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A reconstruction of Archean biological diversity based on molecular fossils from the 2.78 to 2.45 billion-year-old Mount Bruce Supergroup, Hamersley Basin, Western Australia

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Abstract—Bitumens extracted from 2.7 to 2.5 billion-year-old (Ga) shales of the Fortescue and Hamersley Groups in the Pilbara Craton, Western Australia, contain traces of molecular fossils. Based on a combination of molecular characteristics typical of many Precambrian bitumens, their consistently and unusually high thermal maturities, and their widespread distribution throughout the Hamersley Basin, the bitumens can be characterized as 'probably of Archean age'. Accepting this interpretation, the biomarkers open a new window on Archean biodiversity. The presence of hopanes in the Archean rocks confirms the antiquity of the domain Bacteria, and high relative concentrations of 2α-methylhopanes indicate that cyanobacteria were important primary producers. Oxygenic photosynthesis therefore evolved $>$ 2.7 Ga ago, and well before independent evidence suggests significant levels of oxygen accumulated in the atmosphere. Moreover, the abundance of cyanobacterial biomarkers in shales interbedded with oxide-facies banded iron formations (BIF) indicates that although some Archean BIF might have been formed by abiotic photochemical processes or anoxygenic phototrophic bacteria, those in the Hamersley Group formed as a direct consequence of biological oxygen production. Biomarkers of the 3β-methylhopane series suggest that microaerophilic heterotrophic bacteria, probably methanotrophs or methylotrophs, were active in late Archean environments. The presence of steranes in a wide range of structures with relative abundances like those from late Paleoproterozoic to Phanerozoic sediments is convincing evidence for the existence of eukaryotes in the late Archean, 900 Ma before visible fossil evidence indicates that the lineage arose. Sterol biosynthesis in extant eukaryotes requires molecular oxygen. The presence of steranes together with biomarkers of oxygenic photosynthetic cyanobacteria suggests that the concentration of dissolved oxygen in some regions of the upper water column was equivalent to at least ~1% of the present atmospheric level (PAL) and may have been sufficient to support aerobic respiration. *Copyright © 2003 Elsevier Ltd*

1. INTRODUCTION

Microfossils, stromatolites and isotopes of sedimentary carbon and sulfur all indicate that microorganisms inhabited Earth during the Archean, the time before 2.5 billion years ago (Ga) (Fig. 1). The fossils and isotopes also provide circumstantial evidence for the early evolution of some physiological attributes and metabolic pathways. Sedimentary organic matter strongly depleted in the carbon isotope ^{13}C implies the presence of archaeal methanogens and bacterial methanotrophs (Hayes, 1983), fractionated sulfide isotopes in sulfate crystals suggest the existence of mesophilic bacterial sulfate-reducers (Shen et al., 2001), and palimpsest filament tufts in lacustrine stromatolites are consistent with phototropic cyanobacteria (Buick, 1992). However, these lines of evidence depend on chains of inference with concomitant compounding uncertainties. The oldest fossils well enough preserved to be recognized as a certain member of an extant clade only appear in the mid-Paleoproterozoic, providing diagnostic and unequivocal evidence for organisms of the phylum Cyanobacteria at 2.15 Ga (Hofmann, 1976). The oldest remains of possible eukaryotes were discovered in iron deposits 1.87 Ga old (Han and Runnegar, 1992; Schneider et al., 2002), while morphologic evidence for the domain Archaea is not known from any part of the fossil record. Therefore, the phylogenetic position of life in the Archean remains largely obscure. Potentially, a better insight into early Precambrian microbial diversity can be obtained from molecular fossils (biomarkers) preserved in sedimentary rocks.

This paper discusses the paleobiological and paleoenvironmental significance of biomarkers detected in late Archean sedimentary rocks from the Pilbara region of Western Australia (Brocks et al., 1999, 2003). Biomarkers, their distribution in the Archean samples, their occurrence in other Precambrian strata and their potential biologic source will be discussed according to hydrocarbon class in the following order: normal and branched alkanes, cyclohexylalkanes, adamantanes, tricyclic terpanes, hopanes and the diverse range of steranes and steroids.

A companion paper in this issue (Brocks et al., 2003) gives a detailed assessment of syngeneity of the biomarkers and addressing the possibility that the Archean host rocks were contaminated during drilling and storage, or were adulterated by migrating fluids during post-Archean history. It is concluded that adamantanes and polyaromatic hydrocarbons of the Hamersley Group are 'certainly syngenetic', and aliphatic hydrocarbons and polycyclic biomarkers of the Hamersley and For-

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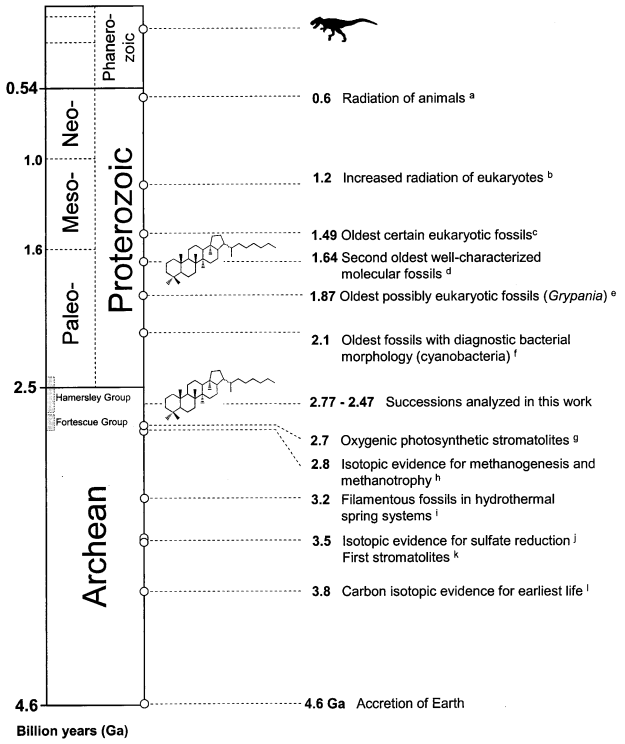

