Structural studies on Miocene kerogen

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The different characteristics of organic matter in Miocene sediment from Portazgo (Madrid, Spain) were studied by several physicochemical techniques. Then g.c.-m.s. was applied to bitumen and to oxidative degradation products of the insoluble organic residue. Both the palaeobotanic study and the composition of the bitumen fraction suggest an important contribution of vascular plants in the sediment; a predominance of odd-numbered alkanes, of straight-chain aliphatic compounds, and of high molecular weight homologues of the series were observed. The degradation methods employed yielded large proportions of aliphatic chains, but aromatic compounds amounted to ≈ 25 wt% of the degradation products, suggesting a significant contribution of lignin. In addition to the degradation with potassium persulphate followed by alkaline permanganate oxidation, by depolymerization perborate was also applied. The latter degradation method yielded aromatic acids and polymethylene compounds, and residual kerogen was transformed into an alkali-soluble polymer, which can be studied by the usual techniques for humic acids.

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The complex nature of kerogens has been investigated by several physical and chemical methods, but their exact composition and macromolecular structure remains uncertain¹. This circumstance is mainly derived from their three-dimensional heterogeneous structure, consisting of a complex system of randomly associated 'building blocks'. The linkages between the structural units are different in both nature and chemical stability, ranging from chemical bonds, such as polymethylene bridges, to physical forces. In addition, a significant amount of solvent-extractable compounds is trapped within the pores of the kerogen matrix at different levels of mobility or stability. These latter compounds are of recognized importance for establishing the nature of the original material, the diagenesis of the sediment, the palaeoenvironment and the oil migration processes^{1,2}.

As a consequence of the high insolubility of kerogens in organic and inorganic solvents (by definition), these materials are not easily fractionated into different colloidal or molecular species. Therefore, they are usually studied as a whole, and present a chemical composition that is highly variable in terms of the nature of the original material and the degree of diagenesis. Consequently, 'kerogen' includes several types of complex materials, ranging from highly aliphatic sediments that are in some respects similar to lipid polymers, to materials with major aromatic moieties that have been compared with sapropelic peats or brown coals¹.

In the present study, the chemical composition of representative organic layers in evaporitic sediments of the Middle Miocene (≈ 15 million years old) was investigated by means of specific degradation techniques that focused on obtaining an insight into some of the above-mentioned features of formation, heterogeneity and skeletal structure of kerogen.

EXPERIMENTAL

Samples were taken from a stratigraphic series (Middle Aragoniense) representing the evaporitic sediments from Portazgo (Madrid), which consisted of alternating layers of gypsum, calcareous marls, chert, micaceous sand, clays, sandy clays or sepiolitic clays. The three levels with most organic matter were selected for this study and labelled as A (depth 41 m), B (114 m) and C (141 m).

Lipid extraction and isolation of kerogen were carried out following the methods described by Robinson³. The rock was pulverized to pass a 100 μ m screen and oven-dried at 60°C. Bitumen was extracted with toluene in 500 cm³ Soxhlet extractors for 50 h, the content of the extraction thimbles being homogenized every 12 h to prevent channelling. The extracted rock was dried and treated with 1M HCl to destroy the carbonates and afterwards washed first with 0.1M Na₄P₂O₇ and then with 0.1M NaOH to remove any possible humic colloids. Although the alkaline extracts were practically colourless, their carbon content was estimated in 50 cm³ desiccated acidified aliquots, using a 'Carmhograph-12' carbon analyser. This instrument was also used for measuring the carbon content of the decarbonated rock.

The separation of kerogen from the extracted rock was carried out by densimetry⁴, using the mixture CHBr₃ + CH₃CH₂OH adjusted to a density of 2 g cm⁻³. The floating particles were separated from the mineral residue by centrifugation, and the mineral content of the isolated kerogens was subsequently reduced by two treatments with HCl:HF (1:1; 1%) for 24 h at room temperature.

