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Identification of very-long-chain acids from peat and coals by capillary gas chromatography–mass spectrometry

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ABSTRACT

Long-chain monocarboxylic (fatty) and dicarboxylic acids, up to 36 and 33 carbon atoms, respectively, were identified in peat, lignite, brown coal, bituminous coal, anthracite and graphite using capillary gas chromatography–mass spectrometry (60-m polar capillary column with Supelcowax in the quadrupole mass spectrometer, *i.e.* QP-1000 from Shimadzu). The concentrations of acids decreased as coal rank increased. Branched and unsaturated acids disappeared sooner than alkanoic acids.

INTRODUCTION

The occurrence of very-long-chain fatty acids (VLCFAs) in nature has recently been reviewed [1]. In the animal kingdom VLCFAs are found in tissues ranging from the most primitive life forms (sponges) to the most advanced ones (mammalian skin or brain). In plants they appear mostly in waxes on the plant surface [2]. Several studies have dealt with the presence of VLCFAs in peat [3–5] or, exceptionally, in coal [6,7]. Studies by Ekman and Fagernäs [3] succeeded in determining a variety of acyclic acids, including dicarboxylic ones, with chains longer than 22 carbon atoms; all the acids were unbranched and saturated.

Our experience with analysis of VLCFAs in biological materials and the results of analyses of VLCFAs from unusual sources [8–11] formed the basis of our attempts at identifying VLCFAs in samples ranging from peat to coal to graphite.

EXPERIMENTAL

Chemicals

Dicyclohexylcarbodiimide and 2-amino-2-methylpropanol were obtained from Sigma (St. Louis, MO, USA); other chemicals and solvents were from Lachema (Brno, Czechoslovakia).

Lipid extraction and derivatization

The samples, peat, lignite, brown coal, bituminous coal, anthracite and graphite (see Table I), were dried and ground to less than 0.1-mm-diameter particles. In free form fatty acids occur in concentrations from approximately 1.3% (peat) to 3.8% (anthracite); in graphite free fatty acids were not identified because their concentration is very low. In this paper only total fatty acids after hydrolysis are analysed. We do not examine TLC of some esters. Alkaline extraction was carried out by suspending 10 g of ground sample in 100 ml of ethanolic (90%) 0.5 *M* potassium hydroxide. The suspension was kept at 70°C for 4 h with continuous stirring. The suspension was diluted with water (1:1, v/v) and extracted three times with 10 ml of diethyl ether to remove non-acidic compounds. The suspension was acidified (pH 2) with 0.5 *M* sulphuric

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acid and extracted three times with 100 ml of diethyl ether. The combined ether extracts were washed and evaporated. One half of the extracted acids was treated with a solution of diazomethane in diethyl ether, and the resulting methyl esters were separated by solid-phase extraction (SPE). Free acids (*i.e.* fatty acids and dicarboxylic acids) are very poorly resolved by TLC (see ref. 12); their methyl esters give better resolution, but this TLC is useless.

Oxazolines were prepared from the second half of the extracted acids using a modified method of Yu *et al.* [13]: 5 mg of dicyclohexylcarbodiimide were added to a solution of 5 mg of fatty acids in 1 ml of dichloromethane. After stirring for 10 min, 2-amino-2-methylpropanol (5 mg) was added at 20°C and stirred for 4 h. The evaporated mixture was dissolved in 1 ml of diethyl ether and treated with 0.5 ml of $SOCl_2$ (20°C, 1 h), then washed with ice-cold water and dried by anhydrous sodium sulphate.

Solid-phase extraction

The silica-cart plastic cartridge system, Separon SGX C_{18} (1-ml tubes) (Tessek, Prague, Czechoslovakia), was used. A total amount of 1–2 mg of methyl esters was applied to the column in a small volume of dichloromethane. A methanol–isopropanol (98:2) mixture was allowed to flow under slight syringe pressure, at a rate of approximately 0.5 ml/min; 0.1-ml fractions were collected manually. The solvent was then evaporated in a vacuum and each fraction was analyzed by GC–MS. The retention volume for fatty acid methyl ester (FAME) 22:0 was 2.1 ml. For scanning infrared spectroscopy of the methyl esters after SPE (in carbon tetrachloride) a Perkin-Elmer Model 1310 (Perkin-Elmer, Norwalk, CT, USA) infrared spectrophotometer was used. The methyl esters and oxazolines were silylated before GC–MS according to the literature [5].