Fig. 1. Geological time-scale with important biologic events in the Precambrian. (a) Xiao et al. (1998); (b) Knoll (1992); (c) Javaux et al. (2001); (d) Jackson et al. (1986); (e) Han and Runnegar (1992), revised age of the host rocks in Schneider et al. (2002); (f) Hofmann (1976); (g) Buick (1992); (h) Hayes (1983); (i) Rasmussen (2000); (j) Shen et al. (2001); (k) Buick et al. (1981), Walter et al. (1980); (l) Rosing (1999).

tescue Group 'probably syngenetic'. Therefore, the age of the molecules that contain the biologic information is not fully resolved. Hence, paleobiological interpretation presented in this paper should be cited cautiously and with reference to the remaining uncertainty of syngeneity (Brocks et al., 2003).

1.1. The Interpretation of Archean Biomarkers: Problems of an Actualistic Approach

The interpretation of Archean and Proterozoic biomarkers is complicated by two factors. One is the fragmentary knowledge of biomarker distributions across the great diversity of extant organisms and the second is the problem of how reasonable it is to extrapolate modern biomarker relationships back in time over several billion years. The biological interpretation of geolipids is almost exclusively based on the distribution of biolipids in extant organisms. However, the full biolipid repertoire is only known for a small percentage of microorganisms that have been cultured (Volkman et al., 1993). It is possible, therefore, that many lipids might have a broader biological distribution and more or less taxonomic value than currently known. More problematic, however, are the uncertainties associated with attributing biological sources to biomarkers from rocks several hundred million to billions of years old. Particular pathways of lipid biosynthesis, such as the modification of sterol side-chains, might have evolved independently in different lineages or could have been passed by lateral gene transfer between

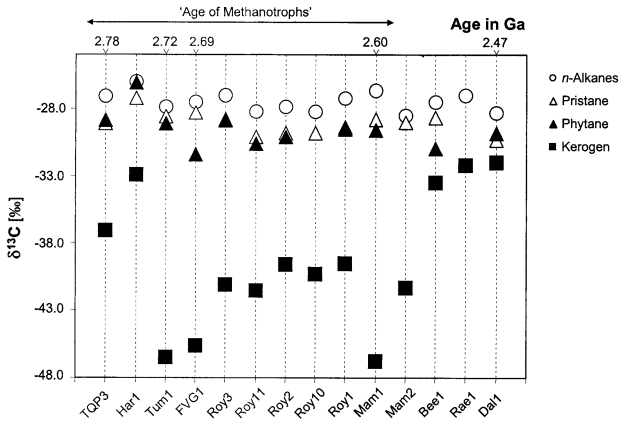

Fig. 2. Comparison of $\delta^{13}C$ of kerogen (squares), average of all *n*-alkanes (circles), and acyclic isoprenoids pristane and phytane (triangles) for samples of the Fortescue and Hamersley Group. Sample names and ages refer to Brocks et al. (2003a). 'Age of Methanotrophs' refers to Hayes (1994).

lineages. So lipids believed to be diagnostic of extant taxonomic groups could also have been prevalent in extinct clades.

2. ARCHEAN BIOMARKERS AND THEIR BIOLOGICAL SOURCES

2.1. *n*-Alkanes

By far the most abundant hydrocarbons in all late Archean bitumens are *n*-alkanes (Brocks et al., 2003). Homologues with chain lengths from 9 to 23 carbon atoms and in one case 28 carbon atoms were detected under GC-FID (gas chromatography–flame ionization detection) conditions. The *n*-alkane distribution is commonly unimodal with a strong predominance of low-molecular weight homologues, a composition generally controlled by the high thermal maturity of the samples. Odd or even predominance of carbon numbers was not observed, as expected from bitumen of such high maturity.

Unbranched lipids in Recent sediments predominantly reflect input from plant waxes (Hedberg, 1968), from polymethylenic biopolymers such as algaenans in eukaryotic algae (Tegelaar et al., 1989), and from membrane lipids in bacteria and eukaryotes such as phospholipids and sphingolipids. A plant wax source for *n*-alkanes can obviously be ruled out for pre-Silurian sedimentary rocks, and polymethylenic biopolymers probably played only a minor role before the Neoproterozoic (Brocks et al., in press). Straight chain lipids from bacterial and eukaryotic membrane constituents are therefore the most likely source for Archean *n*-alkanes.

The carbon isotopic composition of the *n*-alkanes (Fig. 2) suggests that they were predominantly derived from heterotrophic organisms recycling photosynthetic primary products. *n*-alkanes in all Archean bitumens are enriched in ^{13}C by up to 3‰ relative to pristane and phytane. This isotopic relationship is typical for many Precambrian bitumens but different from most Phanerozoic marine samples where *n*-Alkanes are commonly isotopically depleted relative to pristane and phytane (Fig. 3). An explanation for the inversion of this isotopic relationship in the transition from the Precambrian to the Pha-

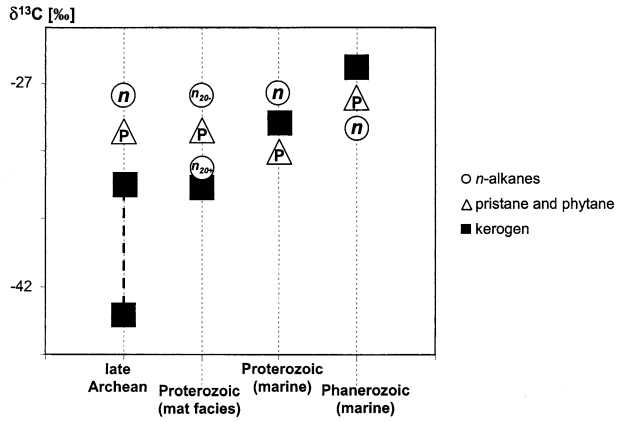

Fig. 3. Typical relationships between $\delta^{13}C$ of *n*-alkanes (*n*), acyclic isoprenoids pristane and phytane (P), and kerogen (K) in the late Archean bitumens, a Proterozoic mat facies, and Proterozoic and Phanerozoic marine samples. Data based on Logan et al. (1999), Logan et al. (1997), and Brocks et al. (2003a).

