



doi:10.1016/S0016-7037(00)01302-9

Release of bound aromatic hydrocarbons from late Archean and Mesoproterozoic kerogens via hydropyrolysis

JOCHEN J. BROCKS,^{1,*} GORDON D. LOVE,² COLIN E. SNAPE,³ GRAHAM A. LOGAN,⁴ ROGER E. SUMMONS,⁵ and ROGER BUICK⁶¹Department of Organismic and Evolutionary Biology and Department of Planetary and Earth Sciences, Harvard University, Cambridge, MA 02138, USA²School of Civil Engineering and Geosciences, Drummond Building, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, United Kingdom³School of Chemical, Environmental and Mining Engineering, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom⁴Geoscience Australia, GPO Box 378, Canberra ACT 2601, Australia⁵Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA⁶Department of Earth and Space Sciences and Astrobiology Program, University of Washington, Seattle, WA 98195-1310, USA

(Received March 28, 2002; accepted in revised form October 9, 2002)

Abstract—Hydrogen-lean kerogens (atomic H/C < 0.4) isolated from the 2.5-billion-year-old (Ga) Mt. McRae Shale, Hamersley Group, at Tom Price, Western Australia, were studied via hydropyrolysis, a continuous-flow technique that degrades organic matter in a stream of high-pressure hydrogen assisted by a dispersed Mo catalyst. The hydropyrolysates yielded predominantly phenanthrene and pyrene, and higher polyaromatic hydrocarbons and alkylated homologues were generated in low relative concentrations. Saturated hydrocarbons were not detected. The molecular and carbon isotopic compositions of the hydropyrolysates are very similar to aromatic hydrocarbons obtained by solvent extraction of the host rocks. Because molecular structures covalently attached to kerogen are unaffected by contamination, this indicates that both the bound and extractable aromatic fractions are syngenetic with the host rocks. Therefore, the results of the hydropyrolysis experiments provide compelling evidence for preserved bitumen of Archean age. The very high proportion of nonalkylated polyaromatic hydrocarbons in the hydropyrolysates is consistent with hydrothermal dehydrogenation of the kerogen, and a marked concentration difference of pyrene in rock extracts and hydropyrolysates might be explained by hydrothermal redistribution of the bitumen. The kerogen and bitumen composition is therefore consistent with models suggesting a hydrothermal origin for the giant iron ore deposits at Mt. Tom Price. Comparison of the Archean samples with hydropyrolysates from immature Mesoproterozoic kerogens from the Roper Group, McArthur Basin, Northern Territory, and with pyrolysis experiments on Proterozoic kerogens in the literature suggests that Precambrian kerogens are frequently highly aromatic and lipid-poor regardless of their degree of thermal preservation. Copyright © 2003 Elsevier Science Ltd

1. INTRODUCTION

Solvent extracts of 2.78- to 2.45-Ga, unweathered, low-grade metasedimentary shales from the Hamersley Basin in Western Australia contain traces of molecular fossils (biomarkers) and other hydrocarbons (Brocks et al., 1999). The bitumens are probably of Archean age on the basis of their typical Precambrian characteristics, high thermal maturities that appear consistent with the thermal history of the host sediments, the absence of Phanerozoic biomarkers, and the wide geographic distribution of similar bitumens within the Hamersley Basin (Brocks, 2001). If the molecules are indeed syngenetic, then they bring unprecedented clarity to our understanding of the metabolic diversity of Archean life and might also help to unravel the mechanism that formed the giant iron ore deposits of the Hamersley Basin. However, the evidence for their Archean age, although strong, is circumstantial, and absolute proof of their antiquity has not yet been obtained (Brocks, 2001). The remaining room for doubt lies in the fact that it can be extremely difficult to distinguish among indigenous hydrocarbons, anthropogenic contamination, and migrated petroleum.

However, molecular structures covalently attached to kerogen should largely be unaffected by mobile contaminants introduced after peak metamorphism. Therefore, the analysis of organic structures by pyrolytic degradation of Archean kerogens might yield the information necessary to confirm or reject the indigenous and syngenetic nature of the solvent extracts. However, standard pyrolytic techniques do not necessarily discriminate between adsorbed and bound organic species. Only methods that remove all adsorbed molecules from the kerogen matrix before release of covalently bonded moieties are suitable for definitively resolving the contamination issue. Thus, this article discusses the hydropyrolytic degradation of over-mature Archean kerogens and correlates the products with the corresponding solvent-extractable bitumens. For comparison, hydropyrolysates of thermally well preserved Mesoproterozoic kerogens (~1.45 Ga) were also generated and examined.

1.1. Previous Studies on Precambrian Kerogens

Pyrolytic or chemical degradation techniques for kerogen are well known and have been used to search for biologic information in Precambrian rocks for almost 40 years. The difficulty in distinguishing indigenous molecular fossils from contaminants stimulated exploration, mainly in the 1960s and 1970s,

* Author to whom correspondence should be addressed (jbrocks@oeb.harvard.edu).

for structural components covalently bound to Paleoproterozoic and Archean kerogens. A detailed overview of all degradative analyses of Precambrian kerogens reported before 1980 is given by Hayes et al. (1983). The early pyrolysis experiments yielded aromatic as well as aliphatic hydrocarbons with up to 15 carbon atoms. The fact that the hydrogen-rich products were generated from 2.5- to 3.5-Ga kerogens with bulk atomic H/C ratios <0.2 led Hayes et al. (1983) to the conclusion that the pyrolysis products were not representative of the kerogen but contaminants trapped within the kerogen structure. Artificial contamination experiments, in which isolated Precambrian kerogen was stained with a series of ^{14}C -labeled compounds and then exhaustively extracted, showed that this explanation was reasonable (Oehler, 1977). The amount of contaminants remaining in the kerogen after the extraction was determined by the detection of residual radioactivity, revealing that up to $60\ \mu\text{g}$ hydrocarbons per gram of kerogen was apparently “irreversibly” adsorbed. Because organic contamination of geological samples appears to be a common phenomenon, most pyrolysis experiments on Archean samples will have detected thermal desorption products rather than covalently bound hydrocarbons. Desorption of volatiles was probably also responsible for most later discoveries of *n*-alkanes and isoprenoids in Paleoproterozoic and Archean kerogens from North America, Africa, and Australia (Philp and van de Meent, 1983).

Hoering and Navale (1987) revisited the pyrolysis of Precambrian kerogens with more rigorous and cleaner methods and made an interesting observation: pyrolysis yields diminished with increasing age of the analyzed samples irrespective of the low thermal maturity of some of the analyzed kerogens. Samples older than 1.7 Ga did not yield any hydrocarbons at all, even when up to 250 g of rock powder was pyrolysed. The authors attributed the lack of pyrolysis products in thermally well preserved kerogens to the destructive effects of geological time. An alternative and more appropriate explanation for low pyrolysis yields from Precambrian kerogens is discussed in section 4.3.