Gas chromatography–mass spectrometry

For identification of FAMEs, a Shimadzu QP-1000 (Shimadzu, Kyoto, Japan) GC–MS system with a 60 m × 0.32 mm I.D. (0.25 μm particle size) SPB-1 fused-silica capillary column (Supelco, Gland, Switzerland), split/splitless injector and helium as a carrier gas was used. Oven temperature was programmed from 100 to 320°C at a rate of 4°C/min. Ionization energy was 70 eV; the electron multiplier voltage was 2.5 kV.

Oxazolines were identified under conditions similar to the methyl esters, with the only difference being in the column temperature. In this case the oven was progammed from 150 to 330°C at a rate of 5°C/min. Separate peaks were identified by total mass spectra (from m/z 50 to 500) according previous papers [11,13].

RESULTS AND DISCUSSION

Peatification of plant tissues involves physicochemical and microbial processes that cause loss of carbon dioxide, water and ammonia and the formation of peat. Further coalification produces different types of coals. Coals are products of solely physico-chemical processes acting on plant remnants. The biochemical and microbial phase ends with the formation of peat; it is followed by the geochemical phase, which involves a rise in temperature and pressure in the deposit.

Table I shows that increasing age (coalification) of the deposit is accompanied by a sharp drop in the content of total extractable carboxylic acids. In peat their content is of the order of one part per hundred, whereas in graphite it amounts to one part per million. It should be noted here that Bouška [14] graphite is composed solely of carbon without any organic compounds, although other elements or inorganic compounds can naturally be present. The presence of trace amounts of carboxylic acids in

TABLE I

COMPOSITION OF LIPIDS AND FATTY ACIDS FROM VARIOUS COALS

Sample	Source	Amount of acid (μg/g of coal)
Peat	Volary	65480[a]
Lignite	Mydlovary	9740[b]
Brown coal	Sokolov	2130
Bituminous coal	Nýřany	863
Anthracite	Úsilné	19
Graphite	Černá	0.9

[a] Ekman and Fagernäs [3,4] described amounts in the range of tens of milligrams (60.1 mg per g of dry peat and/or 85–170 mg/g, depending on the type of extraction procedure.

[b] Kiya *et al.* [6] described yields of 0.98–1.20% from original coals.

TABLE II

FATTY ACID COMPOSITION (μg/g) FROM DIFFERENT SAMPLES

Fatty acid[a]	Peak No.	Peat	Lignite	Brown coal	Bituminous coal	Anthracite	Graphite
i-22:0	1	104.76[b,c]	39.93	0	0	0	0
22:1	2	65.48	0	0	0	0	0
22:0	3	497.64	117.85	37.70	12.60	0.07	0
di-19:0	4	19.64	34.09	1.06	0.08	0	0
i-23:0	5	72.02	15.58	1.06	0	0	0
23:1	6	91.67	0	0	0	0	0
ai-23:0	7	39.28	28.24	1.06	0	0	0
23:0	8	150.60	38.96	4.68	0	0	0
di-20:0	9	78.57	6.81	4.68	3.02	0.06	0
i-24:0	10	52.38	11.68	1.06	0	0	0
24:1	11	72.02	0	0	0	0	0
24:1	12	150.60	18.50	0	0	0	0
24:0	13	1080.42	192.85	57.08	25.80	0.71	0.06
di-21:0	14	6.54	1.94	1.27	0.25	0	0
i-25:0	15	32.74	11.68	2.55	0	0	0
ai-25:0	16	52.38	15.58	0	0	0	0
25:1	17	26.19	0	0	0	0	0
25:0	18	98.22	32.14	5.75	0	0	0
di-22:0	19	183.34	27.27	6.39	4	0.13	0
i-26:0	20	85.12	11.68	1.06	0	0	0
26:1	21	104.76	0	0	0	0	0
26:0	22	772.66	128.56	41.53	18.38	0.57	0.02
ai-23:0	23	137.50	22.40	1.70	0.08	0	0
i-27:0	24	32.74	6.81	0	0	0	0
ai-27:0	25	52.38	7.79	0	0	0	0
27:0	26	65.48	15.58	2.55	0	0	0
di-24:0	27	294.66	45.77	5.96	5.35	0.21	0.01
i-28:0	28	72.02	3.89	0	0	0	0
28:1	29	52.38	0	0	0	0	0
28:0	30	366.68	7.79	10.22	12.08	0.08	0
di-25:0	31	19.64	4.87	1.91	0.08	0	0
i-29:0	32	32.74	3.89	0	0	0	0
29:0	33	72.02	11.68	2.76	0	0	0
di-26:0	34	242.27	30.19	5.75	3.10	0.07	0
i-30:0	35	78.57	3.89	0	0	0	0
30:0	36	189.89	15.58	4.68	0	0	0
di-27:0	37	85.12	4.87	1.70	0	0	0
i-31:0	38	32.74	7.79	0	0	0	0
ai-31:0	39	39.28	0	0	0	0	0
31:0	40	65.48	11.68	0	0	0	0
di-28:0	41	170.24	6.81	3.62	1.03	0	0
32:0	42	124.41	11.68	2.55	0	0	0
di-29:0	43	39.28	2.92	0.21	0	0	0
di-30:0	44	117.86	1.94	1.49	0.08	0	0
i-34:0	45	13.09	0	0	0	0	0
34:0	46	72.02	3.89	0	0	0	0
di-31:0	47	52.38	0.97	0.21	0	0	0
di-32:0	48	85.12	2.92	0.42	0	0	0
i-36:0	49	13.09	0	0	0	0	0
36:0	50	85.12	3.89	0	0	0	0
di-33:0	51	6.54	0.97	0.21	0	0	0

