



Molecular evidence for life in the 3.5 billion year old Warrawoona chert

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ABSTRACT

The biological origin of organic matter in the oldest siliceous sediments (cherts) is still debated. To address this issue, the insoluble organic matter (kerogen) was isolated from a chert of the Warrawoona group. The chemical structure of the kerogen was investigated through a combination of analytical techniques including solid-state ¹³C nuclear magnetic resonance and pyrolysis. Although dominated by aromatic hydrocarbons, the pyrolysate comprises a homologous series of long chain aliphatic hydrocarbons characterized by odd-over-even carbon number predominance. This distribution is only consistent with a biological origin. As kerogen must be contemporaneous of the solidification of the chert, this observation should be regarded as an evidence for the presence of life on Earth, 3.5 By ago.

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1. Introduction

Cherts are amongst the oldest sedimentary rocks on Earth and offer a remarkable record of well-preserved microfossils throughout the Precambrian. Based on spectroscopic and morphological observations, microstructures in the 3.5 By old Apex chert from the Warrawoona group in Australia have been attributed to fossil cyanobacteria and thus would constitute the oldest evidence for life on Earth (Schopf, 1993). In addition to morphological criteria, the carbonaceous nature of the microstructures established by Laser Raman microspectroscopy was considered as an evidence for their biogenicity (Schopf et al., 2002). However, the validity of this criterion as evidence for life has been questioned and it is now accepted that Raman spectroscopy alone cannot assess biogenicity (Pasteris and Wopenka, 2002). Moreover, it was shown that abiotic synthesis (Fischer–Tropsch) is able to yield organic microstructures exhibiting similar morphological features and Raman spectra as the putative microfossils from the Apex chert (Brasier et al., 2002; Garcia-Ruiz et al., 2003). Carbon isotope composition was also often put forward as an additional criterion of the biological origin of the organic matter in the Archean cherts (Mojzsis et al., 1996; Schidlowski, 2001). However, once again, this

approach was shown not to be univocal, as abiotic processes can lead to similar fractionation (Horita and Berndt, 1999; Van Zuilen et al., 2002; McCollom and Seewald, 2006). More recently, the wide diversity in the morphological features of the stromatolites from Strelley Pool chert and the continuity in the carbonaceous matter record in rocks >3.0 By suggested that most OM in these rocks was produced by living organisms (Allwood et al., 2006; Tice and Lowe, 2006).

A new criterion, in the form of an indisputable biomarker, is therefore needed in order to resolve the debate about when life first appeared on Earth. Biomarkers characteristic of cyanobacteria were reported in the soluble organic fraction of the ca. 2.7 By old shales from Hamersley Basin in Australia (Brocks et al., 1999). However, evidence drawn from soluble organic matter is disputable because there are several potential sources of post-depositional contamination, notably subsurface biological activity and groundwater penetration. Although such issues have been carefully evaluated in the case of Hamersley Basin shales (Brocks et al., 1999), the synchronism between the formation of soluble molecular fossils and the host rock remains difficult to demonstrate (Brocks et al., 2003a; Brocks et al., 2003b). In contrast, it is generally accepted that the insoluble macromolecular organic matter characterized by covalent bonds is syngenetic with the host rock hence an increasing interest for this organic fraction (Brocks et al., 2003a; Marshall et al., 2006; Marshall et al., 2007). We have therefore isolated the kerogen from a chert of the lowest metamorphic grade from the Warrawoona Group and investigated its chemical structure using a combination of spectroscopic and pyrolytic tools.

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2. Methods

The sample (PPRG 006 from Precambrian Palaeobiology Research Group collection, courtesy of W. Schopf) was selected for this study from a large collection of cherts on the basis of its isotopic composition (see below). It was collected in the lower chert horizon of the Towers Formation in the North Pole B Deposit Mine from the upper lip of the open cut on the west side; Marble Bar 1:250,000 map sheet grid ref n°. 223357 (Walter et al., 1983). The North Pole Dome, from which the sample was collected, is situated 30 km NW of the Apex cherts in the Marble Bar region. Since 1983, the Towers Formation cherts at The North Pole Mine have been reassigned to the Dresser Formation, which has an age of ca. 3.490 By (van Kranendonk, 2006).