1.2. Hydropyrolysis

The low H/C ratios of Archean kerogens and the difficulty of distinguishing between adsorbed contaminants and covalently bound indigenous structures make conventional chemical degradation and pyrolysis techniques unsuited to this problem. The development of a more effective hydropyrolysis technique (HyPy) has made it possible to reexamine the nature of hydrogen-lean kerogens. Hydropyrolysis refers to pyrolysis under high hydrogen gas pressures ($>10\ \text{MPa}$). Fixed-bed hydropyrolysis in the presence of a dispersed sulfided molybdenum catalyst gives rise to overall carbon conversions greater than 85% for Phanerozoic Type I and Type II sulfur-rich kerogens and high volatile Type III coals (Roberts et al., 1995; Maroto-Valer et al., 1997; Rocha et al., 1997). By use of pressurized H_2 gas and a catalyst, covalent bonds in kerogen are cleaved at lower temperatures than in more conventional pyrolytic techniques. Thus, hydrocarbons generated by hydropyrolysis preferentially retain their original structures and stereochemistry (Love et al., 1995, 1997, 1998; Bishop et al., 1998; Murray et al., 1998). Moreover, because the hydropyrolysis system is hydrogen saturated, char formation is suppressed leading to increased yields of *n*-alkanes, hopanes, and steranes compared with traditional pyrolysis (Love et al., 1995).

The greatest challenge in the analysis of trace amounts of pyrolysis products is to discriminate between background contamination, caused by thermal desorption of residual bitumen, and genuine kerogen degradation products. Residual hydrocarbons might be intercalated between layers of kerogen or adsorbed to its surface (Oehler, 1977). Hydropyrolysis allows desorption of these contaminants by flushing the kerogen with hydrogen in a relatively low-temperature step preceding the actual hydropyrolysis stage when bond cleavage occurs. As well, it is possible to isotopically label genuine cleavage products by switching the hydropyrolysis reaction gas to high-pressure deuterium (deuteropyrolysis).

2. EXPERIMENTAL METHODS

2.1. Kerogen Preparation

Kerogen was isolated from preextracted rock powder by HCl/HF digestion (concentrated HCl for 24 h and then 48% HF several times for 48 to 72 h at room temperature). Acid resistant minerals were removed by floatation (ZnBr_2 solution; $\rho = 2.0\ \text{g/mL}$). The concentrated kerogen was washed with distilled water, dried for 72 h at 50°C in porcelain crucibles, and then extracted by ultrasonication, twice in dichloromethane (DCM) (10 g, 10 min) and *n*-hexane (10 g). To remove residual trapped bitumen, the kerogen was swelled twice by ultrasonication in pyridine (10 g, 80°C , 2 h). The pyridine was removed by centrifugation and the kerogen reextracted with methanol (10 g) and three times with DCM (10 g). The cleaning steps were repeated if the last extract did not prove clean by gas chromatography–mass spectroscopy–selected iron monitoring (GC-MS SIR).

2.2. Hydropyrolysis

Kerogens were impregnated with an aqueous solution of ammonium dioxodithiomolybdate $[(\text{NH}_4)_2\text{MoO}_2\text{S}_2]$ to give a nominal loading of 2 wt% molybdenum as described previously (Roberts et al., 1995; Rocha et al., 1997). Dioxodithiomolybdate reductively decomposes in situ under hydropyrolysis conditions above 250°C to form a catalytically active sulfided molybdenum phase. Hydropyrolysis was performed in a temperature-programmed open-system reactor described elsewhere (Love et al., 1995). In this investigation, catalyst-loaded samples (50 to 200 mg of kerogen per run) were initially heated in a stainless steel (316 grade) reactor tube from ambient temperature to 220°C at $300^\circ\text{C}/\text{min}$ and then to $325 \pm 5^\circ\text{C}$ at $8^\circ\text{C}/\text{min}$ under a hydrogen pressure of 15.0 MPa and a flow rate of $6\ \text{dm}^3/\text{min}$ (measured at ambient temperature and pressure). This low-temperature desorption step was incorporated to drive off residual bitumen and volatile contaminations. The temperature was then increased to 520°C at $8^\circ\text{C}/\text{min}$ with the same hydrogen pressure and flow rate. Under these conditions, the residence times of generated volatile compounds in the reactor were only a few seconds. The products were collected in a trap cooled with dry ice and recovered in DCM/methanol (4:1 v/v). The solvent was reduced, and activated copper turnings were added to remove all traces of elemental sulfur formed mainly from the iron sulfides in the shales. Solvent extraction of the residual tar and kerogen in the reactor tube did not yield any volatile components, confirming that all products were quantitatively recovered during the heating cycle.

To obtain low levels of background contamination, the reactor tube and trap were rinsed with DCM and annealed at 400°C for 2 h. Any residual contaminants in the reassembled reactor were thermally desorbed by two hydropyrolysis heating cycles (520°C , $300^\circ\text{C}/\text{min}$). The products of the second control cycle were trapped, analyzed and compared with the actual hydropyrolysates to obtain quantitative information about background levels.

2.3. Product Analyses

Total organic carbon and ROCK-EVAL parameters were determined on a VINCI ROCK-EVAL 6 instrument according to established procedures (Espitalié et al., 1977).

GC was performed with a Hewlett-Packard HP6890 gas chromatography–mass spectroscopy system.

Table 1. Bulk characteristics and maturity data.

Sample	Group	Well	Depth (m)	TOC (%)	T _{max} (°C)	HI	Bitumen maturity ^a
<i>Man1</i>	Roper ^b	McManus1 ^c	1150.00–1151.10	1.6	444	90	Late stage oil generation
<i>Man2</i>	Roper ^b	McManus1 ^c	1542.95–1543.00	6.3	468	47	Late stage oil generation
<i>Bee1</i>	Hamersley	G906	418.90–419.20	7.8	n.m. ^d	~0	Wet-gas zone
<i>Rae1</i>	Hamersley	G1185-81	324.87–325.05	5.8	n.m. ^d	~0	Wet-gas zone
<i>Rae2</i>	Hamersley	B26-7	140.75–141.05	6.0	n.m. ^d	~0	Wet-gas zone
<i>Roy11</i>	Fortescue	WRL-1	695.75–695.80	11.4	n.m. ^d	~0	Wet-gas zone

^a Based on Summons et al. (1994) for samples from the Roper Group and on Brocks (2001) for Archean samples.

^b Velkerri Formation, Roper Group, McArthur Basin, Northern Territory.

^c Location described in Summons et al. (1994).

^d Not measurable.

graph fitted with a flame ionization detector (FID) and a HP Ultra 1 column (25 m × 0.25 mm inner diameter [i.d.], 0.33- μ m film thickness) using manual injection in splitless mode and H₂ as carrier gas. The oven was programmed at 40°C (2 min) and heated to 310°C at 4°C/min, with a final hold time of 15 min. GC-MS in the full-scan mode and by SIR were carried out on a VG AutoSpecQ and a Hewlett Packard 5973 Mass Selective Detector (MSD). The AutoSpecQ was equipped with a Fisons GC 8000 Series gas chromatograph and a DB-5 capillary column (60 m × 0.25 mm i.d., 0.25- μ m film thickness) using H₂ as carrier gas. Samples were injected in splitless mode or on column. The GC oven was programmed at 60°C (2 min), heated to 310°C at 4°C/min, with a final hold time of 25 min. The source was operated in electron ionization (EI) mode at 70 eV ionization energy. The AutoSpec full-scan rate was 0.40 s/decade over a mass range of 50 to 500 Da and a delay of 0.20 s/decade. The HP 5973 MSD was equipped with a HP 6890 gas chromatograph (Hewlett-Packard) and a HP-5 column (50 m × 0.20 mm i.d., 0.11- μ m film thickness). Samples were injected in pulsed splitless mode with He as carrier gas. The GC oven was programmed at 40°C (2 min), heated to 310°C at 4°C/min, and held at the maximum temperature for 25 min. The MS source was operated in EI mode at 70 eV. Biomarkers were analyzed by metastable reaction monitoring (GC-MS MRM) on a VG AutoSpecQ with the same GC conditions as described for SIR experiments. The source was operated in EI mode at 250°C, 70 eV ionization energy, and 8000 kV acceleration voltage. Di- to pentacyclic terpanes and steranes were measured in parent to daughter ($m/z = 123, 177, 191, 205, 217, 231, 245$ Da) transitions with cycle times of 1.4 to 2.1 s.