nerozoic was suggested by Logan et al. (1995). In organic matter derived from oxygenic photosynthetic primary producers the phytyl side-chain of chlorophyll, a precursor to most sedimentary pristane and phytane, is typically slightly enriched in ^{13}C relative to unbranched membrane lipids (Hayes, 1993). In the Phanerozoic, the bulk of this organic matter is transported into the sediment by rapidly sinking particles such as faecal pellets, so the original isotopic signature is preserved. This transport mechanism was absent before the Late Neoproterozoic and so the organic matter was extensively reworked as it sank slowly through the water column. As heterotrophic reworking is frequently associated with isotopic enrichment (Hayes, 1993), straight-chain lipids contributed by the organisms at higher trophic levels become isotopically enriched relative to primary photosynthate including pristane and phytane. A similar process of isotopic enrichment by heterotrophic reworking might also be responsible for the ^{13}C enriched *n*-alkanes in the Archean bitumens. An alternative scenario, contribution of isotopically light acyclic isoprenoids by Archaea, is discussed further below.

2.3. Cyclohexylalkanes

Alkanes with a terminal cyclohexyl ring were detected in low concentrations in all late Archean bitumens, with a relative distribution similar to the *n*-alkane profile (Fig. 4C in Brocks et al., 2003). Low concentrations of cyclohexylalkanes are also common in most bitumens and petroleums from the Proterozoic and Phanerozoic. Although cyclohexylalkanes could have a direct biologic source in the uncommon ω-cyclohexyl fatty acids isolated from some bacteria (Suzuki et al., 1981), the close affinity of the *n*-alkane and cyclohexylalkane homologue series in the Archean bitumens points to diagenetic cyclization of straight chain lipids as the most likely source (Fowler et al., 1986; Hoffmann et al., 1987; Summons et al., 1988b). Therefore, the cyclohexylalkanes probably have the same precursor as the *n*-alkanes.

2.3. Monomethylated Alkanes

Methylalkanes with the same mass-range as the *n*-alkanes are abundant in all Archean bitumens (Fig. 4A in Brocks et al., 2003). Isomers with all possible branching positions were detected in roughly equal concentrations with no predominance of end- or midbranched monomethylalkanes. End-branched monomethylalkanes and lower concentrations of midchain branched isomers are ubiquitous in petroleums and bitumens from the late Paleoproterozoic to the present (Summons and Walter, 1990). Elevated concentrations of midchain branched isomers are mainly present in Proterozoic and lower Paleozoic sediments, for example in the early Cambrian Chandler Formation (Summons, 1987), the terminal Proterozoic to Cambrian Huqf Formation of Oman (Klomp, 1986; Höld et al., 1999), in Neoproterozoic oils from eastern Siberia (Fowler and Douglas, 1987) and in bitumens from the Mesoproterozoic McArthur Basin, Northern Territory (Summons et al., 1988b). Exceptionally high concentrations of midchain branched methylalkanes of carbon numbers $> C_{24}$ were observed in immature terminal Proterozoic microbial-mat facies (Logan et al., 1999). Bitumens with a non-specific isomer distribution almost identical to the Archean samples have been detected in the late Paleoproterozoic Barney Creek Formation (Summons et al., 1988b) and Mesoproterozoic Velkerri Formation (Summons et al., 1994), McArthur Basin, Northern Territory.

Biological precursors of methylalkanes in extant organisms are widespread in the domain Bacteria (Summons, 1987) but are also present in Eucarya (Höld et al., 1999) and allegedly in Archaea (Brassell et al., 1981). In modern to Recent sediments the most common source of end-chain branched methylalkanes are 2- and 3-methylalkanoic acids prevalent in bacterial lipids (Kaneda, 1977; Fulco, 1983). Symbiotic bacteria living in extant demosponges produce high concentrations of end- as well as midchain branched carboxylic acids in a large variety of structural isomers in the C_{15} to C_{25} range (Thiel et al., 1999a). Midchain branched alkanes in the carbon-number range C_{16} to C_{18} are common in cyanobacterial mat-communities (Robinson and Eglinton, 1990; Shiea et al., 1990; Kenig et al., 1995; Köster et al., 1999). An additional potential source of methylalkanes in bitumen are cyclopropyl carboxylic acids that are quite common in Bacteria but are also present in some protists (Fowler and Douglas, 1987; Höld et al., 1999, and references therein).

In the Archean bitumens it is not clear whether the isomeric distribution of methylalkanes is representative of the original input or whether the branching positions were catagenetically 'reshuffled' to yield the observed non-specific isomer mixture. Repositioning of the methyl group along the alkane chain is not a likely mechanism (Summons et al., 1988b; Höld et al., 1999), but different positional isomers can be generated by thermal cleavage of higher midchain branched alkanes at different positions (Summons et al., 1988b). The thermal maturity of the Archean bitumens is so high that branched alkanes $> C_{20}$ would have been almost quantitatively cracked in most samples. It is therefore impractical to attempt to reconstruct the original isomer and homologue distribution of methylalkanes. Clearly, however, biosynthetic pathways leading to branched-chain lipids were present by the late Archean.

