Note

Occurrence of proteinaceous moieties in S- and O-rich Late Tithonian kerogen (Kashpir oil Shales, Russia)

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Abstract

The polar fraction, isolated from the off-line pyrolysate at 400°C of a Late Tithonian, sulphur- and oxygen-rich, kerogen was examined via Raney Nickel desulphurization and TMAH thermochemolysis. Important information on this kerogen, not accessible via conventional pyrolysis, was thus obtained: (i) its structure is not simply based on alkyl skeletons cross-linked by ether and (poly)sulphide bridges, (ii) TMAH thermochemolysis afforded direct evidence of the survival of proteinaceous moieties in this 140 million years old kerogen and (iii) encapsulation within an aliphatic organic matrix was probably the main pathway reponsible for such a conspicuous preservation, also possibly favoured by the presence of numerous sulphur links. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The 140 m.y. sulphur- and organic-rich Kashpir oil shales form a widespread deposit on the Russian plat-form (total area ca. 90,000 km^2). Recent studies (Riboulleau et al., 2000) on the kerogen of the organic-richest level (45% TOC) of the Gorodische section, termed "ftop", showed that intermolecular sulphur incorporation was a major process in its formation. These studies also pointed to a substantial role for oxi-dative cross-linking via ether bridges, reflected by (i) the high O/C atomic ratio (ca. 0.1) of ftop kerogen and (ii) a relatively abundant production of oxygen-containing products, especially *n*-alkanones, upon off-line pyrolysis at 400°C. However, as expected for such a heteroatom-rich kerogen, large amounts of difficult to identify polar products occur in the off-line pyrolysate. Indeed, the

most polar components (methanol-eluate) account for ca. 40% of the total pyrolysate, and the bulk, especially the predominant non-acid components, cannot be iden-tified by gas chromatography/mass spectrometry (GC–MS). Polar pyrolysis products contain important infor-mation which cannot be retrieved through analysis of less polar ones (e.g. Rullkötter and Michaelis, 1990). The present study was therefore focused on the metha-nol-eluted non-acid (MeNA) fraction from the 400°C off-line pyrolysate of ftop. It was submitted to Raney Nickel desulphurization, additional pyrolysis via con-ventional Curie Point pyrolysis at 650°C and thermo-chemolysis with tetramethylammonium hydroxide (TMAH) at 358 and 650°C.

2. Experimental

Isolation of ftop kerogen, off-line pyrolysis at 400°C for 1 h, pyrolysate separation by column chromatography into fractions of increasing polarity using *n*-heptane, toluene and methanol, separation of the latter into an

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acid and a non-acid fraction by extraction, elemental analysis, GC–MS analyses and conventional Curie point pyrolysis-GC–MS (CuPy-GC–MS) were as previously described (Riboulleau et al., 2000).

Raney Nickel desulphurization, and hydrogenation of a part of the MeNA, fraction, was carried out according to Sinninghe Damsté et al. (1988). The desulphurized mixture was separated by thin layer chromatography (TLC) on silica gel plates, developed with cyclohexane, into two subfractions (R_f: 0–0.5 and 0.75–0.9), recovered by CH_2Cl_2 extraction and analysed by GC–MS. CuPy-GC-MS and TMAH thermochemolysis (TMAH/CuPy-GC–MS) of the MeNA fraction were performed on ca 1.5 mg aliquots to which a solution of 200 µl of internal standard (*n*-icosane, 1.7 mg/1600 µl of CH_2Cl_2) was added. Thermochemolysis was performed according to Challinor (1989) by adding 300 µl of a 25% solution of TMAH in methanol to MeNA aliquots. Identifications were based on elution orders and mass spectra previously published (e.g. Knicker and Hatcher, 1997; Zang et al., 2000). An analytical flow chart is given in Fig. 1.

3. Results and discussion

The MeNA fraction accounts for ca. 25% of the total 400°C off-line pyrolysate of ftop kerogen. Elemental analysis of this fraction showed high contents of oxygen and sulphur (9.7 and 7.2 wt.%, respectively) and a substantial level of nitrogen (1.5 wt.%). The atomic ratios (O/C: 0.1, S/C: 0.038 and N/C: 0.018) are similar to those of ftop kerogen (Riboulleau et al., 2000), except for S/C which substantially decreased (0.068 in ftop). As observed for other S-rich kerogens (e.g. Mongenot et al., 1999) a part of the (poly)sulfide bridges are cleaved upon pyrolysis

and the MeNA fraction mostly corresponds to building blocks of ftop kerogen freed through the partial cleavage of such thermally labile links. Accordingly, these blocks are rapidly released upon pyrolysis and can retain some potentially labile moieties. The GC trace of the fraction shows a large hump, reflecting a complex composition and extensive co-elutions, with only two well resolved peaks (Fig. 2a). The latter were previously identified as C_{16} and C_{18} *n*-alkanols (Riboulleau et al., 2000) and only C_9-C_{16} *n*-alkylphenols and *n*-alkoxyphenols could be further identified in this complex mixture, via selective detection of characteristic ions.