The elementary composition of kerogen was determined with a Hewlett-Packard CHN-185 microanalyser, and i.r. spectra with a Perkin-Elmer 580B

Table 1 Elementary composition of kerogens^a

Kerogen sample	Strata ^b		
	A	B	C
C (wt %)	60.32	63.38	64.90
H (wt %)	3.91	3.72	4.11
O (wt %)	33.59	31.32	28.70
N (wt %)	2.17	1.59	2.29
H/C atomic ratio	0.78	0.70	0.76
O/C atomic ratio	0.42	0.37	0.33

^a Ash-free, oven-dry basis^b Depth: A, 41 m; B, 114 m; C, 141 m

spectrophotometer. Gel filtration was carried out⁵ using Sephadex G-50 or G-25, and the densitometric curves were recorded at 450 nm.

The degradative studies of kerogen involved two degradation processes: (a) partial acid degradation with potassium persulphate followed by alkaline permanganate oxidation of the kerogen residue^{6,7}; and (b) five-step perborate oxidation⁸. In both cases, the acidified digests were extracted with ethyl acetate, the organic phase was dehydrated, dried under reduced pressure and then methylated with ethereal diazomethane generated from Diazald. The identification of the degradation products was carried out by g.c.-m.s. using a Hewlett-Packard 5992B instrument fitted with a fused silica column coated with OV-101. The oven temperature was programmed between 100 and 270°C, and the helium flow was 1 ml min⁻¹. The relative amounts of the different identified compounds were considered to be proportional to the FID response in the 5730A Hewlett-Packard gas chromatograph on which the samples had previously been separated. Before injection into the gas chromatograph, the mixtures of the oxidation products were purified by elution with methylene chloride on a small column filled with silica gel to remove the coloured, high molecular weight fractions.

RESULTS

The micropalaeobotanic analysis of the organic matter in the three levels studied showed the predominance of residues of continental marshy plants. In the upper layers (A), the most frequent spores were *Stereisporites minor* Kr., *Cicatricosisporites* sp. and *Verrucatosporites* sp., while the pollens were mainly represented by *Pityosporites* sp., *Triporepollenites robustus* Th. and Pf. and *Tetracolporopollenites* sp. In general, the floristic data pointed to the coexistence of forest and brush species decomposing in a relatively aerobic environment with certain algal contribution.

In the deeper layers, the predominant spores were *Triplanosporites* forms, *Leiotriletes* spp., *Polypodiaceosporites* sp. and *Stereisporites minor minor* Kr., and the pollens were mainly represented by *Inaperturopollenites* spp., *Monocolpopollenites* sp., *Caryapollenites* forms, *Graminidites* spp., *Trivestibulopollenites betuloides* Pf., *Liliacidites* forms, palm pollens and *Ephedripites* spp. Epidermal fragments of vascular plants (mainly Monocotyledoneae) were also relatively frequent. The typical palaeofloristic features in these levels are acustrine with an important proportion of vascular plants (Angio- and Gymnospermae). The interpretation of lithofacies

and palinofacies suggests a wide saline lake with a varying water level. The thin-layered stratified clays alternating with gypsum and anhydrite point to an undisturbed aquatic environment, with no streams^{9,10}.

The chemical analyses of the sediments showed a variable proportion of bitumen in the different layers (A, 0.23 wt %; B, 0.18 wt %; C, 0.21 wt %), representing 12–20% of total carbon. Extractable humic substances were practically absent (A and B, 0.01 %; C, 0.04 % of the total C), and most of the organic matter consisted of a kerogen (A, 79.4 %; B, 87.5 %; C, 83.9 % of total carbon).

The elementary composition of kerogen is shown in Table 1. When the atomic H/C and O/C ratios are plotted on a van Krevelen diagram, the position of the points are clearly included in the areas considered to be of the III-type kerogen¹. In addition, with increasing depth (A < B < C) the elementary composition of kerogen followed the specific evolutionary type III path. This is also confirmed by the progressive resistance to persulphate degradation (A, 58.7 wt %; B, 19.9 wt %; C, 13.1 wt % degraded by K₂S₂O₈), which agreed with a higher diagenesis of the sample from the deeper layer.

