the results of chemical investigations permit the conclusion that the composition of the HCs and the carotenoid pigments makes it possible to suggest the initial organisms

producing organic matter in the medicinal mud and determining its genetic type. The results of a study of the antioxidant properties indicate different biological activities of the lipid fractions. The maximum antioxidant activity was found in the polar fraction which was subsequently used in biological trials. It possessed a hepatoprotective effect in severe hepatitis produced by CCl_4. The therapeutic influence of the preparation is due to the suppression of the production of dienic conjugates, Schiff's bases, and malondialdehyde; to a stimulation of the antiradical activity of the liver lipids; to the protection of glutathione from oxidation; to a decrease in the steatosis of the parenchyma and in the formation of detergent phosphatidylcholine; to the retention of a high level of phosphatidylcholine and phosphatidylethanolamine in the membrane; to an acceleration of the oxidation of hexobarbital with a shortening of the sleep caused by it; to an improvement in the excretion of sulfobromophthalein; and to a decrease in hyperenzymemia by the predominant passage into the blood of the liver-specific enzymes urocaninase and the isoenzymes of lactate dehydrogenase, and also of alanine and aspartate aminotransferases. In sum, the polar fraction of the lipids prevents the development of necrosis of the hepatocytes and a disturbances of the histoarchitecture of the liver. The nonpolar fraction has no hepatoprotective properties under the conditions of toxic pathology.

EXPERIMENTAL

The absorption spectra of the carotenoids were taken on a Specord UV-VIS spectrophotometer in hexane, benzene, acetone, ethanol, and chloroform. The thickness of the cell was 1 cm, and the comparison cell contained the corresponding solvent. The mass spectra of the carotenoids were obtained by the direct introduction of samples into the ionization chamber of a MKh-1310 instrument, the temperature of the evaporation chamber being 190°C and that of the ionization chamber 250°C with ionizing energies of 60 and 20 eV.

The GLC of the HCs was carried out on a Biokhrom-1 instrument using a capillary column (0.25 mm × 50 m) containing OV-101. Linearly programmed heating of the column thermostat from 145 to 275°C at three degrees per minute. The pressure at the entry to the column was 1.5 atm. The temperature of the evaporator was 300°C and that of the detector 280°C. The chromato-mass spectra of the HCs were obtained on a LKB-2091 instrument. Analytical conditions: capillary column containing Apiezon at an ionizing energy of 70 eV and a temperature of the ion source of 250°C.

Low-temperature luminescence spectra were obtained to detect PAHs. The luminescence was excited by the radiation of mercury lamp with a wavelength of 365 nm. 3,4-Benzopyrene was identified and its amount was determined by comparison with the spectrum of standard substances and by comparison with literature figures [12]. n-Octane solutions of the polar and nonpolar fractions of the lipids in a concentration of $1 \cdot 10^{-3}$ g/liter frozen at 77 K were analyzed. The 3,4-benzopyrene was determined from the well-defined lines in the luminescence spectrum with maxima at 4030 and 4080 Å. Its concentration was found from a calibration curve plotted for various concentrations of 3,4-benzopyrene. For preparative thin-layer chromatography (TLC) we used silica gel LSL_{254} (containing 13% of gypsum) and Silufol plates.

The medicinal mud was collected in July 1985, and was stored for not more than 10 days in the frozen state. A lipid extract was obtained by the exhaustive extraction of the native mud [13]. The yield of lipids was 2.7 g per 1 kg of mud. The liquid concentrate was treated with hexane (ratio 1:40), the solution of the hexane-soluble lipids was filtered, and the residue of hexane-insoluble lipids was covered with chloroform, and, in this way, from 1 kg of native mud, 1.1 g of hexane (nonpolar) fraction and 1.6 g of chloroform (polar) fraction were obtained.

The antioxidant activities of the fractions were evaluated with the aid of the model reaction of the inhibited oxidation of cumene at 60°C in the presence of the initiator azoisobutyronitrile [14]. The inhibition rate constants and the concentrations of antioxidants were calculated from relations given in the literature [14].