2.4. Acyclic Isoprenoids

Most of the Archean bitumens contain C_{11} to C_{16}, C_{18}, C_{19} and C_{20} acyclic isoprenoids in similar concentrations (Fig. 4B in Brocks et al., 2003). Acyclic isoprenoids with either 17 or $>$ 20 carbon atoms were not detected. The carbon isotopic compositions of the C_{19} and C_{20} isoprenoids pristane (Pr) and phytane (Ph) are in the range $\delta^{13}C \approx -27$ to -30‰, up to 3‰ lighter than the *n*-alkanes. Acyclic isoprenoids with 20 or less carbon atoms are also ubiquitous in bitumens from the late Paleoproterozoic to the present (Summons and Walter, 1990). Higher pseudohomologues are generally less common but have been detected in Proterozoic sediments such as the 850-Ma Chuar Group, Grand Canyon, Arizona (Summons et al., 1988a) and the 1.64-Ga Barney Creek Formation, McArthur Basin, Northern Territory (Summons et al., 1988b). In most younger bitumens and oils a high proportion of the acyclic isoprenoids with 20 carbon atoms or less is derived from the phytyl side chain of chlorophylls from phototrophic organisms with possible minor contributions from tocopherols and carotenoid pigments (Volkman and Maxwell, 1986). A second important source for acyclic isoprenoids in some environmental settings is cell membranes of Archaea (Moldowan and Seifert, 1979; Chappe et al., 1982). Many Archaea synthesize diagnostic acyclic isoprenoids with $>$ 20 and up to 40 carbon atoms (Brassell et al., 1981; Summons, 1987; Grice et al., 1998; Vink et al., 1998) but some halophiles and methanogens mainly produce shorter C_{14} to C_{20} pseudohomologues (Volkman and Maxwell, 1986; Rowland, 1990).

For the Archean bitumens, a mainly phototrophic source of acyclic isoprenoids is likely with a possible minor contribution of $\leq C_{20}$ isoprenoids from Archaea. If the presence of abundant 2α-methylhopanes in the Archean bitumens is correctly interpreted as indicating a significant input of organic matter from cyanobacteria (see below) then the presence of phytane and lower acyclic isoprenoids can be attributed to degradation products of chlorophyll. In addition, the isotopic composition of pristane and phytane is consistent with oxygenic photosynthetic carbon assimilation (Schidlowski et al., 1983). The carbon isotopic depletion of both pristane and phytane relative to *n*-alkanes by up to 3‰ could reflect a contribution of isotopically depleted membrane lipids from methanogenic Archaea (Rowland, 1990; Summons et al., 1998; Thiel et al., 1999b). However, the more diagnostic isotopic fingerprint of methanogens with phytane markedly depleted in ^{13}C relative to pristane (Murray et al., 1998) was not detected in any sample. A significant contribution of $\leq C_{20}$ isoprenoids by thermal degradation of higher Archaeal pseudohomologues can also be ruled out, since random cleavage of higher acyclic isoprenoids creates a whole set of lower pseudohomologues including C_{17}, C_{21} and above. The absence of C_{17} and $C_{20}+$ pseudohomologs, on the other hand, is consistent with thermal degradation of regular C_{20} isoprenoids (Haug and Curry, 1974). Therefore, the acyclic isoprenoids in the Archean bitumens are predominantly derived from phototrophic organisms with a possible contribution of $< C_{21}$ isoprenoids from Archaea.

2.5. Adamantanes

Adamantanes and diamantanes have no known direct biologic precursors. However, they are of interest here because they represent the only saturated hydrocarbons of absolutely unequivocal Archean age (Brocks et al., 2003). Moreover, adamantanes are believed to be derived from thermal rearrangement of biomarkers such as tricyclic terpanes, steranes and hopanes (Chen et al., 1996). As such, they could indicate the presence of complex, polycyclic lipids in the Archean shales even if other biomarkers were non-indigenous. However, it is also conceivable that adamantanes in the Archean bitumens formed as the result of enhanced thermal degradation of non-lipid organic matter. Therefore, it is currently not possible to obtain biologic information from Archean adamantanes.

2.6. Cheilanthanes

Cheilanthanes are tricyclic terpanes with the tricyclohexaprenane skeleton (Neto et al., 1983). In most Archean bitumens C_{19} to C_{25} 13β(H),14α(H)-cheilanthanes were detected by GC-MS MRM (Fig. 5 in Brocks et al., 2003). Tricyclic terpanes $> C_{25}$ were not analyzed but might also have been present. Cheilanthanes with 19 to 30 carbon atoms are ubiquitous in oils and bitumens from the Neoproterozoic to the present. Precambrian cheilanthanes were detected in terminal Proterozoic oils from the Siberian Platform (Summons and Powell, 1992) and Oman (Grantham, 1986), from the 760-Ma Neoproterozoic Xiamaling Formation, Yanshan region, North China (Wang, 1991; Wang and Simoneit, 1995), the 850-Ma Chuar Group, Arizona (Summons et al., 1988a), the 1.1-Ga Nonesuch Formation, Michigan (Summons and Walter, 1990), and the 1.64-Ga Barney Creek Formation, McArthur Group, Northern Territory, Australia (Summons et al., 1988b). Cheilanthanes have been termed 'orphan biomarkers' as they are without known biologic precursors (Ourisson, 1994; Rohmer et al., 1992). However, a study by Greenwood et al. (2000) suggested that cheilanthanes in bitumens from the Late Carboniferous to Early Permian Tasmanites Oil Shale could have an eukaryotic origin. These authors performed laser pyrolysis GC-MS on isolated and preextracted specimens of the fossil alga *Tasmanites* and detected C_{19} to C_{28}-cheilanthanes evidently derived from the algal biomass. Although cheilanthanes might have multiple sources, direct evidence for this assumption does not exist. A more reliable biologic interpretation of cheilanthanes in the Archean bitumens will become possible once extant precursor organisms have been discovered.