The insoluble organic matter was isolated from the ground chert as follows: stirring at room temperature for several hours in dichloromethane/methanol, 2/1, v/v in order to remove soluble organics, followed by demineralization using the classical HF/HCl treatment (Durand and Nicaise, 1980) and further solvent extraction. After each treatment, the insoluble residue was recovered by centrifugation.

Variable amplitude cross-polarization/magic angle spinning (VACP/MAS) solid-state ^{13}C nuclear magnetic resonance (NMR) spectrum was obtained at 100.62 MHz for carbon (Bruker Avance 400 spectrometer, recycle time 5 s, contact time 1 ms), using a high spinning rate (20 kHz) to spin out chemical anisotropy and avoid spectrum disturbance by spinning side bands.

Curie point pyrolysis-gas chromatography/mass spectrometry (CuPy-GC/MS) was performed with a Fischer 0316 flash pyrolyser. The sample (ca. 2 mg) was pyrolysed for 10 s using ferromagnetic tubes with a Curie temperature of 650 °C under a 5 ml min $^{-1}$ He flow. The pyrolyser was directly coupled to the GC/MS: a HP-5890 gas chromatograph (30 m CPSil5CB capillary column, i.d. 0.25 mm, film thickness 0.5 μm) and a HP-5889A mass spectrometer (electron energy 70 eV, ion source temperature 205 °C, scanning from 40 to 650 a.m.u, 0.7 scan/s). The GC oven was programmed from 100 to 300 °C at a rate of 2 °C min $^{-1}$ after a first stage at 100 °C for 10 min.

High resolution transmission electron microscopy (HRTEM) observations were carried out using a Jeol 2011 microscope operating at 200 keV. Image analysis was conducted after skeletonization as described by Rouzaud and Clinard (2002).

3. Results and discussion

Demineralization of 149.5 g of crude rock yielded 32.7 mg of kerogen, i. e. 218 ppm. The carbon content (53.4%) of the isolated kerogen indicates that most of the initial carbon of the rock is recovered in this insoluble fraction, since the crude rock contains 121 ppm of carbon (Beaumont and Robert, 1999). The elemental composition gives an atomic H/C ratio of 0.62, indicating a rather aromatic character. This kerogen concentrate also contains mineral phases, such as titanium and chromium oxides, as revealed by SEM-EDS. These oxides, which are known to survive HF / HCl treatment,

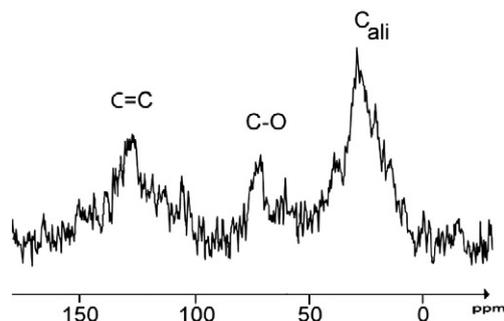


Fig. 1. Solid state variable amplitude CP-MAS ^{13}C NMR spectrum of the kerogen from the Warrawoona chert.

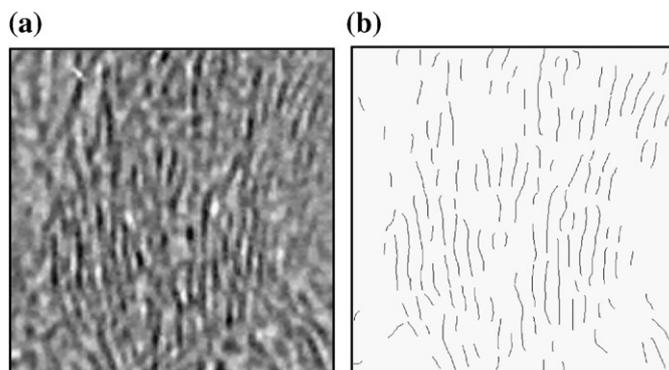


Fig. 2. HRTEM image (10 \times 10 nm) of the kerogen from the Warrawoona chert and corresponding skeletonized image.

were previously observed by Kato and Nakamura (2003) on crude cherts from Warrawoona.