Total extract and pyrolysis yields were determined by GC-FID on the Hewlett Packard 6890 GC system, and individual compounds were quantified by GC-MS (SIR) with d₁₄-para-terphenyl (D₁₄), 3-methylhenicosane (aC₂₂), and d₄- $\alpha\alpha\alpha$ -ethylcholestanol (D₄) used as internal standards.

3. RESULTS

Hydropyrolysis experiments were performed on two kerogens isolated from dark gray to black mudstones from the ~1.45-Ga Velkerri Formation (Roper Group, McArthur Basin, Northern Territory), on three kerogens isolated from black shales from the ~2.5-Ga Mt. McRae Shale (Hamersley Group, Hamersley Basin, Western Australia) and one black shale from the ~2.7-Ga Roy Hill Shale (Fortescue Group, Hamersley Basin, Western Australia). Bulk kerogen data and an assessment of bitumen thermal maturity are summarized in Table 1.

3.1. Hydropyrolysis Experiments on Kerogens from the ~1.45-Ga Roper Group

3.1.1. Bulk Characteristics and Bitumen Composition

The kerogens isolated from the two mudrocks of the Velkerri Formation (samples *Man1* and *Man2*) have a dark brown color consistent with Rock Eval data and aromatic maturity param-

eters that indicate a thermal maturity in the late stages of oil generation (Table 1; Summons et al., 1994). The alkane fractions of the bitumens are dominated by an unresolved complex mixture (UCM) (Fig. 1A). *n*-Alkanes are resolved in the range *n*-C₁₃ to *n*-C₂₈ with a unimodal distribution and a maximum at *n*-C₁₆. Methylalkanes and cyclohexylalkanes are relatively abundant, and acyclic isoprenoids were below detection limit. Also detected were sterane and hopane biomarkers, but signal-to-noise ratios were too low for a reliable interpretation. The bitumens have high concentrations of saturated vs. aromatic hydrocarbons (Sat/Aro ≈ 4) and the aromatic fraction contains predominantly alkylated aromatic hydrocarbons with two or three rings.

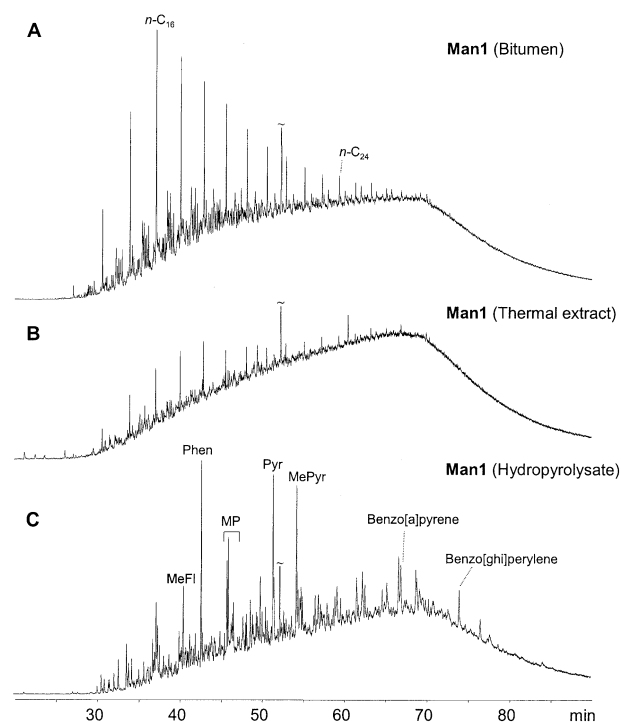


Fig. 1. GC-MS full scans of sample *Man1* from the Mesoproterozoic Roper Group, McArthur Basin. (A) Bitumen. (B) Products of thermal desorption under a continuous hydrogen stream in the hydropyrolysis reactor at temperatures ramped up to 325°C. (C) Hydropyrolysate at 325 to 520°C. ~ = internal standard. Compound abbreviations are listed in Table 2.

Table 2. Solvent extract yields, hydropyrolysis yields, and aromatic hydrocarbon ratios.

Sample	Sat ^a	Aro ^a	Saturated hydrocarbons (ppm) ^b				Aromatic hydrocarbons (ppm) ^c										Phen/ MP ^e	MP/ DMP ^e	Pyr/ MePyr ^e
	mg/g	mg/g	nC16	nC18	nC20	nC22	BiPh	MeBP	Fl	MeFl	Phen	MP ^f	DMP ^d	FlAnth	Pyr	MePyr ^d			
Bitumen																			
<i>Man1</i>	91	25	200	160	100	58	—	—	—	4.4	4.5	41	86	—	—	—	0.11	0.48	—
<i>Bee1</i>	1.6	0.21	8.4	4.5	1.8	0.69	15	2.6	2.5	0.53	4.6	0.58	0.25	0.18	0.13	—	8.0	2.3	—
<i>Rae1</i>	7.5	0.47	19	12	2.3	0.49	20	3.1	2.4	0.4	8.1	0.76	0.43	0.17	0.13	—	11	1.9	—
<i>Rae2</i>	17	0.27	49	15	4.4	2.1	4.7	10	—	—	0.16	0.33	0.34	—	—	—	0.49	0.97	—
<i>Roy11</i>	0.13	0.03	1.0	1.0	0.45	0.14	1.9	4.4	—	—	0.21	0.82	—	—	—	—	0.26	—	—
Thermal extracts (25–325°C)																			
<i>Man1</i>	2.3	—	2.7	2.7	1.5	1.1	—	—	0.03	0.34	0.59	2.8	4.7	—	—	—	0.21	0.59	—
<i>Rae1</i>	—	—	—	—	—	—	0.09	0.04	0.01	0.26	0.60	0.18	0.11	0.02	0.03	—	3.3	1.6	—
Hydropyrolysis products (325–520°C)																			
<i>Man1</i>	1.1	4.6	5.7	6.0	4.5	3.2	1.2	5.8	6.7	20	43	63	52	5.1	27	58	0.68	1.2	0.47
<i>Man2</i>	0.5	—	5.9	5.5	3.8	3.1	—	—	—	—	13	50	56	0.98	3.4	20.5	0.26	0.89	0.17
<i>Bee1</i>	0.2	—	—	—	—	—	1.6	1.4	6.0	8.7	43	12	3.2	4.7	21	5.5	3.6	3.8	3.8
<i>Rae1</i>	0.2	—	—	—	—	—	0.79	0.59	0.66	1.3	31	5.4	0.72	1.2	24	0.47	5.7	7.5	51
<i>Rae2</i>	0.02	—	—	—	—	—	0.04	0.12	0.04	0.20	0.93	0.36	0.07	0.03	0.26	0.01	2.6	5.1	26
<i>Roy11</i>	0.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

^a Concentration of total aromatic and saturated hydrocarbon fractions (mg/g of kerogen) determined by GC-FID using aC₂₂ and D₁₄ as internal standards. Single values refer to unfractionated total pyrolysates.