The infrared spectrum (Figure 1) resembled that of kerogen formed from lignified terrestrial plants¹. The contribution of oxygenated compounds was significant (1720 cm⁻¹ band), the important intensity of which is not a consequence of the previous treatment with aqueous alkali¹¹ since a nearly identical spectrum was obtained from the original, lipid-extracted kerogen samples. The high intensity of the band near 1610 cm⁻¹ may in part be attributed to a relatively large proportion of aromatic or quinonic material¹². Aliphatic structures (2920, 1460 cm⁻¹) were also defined in the spectra. No important differences were found between the intensity of the bands in the three samples studied.

The major bitumen components are shown in Figure 2 and Table 2. These fractions consisted predominantly of *n*-alkanes (C₁₅–C₃₃) and *n*-fatty acids (C₁₂–C₃₀). A certain proportion of methyl dehydroabietate was found in samples B and C. Some dialkyl phthalates (diisobutyl and dicyclohexyl) were also detected, amounting a 3 % of the degradation products, but they are likely to come from sample contamination, and were not included in Table 2.

From the comparison of the alkane and fatty acid series in the three layers studied, several differences were found (Figure 2). In all the cases, the alkane series presented a predominance of the odd-numbered carbon chains, but the proportion of the shorter chains (<C₂₁) predominated in the deeper layer. This tendency was also observed in the case of fatty acids, which presented a

Figure 1 Infrared spectrum of kerogen from Portazgo

Figure 2 Distribution diagram (carbon number *versus* relative abundance) of alkanes (top) and fatty acids (bottom) present in the bitumen fraction of the three layers studied

Table 2 Total percentages^a of the main groups of organic compounds in the sediment

	Bitumen			Degradation products of kerogen C: oxidizing method		
	A	B	C	Successive		Multi-step
				K ₂ S ₂ O ₈	KMnO ₄	NaBO ₂ ·H ₂ O ₂
Total alkanes	41.3	18.4	23.8	11.9	0.7	2.9
Branched or cyclic alkanes	0.5	0.0	0.0	0.0	0.0	0.0
Alkane range	16–33	15–33	15–33	18–31	22–27	19–29
Total fatty acids ^b	52.2	76.0	71.3	71.6	72.6	64.6
Unsaturated fatty acids ^b	1.0	1.0	1.6	6.2	14.2	14.4
Branched fatty acids ^b	2.0	0.0	0.0	5.6	4.5	3.5
Fatty acids range	14–30	12–30	14–30	14–28	14–24	12–26
Total aromatics ^b	0.0	0.0	0.0	5.6	24.4	30.1

^a Percentage of total amount of products present in the gas chromatogram

^b Methyl esters

bimodal distribution with a minimum value near C₂₁. The proportion of the higher molecular weight chains (>C₂₀) diminished with depth; palmitic and stearic acids represent the major fatty compounds in the deeper layer.

The organic compounds obtained after the degradation of kerogen are shown in Figure 3. The same

chromatographic peaks with no important differences were found in the three levels studied; only the data obtained for sample C will be discussed.

Partial degradation with potassium persulphate yielded aliphatic compounds that are thought to be incorporated in kerogen by means of easily removable

Figure 3 Gas chromatographic separation of the products obtained from Portazzo kerogen after: a, perborate degradation; b, degradation with potassium persulphate; and c, alkaline permanganate oxidation of the residue of the persulphate degradation. Major aromatic compounds are indicated above the chromatograms and the aliphatic series of alkanes (ALK.) and fatty acids (F.A.) are indicated below

(physical or chemical) bonds. Palmitic and stearic acids represented the major chromatographic peaks. Unsaturated and branched fatty chains were more common than in the toluene extract, while the relative contribution of alkanes was much lower than in the bitumen fraction.