3.1. Desulfurization with Raney Nickel and hydrogenation

This treatment is commonly used to release and identify hydrocarbon skeletons in S-bound moieties (e.g. Sinninghe Damsté et al., 1988). Elemental analysis showed an almost complete removal of sulphur (>95%) from the MeNA fraction. C_{14}–C_{28} *n*-alkanes, derived from moieties with linear skeletons were formed through the cleavage of sulphur links. Nevertheless, (i) the GC trace still showed a prominent hump (Fig. 2b) and (ii) hydrocarbons separated by TLC only accounted for ca. 6% of the desulphurized fraction which is still dominated (>90%) by a complex, oxygen-rich and nitrogen-containing, mixture of chiefly unidentified compounds. Indeed, GC/MS only showed the presence, in addition to the above *n*-alkanes, of the few alkanols and phenols already detected in the untreated MeNA fraction. In contrast, Raney Nickel desulphurization of the complex MeNA fraction isolated from the off-line pyrolysate at 400°C of a S-rich kerogen of Orbagnoux (Mongenot et al., 1999) resulted in a sharp decrease in hump intensity and in a large production of alkanes. In
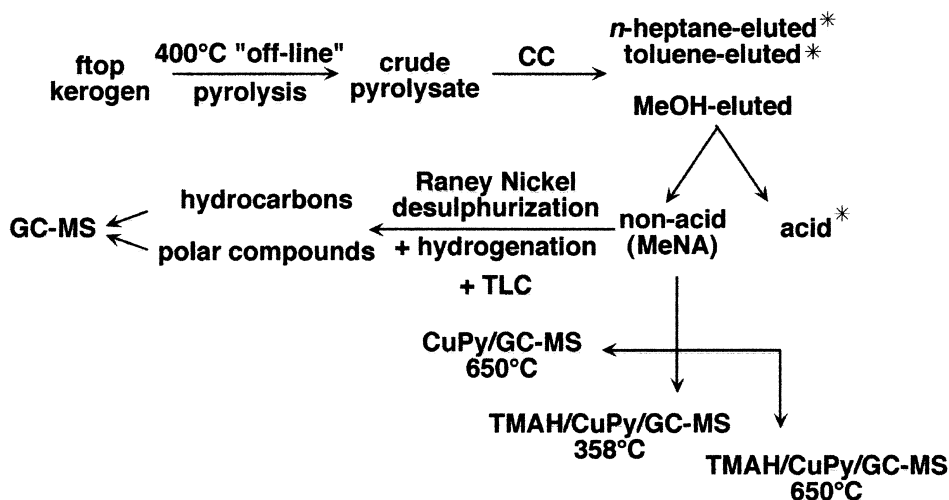

Fig. 1. Analytical flow chart for structural analysis of the methanol-eluted, non-acid pyrolysis products of ftop kerogen. (*) Previously analysed (Riboulleau et al., 2000).

both cases, the MeNA fraction corresponds to sulphur bridge-comprising building blocks. Nevertheless, unlike the Orbagnoux sample, the building blocks of ftop kerogen do not simply correspond to alkyl skeletons cross-linked by such S-bridges since a simple mixture of hydrocarbons is not obtained following desulphurization.

3.2. Conventional pyrolysis

The trace of the pyrolysate obtained via pyrolysis of the MeNA fraction (CuPy-GC–MS at 650°C) still exhibits a large hump. Moreover, all the pyrolysis products identified had already been detected via direct CuPy-GC–MS at 650°C of ftop kerogen and/or GC–MS analysis of the less polar fractions of its off-line pyrolysate (Riboulleau et al., 2000). Pyrolysis at 650°C therefore resulted, as expected, in further cracking of the MeNA fraction but this was not associated with the release of new series affording further information on its composition.

3.3. TMAH thermochemolyses

TMAH thermochemolysis is a powerful tool used to examine the structure of oxygen-containing macromolecules: (i) efficient cleavage is achieved for esters and ethers (Challinor, 1989) and peptide bonds (Knicker and Hatcher, 1997) and (ii) methylation makes easier GC–MS identification of polar pyrolysis products. TMAH thermochemolysis was used for the study of various samples, including kerogens (Kralert et al., 1995), aliphatic biopolymers (McKinney et al., 1996), algal sapropels (Knicker and Hatcher, 1997), peat humic acids (Zang et al., 2000) and Recent sediments (Garcette-Lepecq et al., 2000).