^b Concentrations of individual saturated hydrocarbons in ppm (μg/g of kerogen) determined by GC-FID using aC₂₂ as internal standard.

^c Concentrations of individual aromatic hydrocarbons in ppm (μg/g of kerogen) determined by corrected GC-MS signal areas of the molecular ion in SIR or full scan mode using D₁₄ as internal standard.

^d Sum of all isomers.

^e Calculated using the absolute concentration of all isomers. — = not measurable. Compound abbreviations: Naph = naphthalene; BiPh = biphenyl; MeBP = methylbiphenyls; Fl = fluorene; MeFl = methylfluorenes; Phen = phenanthrene; MP = methylphenanthrenes; DMP = dimethylphenanthrenes; FlAnth = fluoranthene; Pyr = pyrene; MePyr = methylpyrenes.

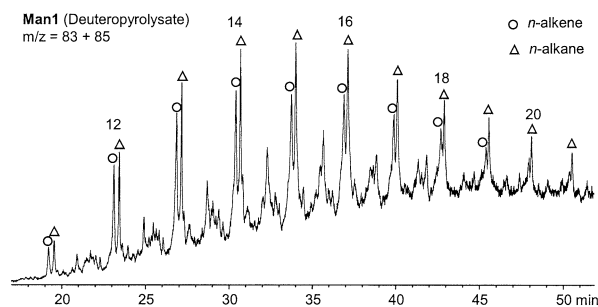


Fig. 2. GC-MS reconstructed ion chromatogram $m/z = 83 + 85$ of the deuteropyrolysis products of sample *Man1*, Roper Group. Compounds are identified by carbon number.

3.1.2. Hydropyrolysis

The hydropyrolysis experiments on both Velkerri Formation samples yielded similar results and only *Man1* will be discussed. Residual bitumen in the kerogen was thermally desorbed in a hydrogen stream at 325°C in the pyrolysis reactor. This thermal extract has a composition similar to the bitumen with a predominant UCM but with relatively lower concentrations of *n*-alkanes (Fig. 1B). The temperature-programmed hydropyrolysis experiment at 325 to 520°C generated mainly aromatic hydrocarbons with molecular masses up to 300 Da (Fig. 1C).

Product yields and ratios of aromatic hydrocarbons are summarized in Table 2. Although the overall hydropyrolysate yield of 5.7 mg per gram of kerogen is ~20 times lower than of bitumen (116 mg), absolute hydropyrolysis yields of individual aromatic compounds are higher. For instance, phenanthrene has a concentration of only 4.5 ppm (1 ppm = 1 µg per gram of kerogen) in the bitumen compared with 43 ppm in the hydropyrolysate (Table 2). Moreover, polyaromatic hydrocarbons (PAH) with four or more aromatic rings such as benzo[*a*]pyrene and benzo[*ghi*]perylene were quantitatively important products of hydropyrolysis but were below detection limit in the bitumen. The UCM of the hydropyrolysate (Fig. 1C) consists to more than 80% of aromatic compounds, whereas the UCM of the bitumen is ~80% saturated (Fig. 1A). Similarly, *n*-alkanes that are abundant in the bitumen occur only in very low relative concentrations in the hydropyrolysate (Table 2).

3.1.3. Deuteropyrolysis

To test whether traces of *n*-alkanes in the hydropyrolysate are desorbed bitumen or degradation products of the kerogen, the hydropyrolysis experiment on *Man1* was repeated in a high-pressure deuterium atmosphere. Figure 2 shows the total alkane and alkene profile ($m/z = 83 + 85$) and Figure 3 the extent of deuterium incorporation into *n*-C₁₄. The relative intensities of the molecular ions in Figure 3 indicate that the extent of deuterium incorporation into *n*-alkanes was of the order $d_1 > d_2 > d_0 > d_3 > d_4 > d_5 > d_6 > d_7$. It seems possible that the modal incorporation of one deuterium atom in *n*-alkane products indicates that *n*-alkyl chains were covalently bound to the kerogen and predominantly attached through one carbon atom. However, it is unlikely that a relatively high ratio of *n*-alkyl chains was attached to kerogen by four and more

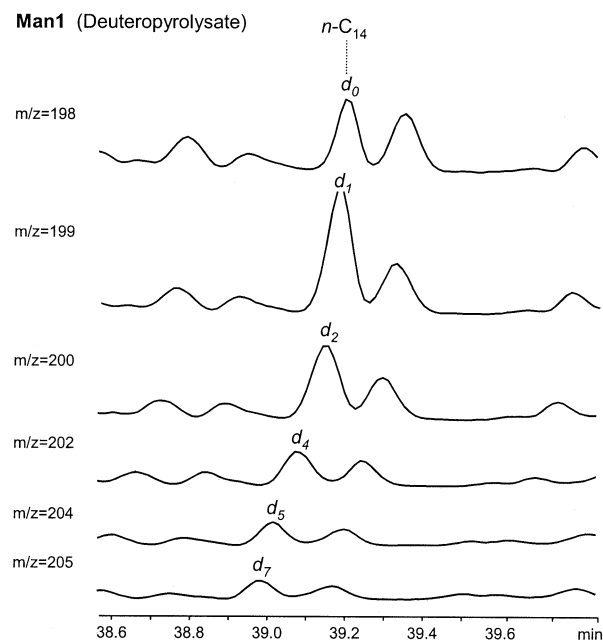


Fig. 3. Scaled GC-MS selected ion chromatograms of the molecular ion of mono- to heptadeuterated tetradecane generated by deuteropyrolysis of sample *Man1*, Roper Group.

covalent bonds. Hence, the presence of higher numbers of deuterium atoms in *n*-alkanes might indicate that random D/H exchange has occurred, probably at the highest temperatures in the programmed hydropyrolysis cycle. Therefore, in this particular experiment, the incorporation of deuterium into *n*-alkanes does not give unequivocal information about covalent bonding. However, also detected in the deuteropyrolysate were monounsaturated *n*-alkenes (Fig. 2). Alkenes were not detected in experiments where H₂ gas was the reaction medium, probably because the more stable D₂ reacts more slowly with unsaturated hydrocarbons or alkyl radicals that form by pyrolytic bond cleavage. Therefore, these structures were not generated by desorption but were probably covalently attached to the kerogen matrix.