2.7. Hopanes

Hopanes were detected in all Archean bitumens (Brocks et al., 2003). Identified structures include the C_{27} to C_{35} 17α(H),21β(H)-hopane series ($\alpha\beta$-hopanes) as well as C_{29} to C_{36} 2α- and 3β-methyl-$\alpha\beta$-hopanes. Several 17β(H),21α(H)-hopane isomers (moretanes) and diagenetically rearranged dia- and neohopanes were also detected in lower concentrations. The isomer and homologue distribution of hopanes resembles that of most other Precambrian bitumens (Pratt et al., 1991; Summons et al., 1988a, 1988b) but differs from most Phanerozoic samples by having higher relative concentrations of A-ring methylated homologues. Hopanes are abundant in all known samples of sedimentary organic matter from the late Paleoproterozoic to the present (Summons et al., 1988b).

Hopanoids have been isolated from a wide range of bacteria and their taxonomic distribution has been reviewed by Ourisson et al. (1987) and Rohmer et al. (1992). Bacteriohopanoids have been detected in some, but not all, cyanobacteria, purple nonsulfur bacteria, gram-negative and gram-positive bacteria, methylotrophs and acetic acid bacteria. Hopanoids appear to be absent from the green and the purple sulfur bacteria and from all anaerobic symbiotic and parasitic forms. Although hopanoid biosynthesis does not require oxygen, it is of some interest that they are generally not present in obligate anaerobes (Rohmer et al., 1984). The most abundant hopanoids in bacterial membranes are C_{35}-bacteriohopanepolyols that carry an extended polyhydroxylated or otherwise functionalized side chain. All bacteria that synthesize C_{35}-bacteriohopanepolyols also contain lower concentrations of the C_{30}-hopanoids diploptene and diplopterol.

While Archaea do not produce hopanoids (Ourisson et al., 1987), they are present in some Eucarya (Rohmer et al., 1992) such as cryptogams, ferns, mosses, lichens, filamentous fungi and, in very low concentrations, in protists of the genus *Tetrahymena*. The rare eukaryotic hopanoids possess only 30 carbon atoms and do not carry the diagnostic polyhydroxy side-chains, so C_{35}-hopanoids are therefore biomarkers for bacteria (Rohmer et al., 1992). The C_{27} to C_{35}-hopanes found in post-Archean bitumens and oils are mostly produced by the diagenetic and catagenetic degradation of C_{35}-bacteriohopanepolyols and so the patterns of hopanes in the Archean bitumens also can be confidently assigned to bacteria.

Taxonomic information below domain level can be extracted from the patterns of hopanes methylated at ring-A. All Archean bitumens contain the series of C_{29} to C_{36}-2α-methyl-$\alpha\beta$-hopanes in high relative concentrations in comparison to Phanerozoic samples. C_{36}-hopanepolyols methylated at C-2 are common and abundant membrane constituents in extant cyanobacteria and prochlorophytes (Rohmer et al., 1984; Simonin et al., 1996; Summons et al., 1999). C_{31}-2-methylhopanoids also occur in pink-pigmented facultative methylotrophs, for example *Methylobacterium organophilum* (Bisseret et al., 1985; Knani et al., 1994), and in nitrogen-fixing bacteria of the genera *Azotobacter* and *Beijerinkia* (Vilcheze et al., 1994). However, 2-methylhopanoids with an extended side chain and $>$ 31 carbon atoms appear to be diagnostic of cyanobacteria (Summons et al., 1999).

The C_{31}-2α-methylhopane index (C_{31}-MHI) measures the concentration of C_{31}-2α-methyl-$\alpha\beta$-hopane relative to C_{30}-desmethyl-$\alpha\beta$-hopane. C_{31}-MHI is frequently high ($>$10%) in sedimentary rocks from playa and saline lakes and in settings of carbonate and evaporite precipitation, environments known to support the growth of cyanobacterial mats. C_{31}-MHI in clastic Phanerozoic sediments is commonly low ($\sim$0 to 9%) while it frequently exceeds 10% in Proterozoic shales (Summons et al., 1999), suggesting that the generally elevated abundance of 2α-methylhopanes in the Precambrian might be explained by an increased component of cyanobacterial primary production. Notably, C_{31}-MHI of the Archean bitumens is also predominantly $>$ 10% with some as high as 20%. Even higher than C_{31}-MHI in all Archean samples are the 2α-methylhopane indices of the C_{32} homolog, a biomarker exclusively generated by cyanobacteria. Thus, these high indices are consistent with an important role for cyanobacteria as primary producers in the late Archean.

The 3β-methylhopane series (C_{31} to C_{36}-3β-methyl-$\alpha\beta$-hopanes) represents a second set of A-ring methylated hopanes detected in Archean bitumen (Brocks et al., 2003). These compounds were detected in much lower concentrations than the 2α-methyl isomers, consistent with observations in most younger bitumens (Summons and Jahnke, 1992). Bacteriohopanepolyols methylated at C-3 have been isolated from methanotrophs, methylotrophs and acetic acid bacteria (Rohmer et al., 1984; Zundel and Rohmer, 1985; Rohmer and Ourisson, 1986; Simonin et al., 1994). Although the biologic source and geochemical significance of 3β-methylhopanes in bitumens and oils is not as well constrained as the 2α-methylhopanes, a methanotrophic source for 3β-methylhopanes in the Archean bitumens is plausible, especially in view of Hayes's (1983) hypothesis concerning the role of methanotrophy to explain the late Archean negative carbon isotope extremes that are found world-wide. Carbon isotopic measurements of individual hopanes might confirm a methanotrophic or methylotrophic origin for these biomarkers if they can ever be isolated in sufficient abundance.