[a] Percentage of total acids longer than C_{18}, see Experimental section. i = *iso*; ai = *anteiso*; di = dicarboxylic acid.

[b] S.D. was in the range 0.005–0.01.

[c] Each value represents the mean ± S.D. from five analyses.

graphite can be the result of contamination. This possibility has been described by Polzer and Bächmann [15], who found VLCFAs in rain water, into which they were carried by wind from plant surfaces.

A variety of carboxylic acids have been described to be present in peat. As seen from earlier studies [3,4] they consist mostly of saturated acids, whether mono- or dicarboxylic, with an even number of carbon atoms, with chain length of up to C_{30}. Coals were found to contain straight-chain fatty acids of up to 32 carbon atoms [6]. A small amount of hydroxy fatty acids, especially in peat, was present, but they were not identified. Unfortunately, the authors were unable to separate and fully identify all methyl esters by GC; they succeeded merely in identifying methyl esters of even-numbered straight-chain fatty acids. Niwa *et al.* [7] used the method of field desorption MS to analyse complex lipids (waxes). They also identified fatty acids and alcohols in the range C_{24}–C_{34}, with a maximum at C_{26}. Our results extend these carboxylic acid compositions.

Table II shows the presence of branched acids (*iso* and *anteiso*), arising probably by microbial biosynthesis during peatification, and monoenoic acids. The presence of unsaturated acids in samples of different geological ages has been shown repeatedly [16]. With increasing age of the peat and coals both the total amount of the acids and the proportion of their different groups are seen to decrease, as expected. Lignite contains practically all the acids identified in peat, while progressive coalification, beginning with brown coal, is seen to be reflected in a marked change in both the quantity and type of acid components. Graphite was found to contain only trace amounts of acids (see the above note on possible contamination).

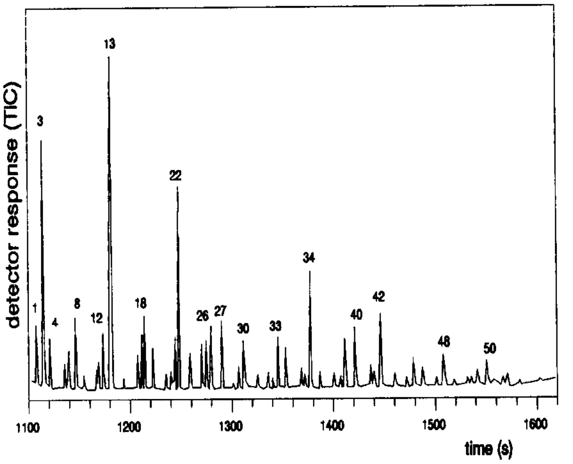

Fig. 1. Capillary gas chromatogram of long-chain fatty acid methyl esters and dicarboxylic acid methyl esters from lignite. For analytical conditions, see the Experimental section. Peaks 1–50 are methyl esters (see Table II).