3.2. Hydropyrolysis Experiments on Kerogens from the ~2.5-Ga Hamersley Group

3.2.1. Bulk Characteristics and Bitumen Composition

Three black shales (*Rae1*, *Rae2*, and *Bee1*) with high kerogen contents from the Mt. McRae Shale, Hamersley Group, were chosen for the hydropyrolysis experiments. The samples were collected from diamond drill cores from different locations from iron ore deposits in the Tom Price area. The regional metamorphic grade of the Hamersley Group near Tom Price is probably middle to upper prehnite-pumpellyite facies (Smith et al., 1982). However, the local metamorphic pattern is complicated by igneous intrusions and possibly by hydrothermal activity. Samples from different locations near Tom Price might therefore have suffered widely different peak temperatures. Consistent with their metamorphic history, kerogens from the Mt. McRae Shale have generally very low hydrogen contents (atomic H/C ≈ 0.1; Hayes et al., 1983).

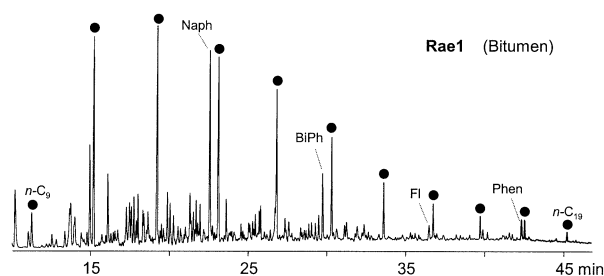


Fig. 4. GC-MS full scan of bitumen *Rae1*, Mt. McRae Shale. *n*-alkanes are marked by circles. Compound abbreviations are listed in Table 2.

The compositions and thermal maturities of bitumens from the Hamersley Group are described in detail in Brocks (2001). In short, the concentrations of saturated hydrocarbons were 120 ppm ($\mu\text{g/g}$ rock) (*Bee1*), 440 ppm (*Rae1*), and 1000 ppm (*Rae2*). The abundance of aromatic hydrocarbons was 16, 27, and 8.8 ppm, respectively. Analysis of the extracts by GC-MS and GC-MS (MRM) revealed the presence of *n*-alkanes, mid- and end-branched monomethylalkanes, ω -cyclohexylalkanes, acyclic isoprenoids, diamondoids, tri- to pentacyclic terpanes, steranes, aromatic steroids, and polyaromatic hydrocarbons. The thermal maturity was ranked in the wet-gas zone on the basis of sterane and hopane ratios at their apparent thermal maxima, condensate-like alkane profiles (Fig. 4), high mono- and triaromatic steroid maturity parameters, high methyladamantane and methyl-diamantane indices and high methylphenanthrene maturity ratios.

The aromatic fractions of bitumens *Rae1* and *Bee1* (Fig. 5A, Table 2) are unusual. The parent aromatic hydrocarbons naphthalene, biphenyl, fluorene, and phenanthrene are abundant, and alkylated homologues occur only in very low relative concentrations, a pattern consistent with pyrolytically altered bitumen (George, 1992). In contrast, in McRae Shale sample *Rae2* alkylated PAH are more abundant than the parent compounds (Table 2). Notably, although phenanthrene is very abundant in *Rae1* and *Bee1*, 4-ring aromatic hydrocarbons such as pyrene and fluoranthene occur only in trace amounts, and higher PAH appear to be absent.

3.2.2. Hydropyrolysis

Figure 6 shows chromatograms of the procedural blank, the thermal desorption products (25 to 325°C), and the hydropyrolysis (325 to 520°C) of Mt. McRae Shale sample *Rae1*. The chromatograms are scaled, and signal heights are approximately proportional to hydropyrolysis yields. Thermal desorption of residual volatiles in the preextracted kerogen yielded even-numbered *n*-alkanes from C₁₆ to C₂₀, fluorene, phenanthrene, and traces of phthalates (Fig. 6B). Fluorene and phenanthrene, both abundant in the original sample, probably represent residual bitumen that was trapped in the kerogen. All other components are obviously recent contaminants that were also detected in the procedural blank, albeit in lower concentrations (Fig. 6A). In the actual hydropyrolysis (Fig. 6C) these contaminants were below the detection limit, indicating that the thermal desorption step had cleaned the kerogen efficiently.

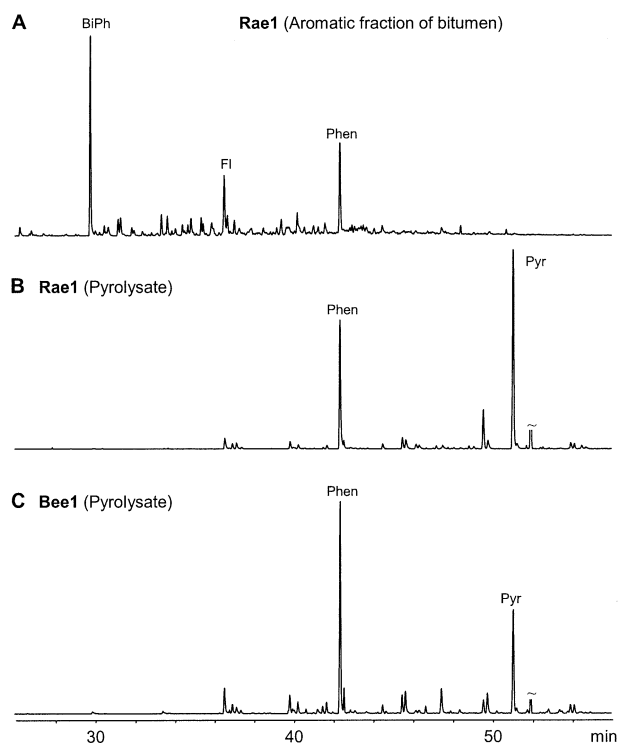


Fig. 5. GC-MS full scans of (A) the aromatic fraction of the bitumen of *Rae1*, Hamersley Group, (B) hydropyrolysis products of *Rae1* and (C) of *Bee1*, Hamersley Group. ~ = internal standard. Compound abbreviations are listed in Table 2

A comparison of the bitumens from the Mt. McRae Shale with the hydropyrolysates reveal some interesting characteristics:

(1) Although saturated hydrocarbons are abundant in the bitumens of samples *Bee1*, *Rae1* and *Rae2* (Fig. 4), they are below the detection limit in the hydropyrolysates in the monitored C₁₂₊ range (Fig. 5).

(2) The absolute yields of individual aromatic hydrocarbons are much higher in the hydropyrolysates than in the bitumens (Table 2). For instance, solvent extraction of *Bee1* yielded only 4.6 ppm phenanthrene whereas pyrolysis yielded 43 ppm.

(3) In all Mt. McRae Shale samples, pyrene and phenanthrene are the by far most abundant hydropyrolysis products. This observation is in strong contrast to the bitumens, where pyrene concentrations are negligible (Fig. 5).

(4) Although Mt. McRae Shale *Rae2* yielded relatively high concentrations of bitumen, the hydropyrolysis yield is one order of magnitude lower than in the other Mt. McRae Shale samples *Rae1* and *Bee1* (Table 2).

(5) The aromatic hydrocarbon distribution in the bitumen of *Rae2* is fundamentally different from the bitumen in *Rae1* and *Bee1*. This is remarkable, as the molecular composition of the aromatic hydropyrolysate of *Rae2* is almost identical to those of *Rae1* and *Bee1* (Table 2).