The solid-state ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectrum of the isolated kerogen (Fig. 1) is dominated by a broad signal centered at 29 ppm due to aliphatic carbon atoms and shows two relatively minor peaks at 70 and 130 ppm, assigned to C–O and unsaturated carbons, respectively. Although solid-state CP/MAS ^{13}C NMR is known to overestimate aliphatic carbons, it must be noted that the presence of alkyl chains is also observed through Fourier transform Infrared (FTIR) spectroscopy (not shown). Indeed, the spectrum of the Warrawoona chert exhibits the typical bands for CH_2 and CH_3 in the 2850–2965 cm^{-1} range. The 2925 cm^{-1} and 2850 cm^{-1} bands are due to asymmetrical and symmetrical stretching vibrations of CH_2 groups. The asymmetrical stretching band of the CH_3 groups can be clearly seen at 2965 cm^{-1} . A band at 1585 cm^{-1} reflects the stretching vibration of aromatic C=C whereas carbonyl functions are detected at 1710 cm^{-1} . The broad band around 1160 cm^{-1} may be due either to ether functions or to residual silica. The NMR spectrum strongly differs from those of mature kerogens, for which the 30 ppm peak is virtually absent due to thermal release of alkyl chains upon maturation (Miknis et al., 1982). As a result, the NMR spectrum of the Warrawoona chert indicates that this material, although 3.5 By old, did not experience any severe, thermally induced chemical modification.

This may appear at variance with the Archean age of the sample. However, the Warrawoona Group has been described as the one which underwent the lowest metamorphism and which contains the best preserved Archean stratigraphic succession on Earth (Van Kranendonk et al., 2002). Moreover, the carbonaceous matter structure, which cannot be used to determine the biological origin (or not) of the sample, can provide powerful information about its thermal history as recently stressed by Tice and Lowe (2006). Indeed, large “graphitic domains” are commonly reported when the sample reached the metamorphic grade of prehnite–pumpellyite (Wedeking and Hayes, 1983). In contrast, our observations of the PPRG 006 sample through high resolution transmission electron microscopy (HRTEM) (Fig. 2) and further image analysis showed relatively large aromatic units but no such “graphitic domains” (PPRG 006 exhibits a mean layer extent about 1.1 nm, corresponding to about 15 fused aromatic rings (Rouzaud et al., 2005)). It must be noted that the occurrence of these large aromatic units is not at variance with the presence of aliphatic carbons in the FTIR and NMR spectra as the latter cannot be detected through HRTEM. However the organization degree is far from having reached the graphite crystalline stage. Indeed, the interlayer spacing is 0.39 nm, significantly higher than the graphite value (0.3354 nm). These observations are in perfect agreement with a recent study of the Apex chert through TEM and electron energy loss spectroscopy (EELS) (de Gregorio and Sharp, 2006). Taken

together, these data point to a rather low metamorphic grade for the Warrawoona sample in agreement with previous reports.

This was confirmed by Curie point pyrolysis-gas chromatography coupled with mass spectrometry, which we performed on the same sample using ferromagnetic wires with a Curie temperature of 650 °C. As shown in Fig. 3a, the trace of the pyrolysate is dominated by a wide range of aromatic compounds along with a series of *n*-alkane/*n*-alk-1-