The Archean bitumens also contain hopanes with rearranged carbon skeletons (Brocks et al., 2003). A series of weak signals was tentatively identified as C_{29} to C_{34}-17α(H)-diahopanes (17α(H)-15α-methyl-27-norhopanes). The diahopane skeleton is derived from the hopane structure by the shift of a methyl group from C-14 to C-15. Direct biologic precursors of diahopanes have not yet been identified and it is almost certain that diahopanes are exclusively formed by diagenetic rearrangement of regular hopanoids (Moldowan et al., 1991). A second group of modified hopanes in the Archean samples are the C_{27} and C_{29}-neohopanes 18α(H)-22,29,30-trisnorneohopane (Ts) and 18α(H),21β(H)-30-norneohopane (C_{29}Ts). Ts and C_{29}Ts are both ubiquitous constituents of crude oils and bitumens of all periods (Moldowan et al., 1991). As for diahopanes, neohopanes have no known biologic source but are probably formed by acid-catalyzed transfer of the methyl group at C-18 to C-19 (Seifert and Moldowan, 1978).

2.8. Steranes and Steroids

Steranes, the diagenetic and catagenetic alteration products of sterols, have been detected in oils and bitumens of all post-Archean periods (Summons and Walter, 1990). Significantly, the Archean bitumens contain most structural isomers and pseudohomologues of C_{26} to C_{30}-regular, rearranged and A-ring methylated steranes as well as many mono- and triaromatic steroids (Brocks et al., 2003), just like sediments from the Proterozoic to the present. Moreover, the Archean steroid distributions are practically indistinguishable from those of late Paleoproterozoic to Cenozoic rocks (Summons et al., 1988b; Summons and Walter, 1990; Pratt et al., 1991; Summons and Powell, 1992).

Sterols are abundant membrane components in most Eucarya. Only few bacteria have the unequivocal capacity for de novo steroid biosynthesis: the methylotrophic bacteria *Methylosphaera sp.* and *Methylococcus capsulatus*, several species of myxobacteria, e.g., *Nannocystis exedens*, and possibly some species of the genus *Mycobacterium*. However, *M. capsulatus*

synthesizes exclusively 4-methyl and 4,4-dimethyl sterols with an uncommon unsaturation pattern and does not have the capacity to alkylate the sterol side chain (Bird et al., 1971; Bouvier et al., 1976; Jahnke and Nichols, 1986; Ourisson et al., 1987; Summons et al., 1992). Potential geolipids derived from *M. capsulatus*, 4-methylcholestanes and 4,4-dimethylcholestanes, have only rarely been observed in bitumen (Chen and Summons, 2001). *Nannocystis exedens* exclusively generates C_{27}-cholestenols (Kohl et al., 1983) and, again, does not have the biosynthetic capacity to alkylate the side-chain. Very low concentrations of C_{27} to C_{29}-sterols have also been reported from cyanobacteria (de Souza and Nes, 1968; Nadal, 1971; Paoletti et al., 1976; Sallal et al., 1987; Kohlhase and Pohl, 1988; Rzama et al., 1994; Hai et al., 1996). However, it has been argued that such trace amounts can generally be linked either to exogenous sterol incorporation or to eukaryotic culture contamination (Bouvier et al., 1976; Ourisson et al., 1987). For example, recent re-examination of some cyanobacterial lineages revealed that all cultures that yielded sterols were contaminated by fungi (Summons et al., 2001). Previous reports of sterol synthesis in cyanobacteria are therefore contentious and need re-examination. Trace quantities (0.001 mg/g dry cell weight) of C_{27} sterols were also detected in a culture of *Mycobacterium smegmatis* and attributed to bacterial biosynthesis (Lamb et al., 1998). However, in the closely related *M. tuberculosis*, enzymes involved in sterol metabolism were demonstrably acquired by lateral gene transfer from a eukaryote (Debeljak et al., 2000; Gamieldien et al., 2002). Thus, even if mycobacteria have the capacity for *de novo* sterol synthesis, it is likely that the genes coding for this process have been secondarily derived in relatively recent geological time.

If sterol biosynthesis in prokaryotes is indeed limited to a few distinctive structural isomers, then the complex sterane distribution generally detected in bitumens and oils can be safely attributed to eukaryotes. Most bitumens throughout post-Archean Earth history contain similar distributions of C_{26} to C_{30}-steranes, A-ring methylated steranes, mono- and triaromatic steroids and their diagenetic rearrangement products (Summons et al., 1988b). The late Archean bitumens studied here also have the same general sterane and aromatic steroid pattern (Brocks et al., 2003). It is highly unlikely that such steranes in Phanerozoic and Proterozoic sediments had an eukaryotic source but had a prokaryotic origin in the Archean. Although it is possible that as yet unknown or extinct prokaryotic organisms produced sterols, the wide structural range of steranes present, their relative abundances like those of younger bitumens, and their marked dissimilarity to known prokaryotic examples is convincing evidence for the existence of eukaryotes in the late Archean (*contra* Cavalier-Smith, 2002).

Although sterane distributions in the Archean bitumens are equivalent to those of younger bitumens, they offer no taxonomic information below domain level. The most abundant steranes in all samples were desmethylsteranes with 27 to 29 carbon atoms (Brocks et al., 2003). Sterols with the cholestane skeleton (C_{27}) are common in extant rhodophytes, precursors of ergostanes (C_{28}) are predominant in many diatoms (Volkman and Hallegraeff, 1987) and precursors of stigmastanes (C_{29}) are abundant in chlorophytes and higher plants (Volkman, 1986; Summons and Walter, 1990). However, C_{27} to C_{29}-desmethylsteranes are not exclusive to any particular taxon (Volkman, 1986). Even closely related species within the domain Eucarya may biosynthesize varying mixtures of different sterols with all three carbon skeletons (Volkman et al., 1980; de Leeuw and Baas, 1986; Volkman, 1986).