The hydropyrolysis yields of *Rae1* and *Bee1* were sufficient to determine carbon isotopic compositions of some individual compounds (Table 3). $\delta^{13}\text{C}$ of individual aromatic hydrocarbons in the bitumens and in the hydropyrolysates (-29‰ to

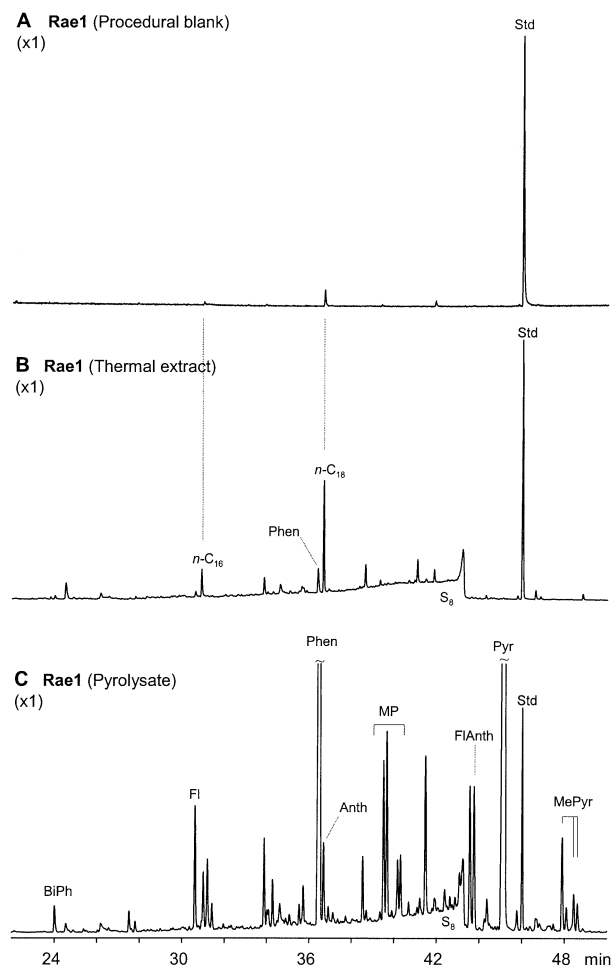


Fig. 6. Scaled GC-MS full scans of pyrolysis experiments on *Rae1*, Hamersley Group. (A) Procedural blank, (B) volatiles desorbed from the kerogen in a continuous hydrogen stream in the hydro-pyrolysis reactor (25 to 325°C) and (C) hydro-pyrolysis products (325 to 520°C). In (C), the major hydro-pyrolysis products phenanthrene and pyrene are truncated (~); a full-scale chromatogram is shown in Figure 5B. S_8 = elemental sulfur; Std = internal standard; anth = anthracene; other compound abbreviations are listed in Table 2.

–33‰ vs. Pee Dee Belemnite [PDB]) are identical within the analytical error and match the bulk isotopic compositions of the kerogens (–32.2‰ to –33.5‰).

A comparison of the hydro-pyrolysates of the Archean Mt.

McRae Shale with the Mesoproterozoic Velkerri Formation reveals two major differences. First, in the hydro-pyrolysates and the bitumens of the Mt. McRae Shale, the ratios of parent PAH to alkylated PAH are unusually high (Fig. 5). In the hydro-pyrolysates of the Velkerri Formation, these ratios are low (Table 2). Second, the concentration of five-ring and larger PAH in the hydro-pyrolysates of the Mt. McRae Shale was close to the detection limit (Fig. 5), in contrast to the Velkerri Formation, where higher molecular weight PAH are abundant (Fig. 1C).

3.3. Hydro-pyrolysis Experiments on Kerogens from the ~2.7-Ga Fortescue Group

3.3.1. Bulk Characteristics and Bitumen Composition

Sample *Roy11* from diamond drill core WRL-1, Jeerinah Formation, Fortescue Group, contains 11% kerogen. The estimated regional metamorphic grade of the Jeerinah Formation at the collection site is lower prehnite-pumpellyite facies (Smith et al., 1982). Therefore, *Roy11* has potentially experienced a milder thermal history than the Mt. McRae Shale at Tom Price (section 3.2.1.). However, the hydrogen content of kerogens from drill core WRL-1 (atomic H/C \approx 0.1 to 0.3; R. Buick, personal communication) is similar to the Mt. McRae Shale, and the saturated hydrocarbon fraction of the bitumen of *Roy11* is almost identical to samples from the Hamersley Group (Brooks, 2001). Bitumens from both the Fortescue Group and Hamersley Group have condensate-like *n*-alkane distributions and very similar biomarker ratios. The aromatic fraction of Fortescue Group sample *Roy11*, with low relative concentrations of parent aromatic hydrocarbons, is similar to Mt. McRae Shale sample *Rae2*.

3.3.2. Hydro-pyrolysis

Although regional models of metamorphism suggest that the thermal stress experienced by Fortescue Group sample *Roy11* might have been lower than for the samples of the Hamersley Group, hydro-pyrolysis experiments failed to detect saturated or aromatic hydrocarbons in the analyzed C_{12+} mass range.

4. DISCUSSION

4.1. Arguments for the Syngeneity of Aromatic Hydrocarbons in Late Archean Rocks

The aromatic hydrocarbons generated by hydro-pyrolysis are demonstrably syngenetic to the late Archean kerogens. First,

Table 3. Carbon isotopic composition ($\delta^{13}C$ in ‰ versus PDB) of kerogens and of hydrocarbons in bitumens and hydro-pyrolysates of samples from the Mt. McRae Shale, Hamersley Group.

Sample	Kerogen	<i>n</i> -alkanes	BiPh	FI	Phen	MePhen	Pyr
<i>Roy11</i>	Bitumen	–27.4 to –29.0	— ^a	—	—	—	—
	Pyrolysate		—	—	—	—	—
<i>Rae1</i>	Bitumen	–26.6 to –27.5	–34 ^b	—	–35	—	—
	Pyrolysate		—	–29	–31.5	–30	–31.5
<i>Beel</i>	Bitumen	–27.0 to –29.0	–33	–29	–32	—	—
	Pyrolysate		—	–29	–32.6	–31	–32.6

^a — = not measurable.

^b Statistical error for aromatic hydrocarbons $\approx \pm 1\%$. $\delta^{13}C$ values in italics are possibly affected by coeluting signals or insufficient baseline separation. For Compound abbreviations, see Table 2.

the rigorous analytical protocol of the hydrolysis experiments, including exhaustive preextraction of the isolated kerogens, procedural blanks, and thermal extraction of residual volatiles adsorbed to the kerogen, minimizes the probability of contamination by hydrocarbons from more recent sources. Second, the concentrations of individual aromatic hydrocarbons generated by hydrolysis are one order of magnitude higher than the yields obtained by solvent extraction of the rock powder. Thus, these molecules are genuine cleavage products of the organic matrix and not adsorbed volatiles.

A comparison of the hydrolysis products with the bitumens indicates that the solvent extractable aromatic hydrocarbons in the Mt. McRae Shale are also syngenetic. Hydrolysis products and bitumens both have unusually high concentrations of parent PAH relative to alkylated PAH and contain no PAH with five or more rings (Fig. 5). Moreover, the carbon isotopic compositions of individual aromatic hydrocarbons in the hydrolysis products and in the bitumens are identical within the analytical error and match the bulk $\delta^{13}\text{C}$ of the kerogen (Table 3). This compositional and isotopic correlation suggests a syngenetic relationship among kerogen, hydrolysis product, and the aromatic fraction of the bitumen.