The nature and relative abundances of TMAH thermochemolysis products can be influenced by temperature (Hatcher and Clifford, 1994). TMAH/CuPy-GC–MS of the MeNA fraction was performed at 358 and 650°C. The traces of the two pyrolysates show a simple profile without any significant hump. The same pyrolysis products, methylated fatty acids and amino acids, are identified in both cases (Figs. 2c and d). However, comparison with the internal standard indicated important quantitative differences for the amino acids. Thus, 358°C is too low a temperature for obtaining an efficient release and much higher yields are observed at 650°C. Furthermore, no significant amounts of secondary products, from the thermal degradation of amino acids, were detected even at 650°C. Thus, the latter temperature is
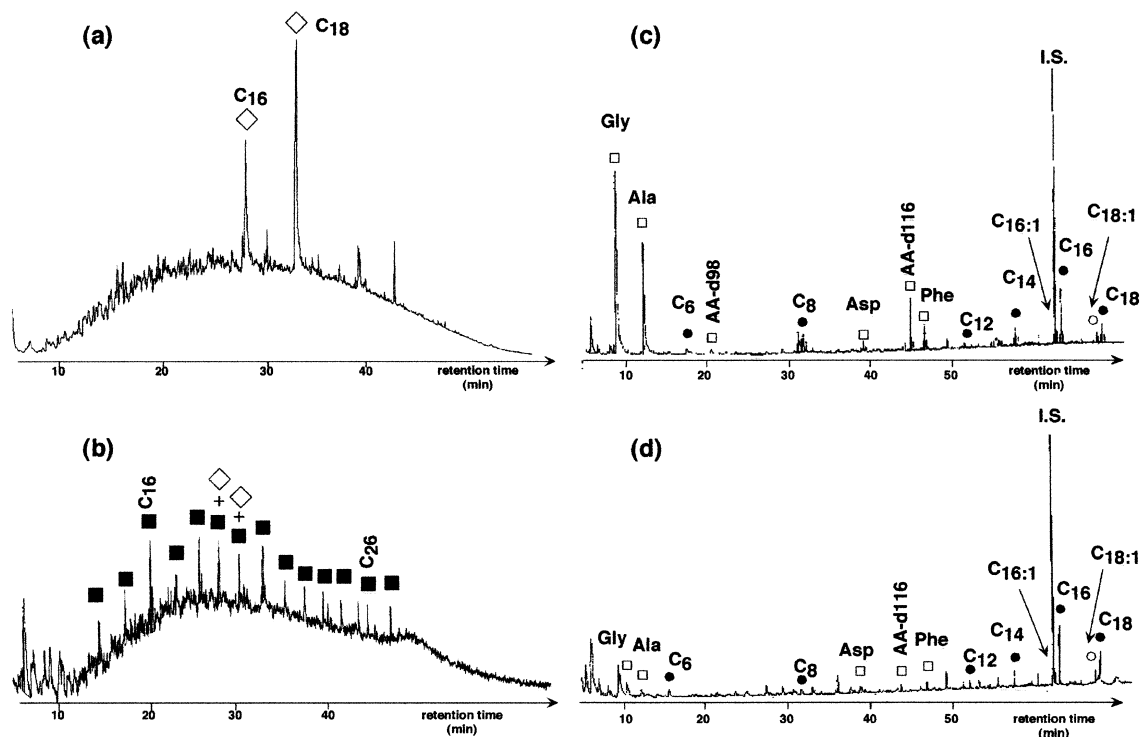

Fig. 2. GC-traces of (a) untreated MeNA and (b) MeNA after Raney Nickel desulphurization and hydrogenation; TIC trace of TMAH/CuPy/GC–MS of MeNA at (c) 650°C and (d) 358°C. ◇, *n*-Alkanols; ■, n-alkanes; □, amino acids and derivatives; ●, saturated fatty acids; ○, unsaturated fatty acids; I.S., internal standard, n-icosane; (C_n), carbon number; Gly, glycine; Ala, alanine; Asp, aspartic acid; Phe, phenylalanine; AA-d98, amino acid derivative with base peak at *m/z* 98; AA-d116, amino acid derivative with base peak at *m/z* 116.

suitable for the release of amino acid moieties from the MeNA fraction. Almost no difference was noted in fatty acid abundance.