Alkaline permanganate oxidation of the persulphate kerogen residue produced additional amounts of fatty

acids with a different distribution pattern. The relative contribution of unsaturated acids ($C_{16:1}$ and $C_{18:1}$) was higher than in the former cases, and the alkane proportion was of little significance. In contrast with the alkanes, the relative content of fatty acids was similar in the persulphate-removable forms and in those strongly associated types obtained after permanganate oxidation. The aromatic compounds represented 24 wt % of the total volatile products and consisted of benzenepolycarboxylic acids. In addition, dicarboxylic aliphatic and phenolic acids were also present in the permanganate digest, but in very small amounts. A comparable composition was reported when similar degradative techniques were used on the pseudokerogen fraction of brown coal¹³.

The effects of perborate degradation on kerogen are very interesting. When used on soil humic acids, this mild reagent induces depolymerization of the high molecular weight structures, which yield a series of acid-soluble, oligomeric fractions, including individual molecules that may be studied by gas chromatography⁸. The perborate treatments were repeated five times with a fresh perborate solution to achieve total solubility of the original sample (Figure 4). Kerogen fractionation was observed after acidification of the solution obtained in the progressive steps: the sample was transformed into a yellow, acid-soluble fraction, a brown, acid-insoluble fraction and residual perborate-insoluble kerogen. After a single perborate treatment, the first two fractions amounted to 12 and 53 wt %, respectively. For qualitative studies, the yellow fraction obtained after each step was recovered, aggregated and stored, and the other two fractions were mixed and degraded by the same method. The differences of the yields between the second and fifth step were found to be moderate, the first step being the most productive in terms of the relative yields of the soluble products. After the fifth step, the results for the fractions obtained showed that 4% of the original kerogen remained insoluble, 65% was transformed into a humic acid-like colloid and the remainder was transformed into acid-soluble oligomers and individual molecules, and also included losses of CO_2 estimated at $\approx 15\%$ of the final C.

The gel filtration of the coloured fractions obtained by kerogen depolymerization with perborate showed that these fractions consisted of mixtures of polymeric (macromolecular or oligomeric) systems of alkali-soluble products. After using Sephadex G-50 to separate the acid-

Figure 4 Progressive degradation and solubilization of kerogen during a multi-step perborate treatment: □, acid-soluble components; ▨, humic acid-like components; ▩, kerogen residue

insoluble colloids from kerogen, the following results were obtained: 6% ($K_{av}=0.00$), 25% ($K_{av}=0.55$), 69% ($K_{av}=1.05$). For the acid-soluble oligomeric fraction (Sephadex G-25), the distribution was 3% ($K_{av}=0.00$), 97% ($K_{av}=1.12$). Assuming that the exclusion limits for Sephadex G-50 and G-25 were 10000 and 5000, respectively, the molecular size distributions of the acid-insoluble and acid-soluble fractions of the digests may be considered similar to those of humic and fulvic acids. Gel filtration was also performed after each of the degradation steps to monitor the depolymerization extent, and it was observed that the magnitude of the excluded fraction of the acid-insoluble colloid was $\approx 35\%$ after the first step, a percentage which diminished after successive treatments, finally reaching the value indicated above.

The infrared spectrum of the acid-insoluble fraction (not shown here) was very similar to that of original kerogen, although the bands corresponding to oxygen-containing functional groups were a little more pronounced. This is in agreement with its elementary composition (C, 42.1%; H, 2.2%; O, 55.6%; N, 0.1%; atomic H/C = 0.63; atomic O/C = 0.99).

After the g.c. separation of the compounds with the lowest molecular weight, present in the acid-soluble fraction (Figure 3), several aromatic and aliphatic substances were identified: the most prominent peaks corresponded to methoxy-substituted benzenedicarboxylic acid, palmitic acid and stearic acid. Benzenedi- and tri-carboxylic acids were also detected. A high number of alkanes and fatty acids were present, the latter in relatively small amounts.