4.2. Possible Hydrothermal Influence on Bitumen and Kerogen Composition

The unusual composition of bitumens from the iron ore mine at Mt. Tom Price has been interpreted as a result of the interaction of organic matter with hydrothermal fluids and/or oxidizing brines overprinted by regional low-grade metamorphism (Brocks, 2001). The hydrolysis experiments support this suggestion. First, although all Hamersley shales were collected in the Tom Price area, the hydrolysis products show order of magnitude differences in the absolute concentrations of individual hydrocarbons (Table 2). This might indicate that organic matter at different locations has suffered different temperatures and/or redox conditions, consistent with hydrothermal activity. Second, the complex temperature regime of a hydrothermal system could have led to partial bitumen dissolution in hot areas of the incipient ore body and to bitumen precipitation in cooler locations (Kawka and Simoneit, 1990; Wing and Bada, 1991). Such redistribution was possibly responsible for the compositional differences between bitumens and hydrolysis products. The bitumens contained, for example, high concentrations of phenanthrene but only traces of pyrene, whereas hydrolysis products yielded very high concentrations of phenanthrene as well as pyrene (Fig. 5). The pyrolytic precursors of pyrene were covalently bound to the kerogen and obviously protected against hydrothermal dissolution, whereas volatile components of the bitumen were possibly subjected to redistribution by circulating hydrothermal brines (Blumer, 1975; Wing and Bada, 1991).

4.3. Lack of Aliphatic Hydrocarbons in the Archean Pyrolysis Products

Most bitumens extracted from late Archean shales of the Hamersley and Fortescue Groups contain a high proportion of saturated hydrocarbons, whereas hydrolysis in the monitored C_{12+} range exclusively yielded aromatic compounds. The

compositional disparity between bitumens and hydrolysis products might be interpreted as the results of contamination that introduced aliphatic hydrocarbons from younger sources into the Archean rocks. However, similar differences between kerogen degradation products and extractable hydrocarbons have also been observed for many other Precambrian kerogen/bitumen pairs and could principally be a function of the original kerogen composition, and thus of biologic source input. For instance, hydrolysis of the moderately mature kerogens from the Mesoproterozoic Roper Group yielded significant amounts of polyaromatic hydrocarbons, whereas the concentrations of aliphatic components were comparatively low (Fig. 1; Table 2). In bitumens of the Velkerri Formation, on the other hand, saturated hydrocarbons were more abundant than aromatics.

Similar observations were made for the 1.1-Ga Nonesuch Shale, Michigan, USA, which contains immature kerogen and a high concentration of strongly aliphatic bitumen (Pratt et al., 1991). However, pyrolysis of the well-preserved kerogens merely yielded minute quantities of *n*-alkanes (Hoering and Navale, 1987) and large amounts of aromatic or phenolic products (Imbus et al., 1988). Additional examples of well-preserved macromolecular matter of Precambrian age that yielded no or only low quantities of aliphatic hydrocarbons when pyrolyzed have also been observed by Arouri et al. (2000), Hoering and Navale (1987), Leventhal et al. (1975), and McKirdy (1976). In all these cases, it is clear that the aromaticity of the macromolecular matrix is not a function of thermal maturity alone. The second crucial parameter that determines the pyrolytic behavior of kerogen is the composition of organic matter in the biologic source. Pyrolysis of Type I kerogen, according to the classic scheme of Tissot et al. (1974), produces high yields of aliphatic hydrocarbons and the biologic source is usually described as prokaryotic or algal. Oxygen-rich Type III kerogens, in post-Silurian sediments commonly lignin rich and derived from terrestrial plants, have much lower pyrolysis yields and tend to be aromatic. Although Precambrian organic matter has an exclusively microbial source, many Proterozoic kerogens, according to the observations above, appear to be more similar to modern Type III kerogens than to the aliphatic-rich Type I. Therefore, many Precambrian source organisms might have produced predominantly biodegradation-resistant nitrogen, sulfur and oxygen-rich (NSO) biopolymers, such as the cell wall material of some Neoproterozoic acritarchs (Arouri et al., 2000). Moreover, polymethylene biomolecules such as algaenans, responsible for a large proportion of the aliphatic material in Phanerozoic kerogens (de Leeuw and Largeau, 1993), were possibly less important in the Precambrian or evolved later in Earth history.

Hence, many Neoproterozoic and Mesoproterozoic sedimentary rocks with a mild thermal history contain kerogen that is predominantly aromatic, together with bitumen rich in saturated hydrocarbons. The main control for this phenomenon is probably the composition of organic matter in the original biota. The same might be true for Archean kerogens. The late Archean shales from Mt. Tom Price also contain aliphatic bitumens but the hydrolysis products are aromatic. The coexistence of saturated hydrocarbons and graphitic kerogens is therefore not necessarily a result of contamination but possibly the fingerprint of an NSO-rich and aliphatic-poor protokerogen.

5. CONCLUSIONS

In this work, hydropyrolysis was employed for the first time to study Mesoproterozoic and late Archean kerogens, leading to the following observations:

(1) Hydropyrolysis is a particularly powerful technique for trace analysis of overmature kerogens. It is characterized by high yields compared with traditional pyrolysis techniques and allows clean kerogens to be obtained by thermal desorption of residual bitumen.

(2) Pyrolysis of 2.5-Ga metakerogens from the Mt. McRae Shale, Hamersley Group, yielded up to 200 ppm aromatic hydrocarbons. This is a comparatively high yield considering that demonstrably indigenous pyrolysis products have never been obtained before from samples older than 1.7 Ga.

(3) The late Archean hydropyrolysates and the aromatic fraction of cooccurring bitumens have very similar molecular compositions. Moreover, the carbon isotopic signatures of kerogens, hydropyrolysates, and bitumens are almost identical. This correlation indicates that not only the hydropyrolysis products but also the aromatic hydrocarbons in the bitumens are syngenetic.

(4) The high concentrations of dealkylated aromatic hydrocarbons in the late Archean hydropyrolysates from Tom Price, the differences in hydropyrolysis yields between closely spaced samples, and the compositional differences between bitumen and kerogen are consistent with hydrothermal alteration of kerogen and hydrothermal redistribution of bitumen.

(5) Hydropyrolysis of Archean kerogens exclusively yielded aromatic hydrocarbons despite the fact that the bitumens are strongly aliphatic. Similarly, kerogens of the Mesoproterozoic Roper Group, along with many other thermally well preserved Precambrian kerogens, yielded predominantly aromatic pyrolysis products, although the corresponding bitumens are rich in saturated hydrocarbons. Because this phenomenon occurs in kerogens of high and low thermal maturity, the main control appears to be the composition of the protokerogen. The coexistence of saturated hydrocarbons and graphitic kerogens in the Archean samples is therefore not necessarily the result of contamination but might indicate the preservation of aliphatic-poor macromolecular matter from Archean source organisms.