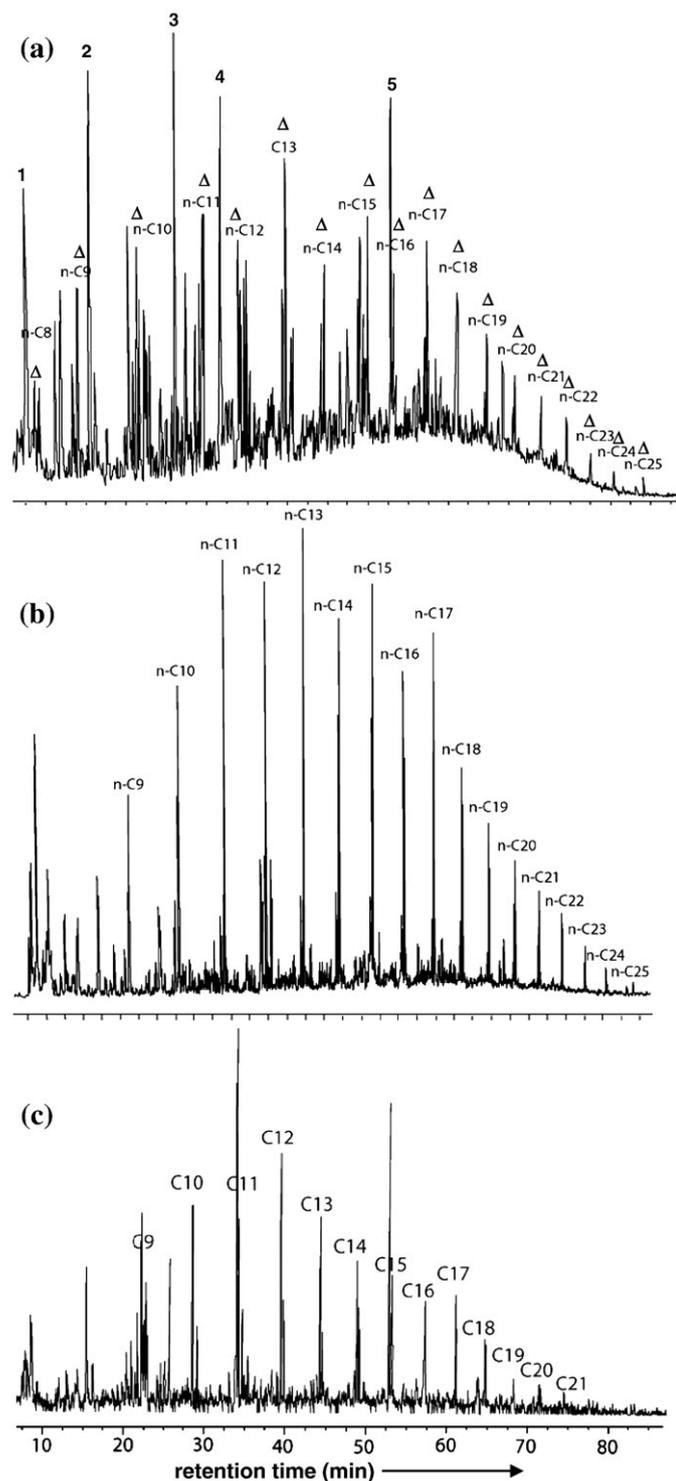


Fig. 3. Curie point pyrolysis at 650 °C: a) Total ion current trace of the pyrolysate. Δ refers to the *n*-alkane/*n*-alkene doublets, 1: toluene, 2: styrene, 3: methylphenol, 4: dimethylphenol, 5: fluorene b) Ion chromatogram at m/z 57 showing the distribution of the *n*-alkanes. c) Ion chromatogram at m/z 58 showing the distribution of the *n*-alkanones.

ene doublets up to C₂₅. The aromatic compounds comprise hydrocarbons (PAHs) along with thiophenic and phenolic products. PAHs are dominated by toluene, styrene, naphthalene, methyl-naphthalenes and fluorene. Significant contributions of indene and anthracene/phenanthrene derivatives are also noted in the pyrolysate. The largest PAHs detected under our analytical conditions is fluoranthene. This rather large production of PAHs upon pyrolysis is expected due to the aromatic character of the sample as revealed by spectroscopic analyses and HRTEM. Thiophenic compounds include C₁ to C₄-thiophene, C₁ to C₂-benzothiophene and C₀ to C₁-dibenzothiophene. C₀ to C₃-phenol are also identified in the pyrochromatogram. These phenols are distinct from those commonly observed in pyrolysates of lignin-derived material. They are likely formed through the cleavage of aromatic ether bonds, as observed in meteorite pyrolysates (Remusat et al., 2005b).