3.3.1. Fatty acids

The fatty acids (C_6–C_{18}, maximum at C_{16}) show a strong even-over-odd predominance and comprise unsaturated compounds. Re-examination of the pyrolysate from conventional CuPy at 650°C, via selective detection of characteristic fragments, confirmed that fatty acids are not generated under these conditions from the MeNA fraction. This production of acids via thermochemolysis at 650°C, and even at 358°C, illustrates the high efficiency of TMAH for cleaving the ester bonds that linked such moieties to non-GC-identifiable structures in this fraction. The distribution and nature of these acids is consistent with the early incorporation, and very efficient protection against subsequent degradation, of weakly altered acids of microalgal and/or cyanobacterial origin in the macromolecular structure of ftop kerogen

3.3.2. Amino acids

Several amino acids were identified in the TMAH pyrolysates of the MeNA fraction (Fig. 2c and d). An amino acid derivative (base peak: m/z 98) possibly related to lysine or phenylalanine (Knicker and Hatcher, 1997; Zang et al., 2000) and an α-amino acid (base peak: m/z 116) were also observed. Parallel control experiments did not show any significant amount of such compounds. Diketopiperazines have been observed previously in conventional pyrolysates of various proteinaceous materials. However, it seems that such a production is restricted to proline-containing materials and do not occur in the presence of TMAH (e.g. Zang et al., 2000).

Amide-N in proteinaceous structures is generally considered as highly sensitive to diagenetic degradation. Nevertheless, recent studies using solid state ^{15}N NMR showed that the bulk of the refractory nitrogen in algal compost (Knicker et al., 1996), algal sapropels of Mangrove Lake sediments (Knicker and Hatcher, 1997), degraded biomass of *Botryococcus braunii* deposited under highly oxic conditions (Derenne et al., 1998), Everglades peat (Zang et al., 2000) and Black Sea surface sediments (Garcette-Lepecq et al., 2000) corresponds to amide groups. Moreover, TMAH thermochemolysis demonstrated that this amide-N is mostly proteinaceous in the 4000 years old sapropel of the Mangrove Lake (Knicker and Hatcher, 1997), in the Everglades peat (Zang et al., 2000) and in the Black Sea surface sediments (Garcette-Lepecq et al., 2000). Phytoplankton decay in laboratory incubations (Nguyen and Harvey, 1998) also showed that proteinaceous material can survive early diagenesis. All these observations were related to recent materials. In contrast, the present study deals with a ca. 140 million years old kerogen and indicates that protein-derived moieties also survived in this ancient material. To the best of our knowledge, this is the first time that TMAH thermochemolysis has provided evidence of the survival of proteinaceous material over such a time scale.

The cause of the recalcitrance of proteins, or derived moieties, in organic-rich materials is still a matter of debate. Three processes have been considered: Firstly, studies on marine organic matter suggest that some specific proteins exhibit a high resistance to degradation and are selectively preserved (Tanoue et al., 1996). Secondly, refractory, amide-containing materials could be formed via the degradation–recondensation pathway of Tissot and Welte (1984): that is formation of highly resistant geomacromolecules via abiotic random condensation of partly degraded proteins, polysaccharides and lipids. Thirdly, encapsulation of proteins, and derived moieties, into intrinsically refractory organic substances was recently shown to be an important pathway (Knicker and Hatcher, 1997; Zang et al., 2000) which can provide effective physical protection, within three-dimensional structures, to potentially labile compounds. The protective matrix would correspond to aliphatic biopolymers for the Mangrove Lake sapropel (Knicker and Hatcher, 1997) and humic substances for the Everglades peat (Zang et al., 2000). In the latter case, direct evidence supporting this pathway was obtained via simulated encapsulation of proteins in humic acids.

The MeNA fraction corresponds to building blocks of ftop kerogen freed via partial cleavage of sulphide bridges. The nature of its thermochemolysis products points to an important role for the encapsulation pathway in the survival of proteinaceous moieties in this ancient material. Indeed, the presence of aliphatic chains in the organic matrix is probably a major feature for the efficient protection of the encapsulated molecules (Knicker and Hatcher, 1997; Zang et al., 2000). Thus, the production of both fatty acids and amino acids from the MeNA fraction strongly supports the encapsulation process. In addition, Nguyen and Harvey (1998) considered that protection may also be afforded by encapsulation into S-rich macromolecules. Accordingly, for ftop, the efficiency of the encapsulation might be increased by the presence of sulphur links, hence the conspicuous survival, on a geological time scale, observed for proteinaceous moieties in this ancient material.

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