The HCs were isolated from the hexane fraction by TLC on Silufol LSL_{254} plates in hexane. The HC fraction was taken from the finish as far as the band of the coextracted elementary sulfur, absorbing at λ_{max} 254 nm. To isolate the saturated HCs, an aliquot of the total fraction was subjected to bromination (a solution of bromine in CCl_4 was added dropwise until a permanent orange coloration appeared). The HCs were separated from the bromination products by TLC in hexane. A second aliquot of the initial HC fraction was subjected to hydrogen-

ation in a reactor over Raney nickel catalyst at a pressure of hydrogen of 80 atm and a temperature of 150°C for 2 h. The initial, the saturated, and the hydrogenated HCs were analyzed by GLC and by GC-MS.

The carotenoid fraction was isolated from the lipid extract by preparative TLC on silica gel LSL_{254} 5/40 in the hexane–acetone (8:3) solvent system. Individual compounds were obtained by separating the mixture according to solubility and by repeated chromatography on Silufol plates in systems with different polarities. They were identified on the basis of spectral characteristics in comparison with standard substances and in light of literature information [15]. The amount of each pigment was calculated from its optical absorption spectrum in the visible region, using the extinction coefficients given in [15].

The absorption spectra in the visible region taken in various solvents and the low-resolution mass spectra obtained for all-trans-β-carotene, 9-cis-β-carotene, β-zeacarotene, γ-carotene, echinenone, spheroidenone, rhodovibrin, canthaxanthin, anteraxanthin, zeaxanthin, and the myxoxanthophylls corresponded to those given in the literature [15].

The biological trials were carried out on 90 male white rats weighing 200-220 g. Over a period of 4 days the animals were given intragastrically 2.5 ml/kg of a 50% solution of CCl_4 in olive oil and, simultaneously, aqueous suspensions of the chloroform fraction in the previously determined therapeutic dose of 30 mg/kg or of the hexane fraction in the same dose. Control animals were given CCl_4 and an equivolume amount of distilled water. The numbers of necrotized hepatocytes per 2000 cells were counted on overall liver preparations stained with hematoxylin-eosin. The functional state of the liver was judged from the retention of sulfobromophthalein 45 min after the intravenous injection of the dye and from the duration of hexobarbital sleep. In lipid extracts of the livers we estimated whole amounts and the fractions of total lipids and phospholipids by TLC on Silufol plates [16], the amounts of dienic conjugates and of Schiff's conjugates [17], and the antiradical activity of the lipids [18]; in liver harmogenates we determined the amount of reduced glutathione [19] and the kinetics of the formation of malondialdehyde [17]. The activities of urocaninase [20], of the isoenzymes of lactate dehydrogenase [21], and of alanine aminotransferase and aspartate aminotransferase [22] were measured in the blood plasma.

CONCLUSIONS

1. The composition of the carbohydrates and carotenoid pigments in the lipids of a typical ooze-like sulfide mud from Lake Karachi has been studied and this has enabled a hypothesis concerning the genesis of the mud to be made.

2. The carcinogenic 3,4-benzopyrene has been found in the lipids of the ooze-like sulfide mud from Lake Karachi, being concentrated in the nonpolar fraction.

3. A high antioxidant activity of a chloroform fraction of the lipids, which possesses a pronounced hepatoprotective action in CCl_4-induced experimental hepatitis has been found.

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CARBON DIOXIDE EXTRACT FROM WOODY VERDURE OF THE SCOTCH PINE. GROUP COMPOSITION AND ACIDS

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The group and individual compositions of the acids of an extract from the woody verdure of Scotch pine obtained on a pilot plant have been studied. The yield of extract amounted to 4.0% on the weight of the raw material, and 88% of the extract dissolved in petroleum ether. The petroleum-ether-soluble substances of the extract contained 32.8% of free acids and 55.4% of neutral substances. The compositions of the higher fatty acids, the diterpene acids, and the bicyclic acids have been determined. Pinifolic acid and its monomethyl ester, 18-acetoxy- and 18-hydroxyanticopalic acids, an acid of the 4-epiimbricatic series, and oxo and hydroxy acids of the abietic type have been isolated and identified.

In order to study the composition of the extractive substances of woody verdure, even in industry petroleum ether is the main extractant, sometimes being used with gasoline, trichloroethylene, isopropanol, and other solvents [1, 2], while steam is used for the isolation of the essential oil [3]. In these cases, the woody verdure is subjected to the action of high temperatures, which may lead to a change in the chemical composition of the extracts obtained. A promising extractant of the woody verdure that is free from these defects may

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