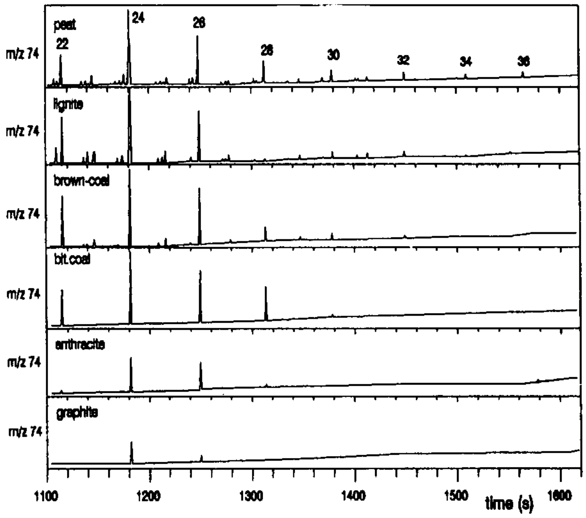

Fig. 2. GC–MS of fatty acid methyl esters from different samples by means of single-ion monitoring of ion *m/z* 74 (base peak for methyl esters).

Identification of FAMEs was performed based on our previous report (11). Double bonds were located in monoenoic acids by splitting the carbon chain of oxazoline in a mass spectrum [11,13]. A common method of determining the configuration of double bond(s) includes infrared spectroscopy. Some peaks at 967 cm^{-1}, *i.e.* the characteristic absorbance for *trans* isomer(s), were not identified. From the above argument it is to be expected that double bond(s) are *cis*. The use of single-ion monitoring (SIM) for base peaks of methyl esters (*e.g.* *m/z* 74 for methyl ester monocarboxylic acids and *m/z* 98 for methyl ester dicarboxylic acids) enabled us to identify many individual acids in the very complex mixture represented by coal extract. Fig. 1 shows the profile of methyl esters of mono- and dicarboxylic acids. All peaks representing individual methyl esters are satisfactorily separated; their sequence (equivalent chain length or relative retention time) corresponds to previously published data

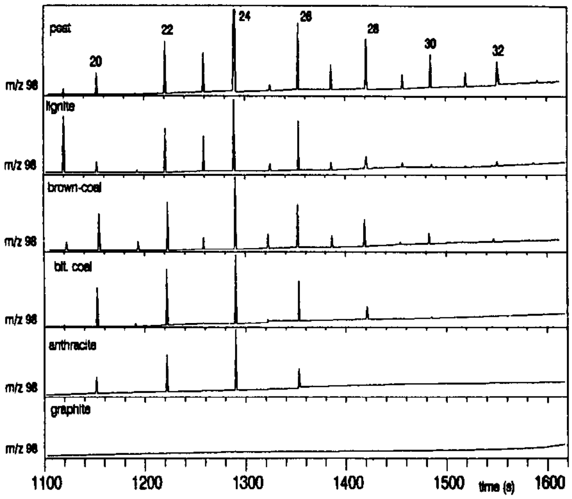

Fig. 3. GC–MS of dicarboxylic acid methyl esters from different samples by means of single-ion monitoring of ion m/z 98 (base peak for dimethyl esters).

[9,12]. With the aid of mass spectra, these results allowed us to identify unequivocally individual components.

Figs. 2 and 3 give the SIM of m/z 74 and m/z 98 ions in different types of coals. Application of capillary GC–MS enabled us to identify even trace amounts of acids. The method is augmented by using SPE as a prior purification and separation step.

In conlusion, it can be stated that the theory [14] (that carboxyl groups are usually present in brown coal and absent from bituminous coal, anthracite and graphite; with 85% carbon in coal, *i.e.* all coals, with exception of peat and lignite, the carboxyl groups are usually lacking) has not been fully substantiated because marked amounts of acids were found even in bituminous coal and anthracite. However, several rules can be used to generalize the overall occurrence. Monoenoic acids are found only in peat and in lignite and are probably thermally degraded at later stages. On the other hand, dicarboxylic acids have a general occurrence. VLCFAs are likely to undergo thermal degradation and their amount decreases with progressive coalification.

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