24-*n*-Propylcholestane (C_{30}) also occurs in all Archean bitumens (Brocks et al., 2003), albeit in low concentrations (~2–4% of the sum of C_{27} to C_{29}-steranes). This biomarker is also known from other Precambrian samples, including the Neoproterozoic of the Siberian platform (Summons and Powell, 1992), the 1.1-Ga Nonesuch Shale (Pratt et al., 1991) and the 1.64-Ga Barney Creek Formation (Summons et al., 1988b; McCaffrey et al., 1994). Biological precursors with the 24-*n*-propylcholestane skeleton have so far only been isolated from several species of pelagophyte algae of the order Sarcinochrysidales (Raederstorff and Rohmer, 1984; Moldowan et al., 1990). However, the biologic origin of 24-*n*-propylcholestane in the Archean bitumens remains unclear.

Other steroids detected in the Archean bitumens are A-ring methylated cholestanes (C_{28}), ergostanes (C_{29}) and stigmastanes (C_{30}). Unequivocally identified were 2α-, 3β- and 4α-methyl-24-ethylcholestanes as well as traces of 4α,23,24-trimethylcholestanes (Fig. 10 in Brocks et al., 2003). 2α- and 3β-methylsteranes have no known biologic counterparts and they probably form by diagenetic methylation of Δ^2-sterenes either in an abiogenic process (Summons and Walter, 1990) or mediated by heterotrophic microorganisms (Summons and Capon, 1988). On the other hand, sterols methylated at C-4, are widespread in eukaryotic organisms. 4-methylsterols and 4,4-dimethylsterols are intermediates in the biosynthesis of desmethyl sterols (Bloch, 1994) and are typically present in at least low concentrations in all organisms that synthesize sterols. However, the concentration of these reaction intermediates is commonly far too low for a significant sedimentary contribution (Volkman et al., 1990). Higher concentrations of sterols with the 4-methylcholestane (C_{28}), 4-methyl-24-methylcholestane (C_{29}) and 4-methyl-24-ethylcholestane (C_{30}) skeletons are present in most dinoflagellates (Robinson et al., 1984) and less commonly in diatoms (Nichols et al., 1990). Sterols with the 4-methyl-24-ethylcholestane skeleton (C_{30}) have also been isolated from prymnesiophyte algae of the order Pavlovales (Volkman et al., 1990), and with the 4-methylcholestane skeleton (C_{28}) from the methylotrophic bacterium *Methylococcus capsulatus* (Bird et al., 1971). Regular 4-methylsteranes are therefore not sufficiently taxon-specific to interpret their occurrence in Archean bitumen below domain level.

4α,23,24-Trimethylcholestanes (dinosteranes) also occur in trace amounts in the Archean bitumens (Brocks et al., 2003). Dinosteranes are abundant biomarkers in almost all Mesozoic and Cenozoic bitumens (Moldowan et al., 1996) but have also been detected in the Paleozoic and Proterozoic, albeit in lower concentrations (Moldowan et al., 1996). So far, the oldest known dinosteranes come from the ~1.1-Ga Nonesuch Shale, Michigan (Summons and Walter, 1990). The biologic precursor of dinosteranes is dinosterol that is commonly regarded as diagnostic for dinoflagellates (Robinson et al., 1984; Summons et al., 1987, 1992; Moldowan et al., 1996; Moldowan and Talyzina, 1998), although it has also been recorded in one diatom species as a minor component (Volkman et al., 1993). As fossil dinoflagellates (and diatoms) first appear in the Mesozoic, dinosteranes discovered in Paleozoic and Proterozoic

sedimentary rocks were described as products of ancestral dinoflagellates (protodinoflagellates) (Moldowan et al., 1996). However, dinosteranes of Archean age have more likely a biologically independent origin, as ancestral dinoflagellates and diatoms almost certainly appeared much later in Earth history.

So, the wide structural range of steranes in the Archean bitumens in relative proportion similar to those of many younger bitumens is evidence for the presence of eukaryotes in the late Archean. But the phylogenetic status of the sterol producers within the domain Eucarya in the late Archean remains to be resolved.

2.9. Low Levels of Change Through 3 Ga of Lipid Biosynthesis

A striking feature of the 2.7-Ga-old Archean bitumens is their similarity to other Precambrian, but also Phanerozoic samples. All isomeric forms of the homologous series of straight and branched alkanes, acyclic isoprenoids, tricyclic diterpanes, methylated and non-methylated hopanes and steranes have previously been detected in similar relative abundances in the Paleoproterozoic (Summons et al., 1988b), Mesoproterozoic (Pratt et al., 1991), Neoproterozoic (Summons et al., 1988a) and throughout the Phanerozoic (Peters and Moldowan, 1993). Significant changes in the molecular fossil repertoire appear only to occur in the post-Silurian with the addition of plant biomarkers. The following conclusions can be drawn. First, the biosynthetic capacity for producing all principal carbon skeletons of membrane lipids had developed by the late Archean. These include straight chain and branched lipids, acyclic isoprenoids, cheilanthanes, hopanoids and steroids. Second, biosynthetic pathways leading to all important modified sterane and hopane skeletons, including A-ring methylation and side-chain modification, also had evolved by 2.7 Ga.

2.10. The Carbon Isotopic Composition of Kerogen and Individual Hydrocarbons

The carbon isotopic composition of kerogen in the early Precambrian is characterized by a global excursion to very low $\delta^{13}C$ values between ~2.8 and ~2.5 Ga. During this period $\delta^{13}C$ dropped to between −35 and −50‰ and occasionally to −60‰ (Des Marais et al., 1992; Strauss and Moore, 1992; Des Marais, 1997). Before and after this interval, kerogen isotopes were predominantly in the range −24 to −35‰ (Strauss and Moore, 1992). The isotopic excursion in the late Archean is also reflected by kerogens analyzed in this work (Fig. 2). $\delta^{13}C$ for kerogens from the 2.78-Ga Mt Roe Formation to the 2.60-Ga Marra Mamba Iron Formation range from −37 to −47‰. Kerogens from the ~2.5-Ga Mt McRae Shale and Brockman Iron Formation are significantly heavier at −32 to −35‰.