In addition to these, dominant, aromatic products, several series of aliphatic compounds contribute to the pyrolysate. A series of *n*-alkane/*n*-alk-1-ene doublets up to C₂₅ is thus identified (Fig. 3b). These alkanes and alkenes originate from the homolytic cleavage of long alkyl chains through capture and elimination of H[•] radicals, respectively. A series of *n*-alkylbenzenes up to C₂₂ can also be identified in the pyrolysate along with its higher homologue (*n*-alkyl, methylbenzenes). These products result from the cyclization of the alkyl radical, thus confirming the presence of long chains covalently linked to the macromolecular network. An additional series of aliphatic compounds, namely *n*-alkan-2-ones, was identified in the pyrolysate of the Warrawoona chert. Its distribution (from C₉ to C₂₁) is shown by the ion chromatogram at m/z 58 (Fig. 3c). Such ketones are formed upon pyrolysis through homolytic cleavage of ether bonds. Their presence in the pyrolysate is therefore consistent with that of C–O groups inferred from spectroscopic data. The formation of such ether bonds through oxidative cross-linking was shown to be favoured by silicification in a 93 million years-old chert from Italy although such linkages also occur in the organic matter from non-cherty rocks (Salmon et al., 2003). Therefore the oxidative formation pathway of the kerogen seems to be mediated by silicification, pointing to syngeneity between the cherty matrix and the kerogen.

Under laboratory conditions, C–C bonds in alkyl chains are known to be cleaved at 400 °C (within a few tens of minutes) and can be cleaved at lower temperatures on longer time scales. As a result, the release of the alkane/alkene doublets upon pyrolysis confirms that the kerogen of Warrawoona was never exposed to high temperatures for any significant time period.

The pyrochromatogram of the Warrawoona chert at 650 °C shows a slight predominance of odd carbon numbered *n*-alkane/*n*-alk-1-ene doublets (Fig. 3a). It must be noted that the alkylbenzenes, which also originate from alkyl chains, exhibit a similar predominance in the same carbon number range. The distribution of the *n*-alkanes in the pyrolysate can be described more accurately by using the ion chromatogram at a mass-to-charge ratio (m/z) of 57 (Fig. 3b). It must be noted that no classical biomarker of recent organic matter such as pristane, phytane, sterane or hopane could be detected in the pyrolysate in spite of a careful search using their characteristic mass fragments. More generally, a virtual lack of branched alkanes is noted. Taken together, the lack of branched compounds and the slight odd-over-even carbon number predominance make the distribution of the alkanes from the Warrawoona chert pyrolysate strongly different from that obtained from organic matter formed abiotically under any known natural or laboratory conditions. Indeed, upon pyrolysis under the same experimental conditions as described above, the abiotic macromolecular organic matter from carbonaceous chondrites yields a virtual lack of aliphatic products (Remusat et al., 2005b; Sephton et al., 2004). Moreover, these abiotic extraterrestrial aliphatic compounds exhibit the maximal diversity of isomers, i.e. all the branched isomers are present for any given carbon number. A similar diversity of isomers in abiotic insoluble organic macromolecules from carbonaceous chondrites is also observed when the aliphatic linkages