DISCUSSION

The organic material in the Portazgo sediments showed a relatively high degree of transformation: humic and fulvic acids were not present, and most of the characteristics of organic matter suggested a formation in a partially aerobic environment with a significant contribution by vascular plants^{14,15}.

Although the contribution of lower plants to the original biomass may be taken into consideration, the study of the bitumen fraction gave no evidence of predominant activity of this type of organism, judging from the very low proportion of branched fatty acids (iso and anteiso), the absence of isoprenoid alkanes and the relatively small proportion of alkanes with a lower boiling point, especially in younger layers¹⁶. Nevertheless, the C_{16} – C_{20} region of the alkane distribution diagram (Figure 2), with no predominance of the odd-numbered carbon chains, might be partly explained by a microbial origin. The chains of more than 25 carbon atoms, however, showed a certain correlation with the fatty acids of the corresponding region of their distribution diagram (which is more pronounced in the layers A and C), and were probably derived from epicuticular waxes in higher plants¹⁷; a higher proportion of dehydroabietic acid in deeper layers indicated the presence of conifer resin constituents².

The chemical data are consistent with palaeobotanical studies and suggest a continental deposit originating from a forest and brushwood mesophyllic species, decomposing with typical marshy plants, including *Sphagnum* and Pteridophyta and algal species (Botryococcaceae, Pyrrophyceae forms); the continental

indicators of palaeoflora were more conspicuous in upper layers.

The degradative techniques yielded several types of aromatic constituents, presumably derived from the lignin of vascular plants¹⁸. The successive degradation with persulphate and permanganate illustrated the different degrees of association of the organic constituents to the kerogen 'matrix'¹⁹. Different aliphatic constituents were found associated in loosely bound forms to kerogen, mainly alkanes and fatty acids (in persulphate degradation), while the most stable kerogen constituents yielded fatty acids and benzenecarboxylic acids (in permanganate degradation). In view of the above-mentioned differentiation and after considering the relative contribution of the different kinds of organic compounds to the composition of the sediment (free compounds in bitumen, easily removable compounds and compounds contributing to stable structures), several differences were observed (Table 2). Alkanes tend to occur free as well as in loosely associated forms. To all appearances, they were physically entrapped in the kerogen structure. The total percentage of alkanes represents a mean value of 28 wt% in the bitumen of the rock from which the kerogen was isolated; 12% was persulphate-removable compounds, and alkanes were practically absent amongst the permanganate degradation products, with values below 1%. In addition, it was observed that long-chain paraffins ($>C_{20}$) tended to be present in the free state, while the kerogen was the chief source of the lower boiling point alkanes.

Very different tendencies were observed in fatty acids: most seem to be chemically associated with kerogen and no important differences were found in their relative amounts in free or stable forms. The distribution diagrams of these bonded lipids are different from those of the bitumen fractions, the former showing a higher predominance of the low molecular weight fraction homologues, presumably derived from the incorporation or condensation of lipids from lower plants in the kerogen matrix²⁰. The above differences between the free and bonded lipids were found in all the layers studied and coincided with those described for soil organic matter after supercritical gas extraction²¹.

Perborate degradation applied to kerogen turned out to be a rapid and interesting method for structural studies, presenting simultaneously several of the advantages of the mild and drastic degradations previously assayed. A single treatment with sodium perborate was sufficient to degrade 65% of this kerogen into soluble products consisting of a humic acid-like polymer and fulvic acid-like molecules. The production of this type of 'regenerated' alkali-soluble substances seems to be a typical feature of mild degradation procedures²² and may be used for different studies on kerogen structure²³. In addition, the results of the analysis of the individual molecules in the acid-soluble fraction was also in agreement with those previously obtained by successive persulphate–permanganate degradations. A low benzenecarboxylic/phenolic ratio was obtained by the perborate treatment, while the very low proportion of phenolic acids in the permanganate degradation may be attributed to the strongly oxidant character of that treatment. Fatty acids and alkanes were released by perborate over a wide range, and their total amount also confirmed those found by permanganate degradation.