(6) Although it was not possible to obtain information from hydropyrolysis about the syngeneity of biomarkers and other saturated hydrocarbons in late Archean rocks, the most significant outcome of these experiments is the confirmation of certain syngeneity of the aromatic fraction. Thus, these molecules represent the oldest clearly syngenetic organic compounds so far obtained from terrestrial geological sources. Although not particularly revealing in themselves about ancient life and its environment, their very existence and preservation over such spans of time under such harsh conditions offer hope that the more complex molecular fossils detected in the same rocks are also Archean.

Acknowledgments—Supported by the American Chemical Society Petroleum Research Fund (R.B.) and a School of Geosciences, University of Sydney, special stipend (J.J.B.). G.D.L. is grateful to NERC for funding a Postdoctoral Research Fellowship (GT5/98/14/ES). We thank Janet Hope, Carolyn Sandison, Christian Thun, and Natalie Johns for technical assistance, and Paul Greenwood and two anonymous reviewers for helpful comments. We thank Rio Tinto Exploration for

samples and Geoscience Australia for research support and samples. G.A.L. publishes with the permission of the CEO of Geoscience Australia.

Associate editor: G. D. Cody

REFERENCES

- Arouri K., Greenwood P. F., and Walter M. R. (2000) Biological affinities of Neoproterozoic acritarchs from Australia: Microscopic and chemical characterisation. *Org. Geochem.* **31**, 75–89.
- Bishop A. N., Love G. D., McAulay A. D., Snape C. E., and Farrimond P. (1998) Release of kerogen-bound hopanoids by hydropyrolysis. *Org. Geochem.* **29**, 989–1001.
- Blumer M. (1975) Curtisite, idrialite and pendletonite, polycyclic aromatic hydrocarbon minerals; their composition and origin. *Chem. Geol.* **16**, 245–256.
- Brocks J. J. (2001) Molecular fossils in Archean rocks. Ph.D. thesis. University of Sydney.
- Brocks J. J., Logan G. A., Buick R., and Summons R. E. (1999) Archean molecular fossils and the early rise of eukaryotes. *Science* **285**, 1033–1036.
- de Leeuw J. W. and Largeau C. (1993) A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal and petroleum formation. In *Organic Geochemistry* (eds. M. H. Engel and S. A. Macko), pp. 23–72. Plenum Press.
- Espitalié J., Laporte J. L., Madec M., Marquis F., Leplat P., Paulet J., and Boutefeu A. (1977) Methode rapide de caracterisation des roches meres, de leur potentiel petrolier et de leur degre d'evolution. *Rev. Inst. Francais Petrole* **32**, 23–42.
- George S. C. (1992) Effect of igneous intrusion on the organic geochemistry of a siltstone and an oil shale horizon in the Midland Valley of Scotland. *Org. Geochem.* **18**, 705–723.
- Hayes J. M., Kaplan I. R., and Wedeking K. W. (1983) Precambrian organic geochemistry, preservation of the record. In *Earth's Earliest Biosphere: Its Origin and Evolution* (ed. J. W. Schopf), pp. 93–134. Princeton University Press.
- Hoering T. C. and Navale V. (1987) A search for molecular fossils in the kerogen of Precambrian sedimentary rocks. *Precamb. Res.* **34**, 247–267.
- Imbus S. W., Engel M. H., Elmore R. D., and Zumberge J. E. (1988) The origin, distribution and hydrocarbon generation potential of organic-rich facies in the Nonesuch Formation, Central North American Rift System: A regional study. *Org. Geochem.* **13**, 207–219.
- Kawka O. E. and Simoneit B. R. T. (1990) Polycyclic aromatic hydrocarbons in hydrothermal petroleum from the Guaymas Basin spreading center. *Appl. Geochem.* **5**, 17–27.
- Leventhal J., Suess S. E., and Cloud P. (1975) Nonprevalence of biochemical fossils in kerogen from pre-Phanerozoic sediments. *Proc. Natl. Acad. Sci. USA* **72**, 4706–4710.
- Love G. D., Snape C. E., Carr A. D., and Houghton R. C. (1995) Release of covalently-bound alkane biomarkers in high yields from kerogen via catalytic hydropyrolysis. *Org. Geochem.* **23**, 981–986.
- Love G. D., McAulay A., Snape C. E., and Bishop A. N. (1997) Effect of process variables in catalytic hydropyrolysis on the release of covalently-bound aliphatic hydrocarbons from sedimentary organic matter. *Energy Fuels* **11**, 522–531.
- Love G. D., Snape C. E., and Fallick A. E. (1998) Differences in the mode of incorporation and biogenicity of the principal aliphatic constituents of a Type I oil shale. *Org. Geochem.* **28**, 797–811.
- Maroto-Valer M. M., Love G. D., and Snape C. E. (1997) Close correspondence between carbon skeletal parameters of kerogens and their hydropyrolysis oils. *Energy Fuels* **11**, 539–545.
- McKirdy D. M. (1976) Biochemical markers in stromatolites. In *Stromatolites* (ed. M. R. Walter), pp. 163–191. Elsevier.
- Murray I. P., Love G. D., Snape C. E., and Bailey N. J. L. (1998) Comparison of covalently-bound aliphatic biomarkers released via hydropyrolysis with their solvent-extractable counterparts for a suite of Kimmeridge clays. *Org. Geochem.* **29**, 1487–1505.
- Oehler J. H. (1977) Irreversible contamination of Precambrian kerogen by ¹⁴C-labelled organic compounds. *Precamb. Res.* **4**, 221–227.
- Philp R. P. and van de Meent D. (1983) Characterization of Precambrian kerogens by analytical pyrolysis. *Precamb. Res.* **20**, 3–16.

- Pratt L. M., Summons R. E., and Hieshima G. B. (1991) Sterane and triterpane biomarkers in the Precambrian Nonesuch Formation, North American Midcontinent Rift. *Geochim. Cosmochim. Acta* **55**, 911–916.
- Roberts M. J., Snape C. E., and Mitchell S. C. (1995) Hydropyrolysis: Fundamentals, two-stage processing and PDU operation. In *Composition, Geochemistry and Conversion of Oil Shales* (ed. C. E. Snape), pp. 277–294. Kluwer.
- Rocha J. D., Brown S. D., Love G. D., and Snape C. E. (1997) Hydropyrolysis: A versatile technique for solid fuel liquefaction, sulphur speciation and biomarker release. *J. Anal. Appl. Pyrolysis* **40–41**, 91–103.
- Smith R. E., Perdrix J. L., and Parks T. C. (1982) Burial metamorphism in the Hamersley Basin, Western Australia. *J. Petrol.* **23**, 75–102.
- Summons R. E., Taylor D., and Boreham C. (1994) Geochemical tools for evaluating petroleum generation in Middle Proterozoic sediments of the McArthur Basin, Northern Territory, Australia. *APEA J.* 692–706.
- Tissot B., Durand B., Espitalié J., and Combaz A. (1974) Influence of nature and diagenesis of organic matter in formation of petroleum. *Am. Assoc. Pet. Geol. Bull.* **58**, 499–506.
- Wing M. R. and Bada J. L. (1991) Geochromatography on the parent body of the carbonaceous chondrite Ivuna. *Geochim. Cosmochim. Acta* **55**, 2937–2942.