were exposed to RuO₄ oxidation, attesting there is no bias induced by this pyrolysis (Remusat et al., 2005a). Moreover, a similar diversity of isomers was observed in the soluble hydrocarbons formed upon thermal decomposition of siderite, i. e. resulting from an abiotic process (McCullom, 2003). It was recently stressed that thermocatalytic reactions yield homologous series of organic compounds with no carbon number preferences (Rushdi and Simoneit, 2001) and that Fischer–Tropsch-type products are dominated by unbranched alkanes with a characteristic linear decrease in abundance (McCullom and Seewald, 2006). In contrast, a weak odd-over-even carbon number predominance is a unique characteristics of organics formed biologically since it reflects biosynthesis using addition of C₂ units (Albro, 1976). The C₁₀–C₁₈ range in the alkanes of the Warrawoona pyrolysate is characterized by such odd-over-even carbon number predominance (Fig. 3b), confirming the involvement of a biosynthetic pathway in the formation of these *n*-alkyl chains (a carbon preference index value of 1.2 is calculated in this range (Bray and Evans, 1961)). As a result, Fig. 3b provides evidence for the presence of molecular markers of life in this Warrawoona sample. It must be noted that the carbon range containing the predominance (C₁₀–C₁₈) is markedly shorter than those commonly observed in Phanerozoic samples (longer than C₂₅) and, as far as we are aware, has never been reported.

As already stressed above, molecular structures that are covalently linked to a kerogen-matrix embedded in a host rock are considered to have formed contemporaneously with the host rock (Brocks et al., 2003a; Marshall et al., 2006). It is therefore crucial to establish that the hydrocarbons released from the Warrawoona chert upon pyrolysis were linked to the kerogen-matrix by such covalent bonds. Indeed, it is a common problem with geological samples that soluble organic compounds, such as hydrocarbons, can be physically trapped in the mineral matrix which prevent them from efficient solvent extraction. These labile compounds would be released upon pyrolysis, but may not have formed contemporaneously with the host rock, as discussed above. Such thermal desorption of soluble organics appears very unlikely for the Warrawoona chert because it would not have yielded *n*-alkane/*n*-alk-1-ene doublets. Indeed, the latter result from the homolytic cleavage of a covalent bond whereas only free alkanes, i.e. without co-occurrence of the corresponding *n*-alk-1-enes, are released upon thermodesorption. Moreover, so as to test whether *n*-alkanes with the characteristic odd-over-even carbon number predominance would be tightly trapped within the macromolecular network, we conducted a pyrolysis at 350 °C. This rather low temperature is commonly used to induce thermal desorption of products that were not released upon bitumen extraction (Brocks et al., 2003a). Thermal cracking of C–C bond does not take place at this low temperature and moieties covalently linked to the kerogen are not released. At 350 °C we did observe the release of thermally desorbed

n-alkanes from the Warrawoona chert, but only in trace amounts and with a distinctly different distribution (C₁₂–C₂₀, maximum at C₁₆ with no odd-over-even carbon number predominance, Fig. 4) from that reported above for the 650 °C pyrolysate. These labile alkanes therefore do not account for the typical biological distribution observed in the 650 °C pyrolysate and the aliphatic chains are thus covalently linked to the macromolecular network.

Although it is widely accepted that kerogen has formed at the same time as the host rock, the issue of the possible laboratory or geological contamination is now addressed in greater details. Three potential types of contamination will be successively discussed: laboratory, Phanerozoic and exogenous brought by the veins.

Laboratory contamination can be firmly ruled out as (i) the generated profile for the hydrocarbons is not the one typically observed for contamination (oil contamination usually results in an unresolved mixture in a lower carbon range), (ii) it should have been released through thermal desorption, (iii) the series of *n*-alkane/*n*-alk-1-ene doublets were never observed upon pyrolysis of other samples or blanks.

Phanerozoic contamination can also be excluded because (i) the commonly observed odd-over-even carbon number predominance in Phanerozoic samples appears in a distinctly higher chain length range, i. e. C₂₇–C₃₁ corresponding to that of the higher plant waxes, (ii) the lack of isoprenoid hydrocarbons (pristane, phytane) in the present pyrolysate, (iii) the carbon isotope composition ($\delta^{13}\text{C} = -32.7\%$) is consistent with – although not characteristic of – a Precambrian age (Beaumont and Robert, 1999), (iv) the nitrogen isotope composition, ($\delta^{15}\text{N} = -4.1\%$), is consistent with an Archean age (Beaumont and Robert, 1999). In case of at least a partial contamination with Phanerozoic organic matter, this value would have been raised up to positive values. This is especially true as nitrogen contents are higher in organic matter from young rocks than from Archean ones (Beaumont and Robert, 1999).

An exogenous contamination by hydrocarbons brought by the millimetre-sized veins observed within the matrix of microcrystalline quartz also seems unlikely. Indeed, these veins consist of sub-millimetre crystals of quartz and account for 7% of the bulk sample (assessed from image analysis of thin section). The oxygen isotopic composition ($\delta^{18}\text{O}$) has been analyzed by ion microprobe spectroscopy (IMS) (Robert and Chaussidon, 2006) and similar values have been obtained for both the matrix and the veins, +16.7 and +17.0‰, respectively. A different $\delta^{18}\text{O}$ signature would have been left in the veins after a late input of silica from a distinct geological formation during a metamorphic event, and here, this is not the case. Moreover, the carbon concentrations in both veins and matrix have also been determined by IMS and they show that carbon in veins only account for 3.7% of the total carbon of the whole rock. Such a low contribution rules out that the veins are the only carrier of the aliphatic chains exhibiting the biological signature.

Our data thus report the occurrence of biological markers in the kerogen embedded in a 3.5 By old chert. The nature of the biological activity must also be examined. As stressed above, the pyrolysate of the Warrawoona chert contains thiophenes. The release of organo-sulphur products upon kerogen pyrolysis is commonly considered as evidence for the involvement of the so-called sulphurisation process, which is a well-established mechanism of kerogen formation (Sinninghe Damsté et al., 1989). It involves the introduction of sulphur into potentially labile lipids and/or carbohydrates. Such intra- and intermolecular sulphur incorporations give rise to sulphur-rich macromolecules. The occurrence of such organo-sulphur moieties in the macromolecular network is consistent with scanning electron microscopy observations coupled with energy dispersive spectroscopy (EDS), which systematically have revealed the co-occurrence of sulphur and carbon in the kerogen. The incorporated sulphur originates from H₂S produced by sulphate-reducing bacteria. The presence of thiophene-containing products in the pyrolysate of the Warrawoona chert may thus point to the involvement of sulphate-

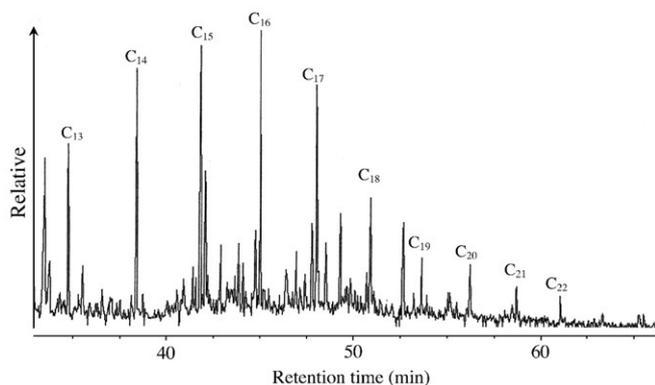


Fig. 4. Curie point pyrolysis at 350 °C: total ion current trace of the pyrolysate showing the release of *n*-alkanes.

bacteria in the preservation of its organic matter. It must be noted that this hypothesis is supported by a recent study that considered microbial sulphate reduction in the 3.5 By old North Pole deposit (Shen and Buick, 2004), based on sulfur isotope measurements although it has been debated even more recently (Philippot et al., 2007). Considering non-organic sulphur, no elemental sulphur could be detected in the kerogen concentrate but barium sulphate was identified. It cannot therefore be excluded that organo-sulphur compounds would be formed upon pyrolysis through a thermo-sulphate reduction process.

Our data report the occurrence of biological markers in the kerogen embedded in a 3.5 By old chert. Several features point to a contemporaneous formation of this kerogen and its siliceous host rock. This observation supports a scenario according to which life was present on Earth 3.5 By ago.

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