A model explaining the secular carbon isotopic excursion in the late Archean has been proposed by Hayes (1983, 1994). This envisages globally elevated rates of methanogenesis with reintroduction of isotopically depleted methane-derived carbon into the kerogen reservoir by methanotrophic activity. Consistent with methanotrophic activity in the late Archean is the presence of 3β-methylhopanes in the bitumens, and methanogenesis is potentially indicated by acyclic isoprenoids. However, the apparent absence of isotopically depleted *n*-alkanes appears difficult to reconcile with the methanogen/methanotroph hypothesis. In the Mt McRae Shale and Brockman Iron Formation, Hamersley Group, *n*-alkanes are *enriched* in ^{13}C relative to kerogen by 2 to 6‰ and in the upper Fortescue Group and lowermost Hamersley Group by 11 to 21‰ (Figs. 2 and 3).

However, a simple quantitative model based on two groups of organisms with different isotopic compositions demonstrates that the co-occurrence of isotopically very light kerogens with isotopically heavier lipids is possible and potentially consistent with the methanogen/methanotroph hypothesis. In this model *L* organisms biosynthesize isotopically light organic matter ($\delta^{13}C_L$) while *H* organisms contribute isotopically heavy biomass to kerogen and bitumen ($\delta^{13}C_H$). It is assumed that lipid and non-lipid biomass in each group has approximately the same isotopic composition and that isotopic shifts during transition to mature kerogen are negligible. If l_K is the mass-fraction of non-lipid carbon contributed to the kerogen by *L* organisms and $1 - l_K$ by *H* organisms then the carbon isotopic composition of the resulting kerogen is

$$\delta^{13}C_{kerogen} = l_K\delta^{13}C_L + (1 - l_K)\delta^{13}C_H \quad (1)$$

Further, if the ratio of lipid to non-lipid carbon contributed to the sedimentary organic matter is *x* times higher in *H* organisms than in *L* organisms, then lipids in the bitumen will have the average isotopic composition

$$\delta^{13}C_{lipids} = l_K/(x - xl_K + l_K)\delta^{13}C_L + [1 - l_K/(x - xl_K + l_K)]\ \delta^{13}C_H \quad (2)$$

Assuming $\delta^{13}C_L = -80‰$ for methanotrophs (Hayes, 1983), $\delta^{13}C_H = -26‰$ for phototrophs, $l_K = 20\%$ and $x = 10$, then the resulting kerogen will have an isotopic composition $\delta^{13}C_{kerogen} = -36.8‰$ and *n*-alkanes in the bitumen $\delta^{13}C_{n\text{-}alkanes} = -27.3‰$. The isotopic difference is 9.5‰, similar to values found in most late Archean kerogen/bitumen pairs. If the relative contribution of organic matter to the kerogen by methanotrophs increases to $l_K = 40\%$ then $\delta^{13}C_{kerogen} = -47.6‰$ and $\delta^{13}C_{n\text{-}alkanes} = -29.4‰$. The resulting isotopic kerogen/bitumen difference is 18.2‰, equivalent to the highest values in the Archean samples. Also, the transition from $\delta^{13}C_{kerogen} = -37‰$ to −48‰ leads, in the model, to a decrease in $\delta^{13}C_{n\text{-}alkanes}$ of only ~2‰. In the Archean samples a systematic variation of $\delta^{13}C_{n\text{-}alkanes}$ with $\delta^{13}C_{kerogen}$ of ~2‰ was not observed (Fig. 2), probably because such a small isotopic fluctuation would have been masked by in situ mixing of gas-condensates.

The main factor that determines the carbon isotopic difference between kerogen and bitumen is *x*, and the existence of mechanisms causing values as high as $x = 10$ is crucial for the validity of the above model. Four biologic mechanisms can be considered:

1. *x* will be high when *H* organisms produce a higher proportion of lipids relative to total biomass than *L* organisms. A lipid content of 20% in *H* organisms and 2.4% in *L* organisms would result in $x \approx 10$. These values are plausible as such differences in lipid contents have frequently been ob-

Table 1. Interpretation of late Archean biomarkers.

Archean biomarker	Interpretation
n-Alkanes	Predominantly membrane lipids of autotrophic and/or heterotrophic Bacteria and Eucarya
Methylalkanes	Predominantly Bacteria
Acyclic isoprenoids $< C_{21}$	Photosynthetic Bacteria; possibly photosynthetic Eucarya; possibly minor contribution from Archaea
Cyclohexylalkanes	Diagenetic cyclisation products of unbranched lipids
Cheilanthanes	Probably Eucarya or Bacteria
Hopanes	Bacteria, including cyanobacteria
2α-Methylhopanes	Cyanobacteria with oxygenic photosynthetic physiology
3β-Methylhopanes	Microaerophilic heterotrophic bacteria, probably methanotrophs and/or methylotrophs
Diahopanes	Diagenetic rearrangement product of hopanoids
Neohopanes	Diagenetic rearrangement product of hopanoids
C_{26} to C_{30} steranes	Eucarya of unknown phylogenetic position and unknown physiology
4-Methylstigmastanes (C_{30})	Eucarya of unknown phylogenetic position and unknown physiology
Dinosteranes	In the Mesozoic and Cenozoic indicative for dinoflagellates, but in the Archean derived from Eucarya of unknown phylogenetic position and unknown physiology
2 and 3-Methylsteranes	Diagenetic methylation products of desmethylsteroids
Diasteranes	Diagenetic rearrangement products of sterenes
Mono and triaromatic steroids	Diagenetic and catagenetic dehydrogenation products of sterenes
^{13}C depleted kerogen	Possible indirect evidence for methanotrophs and methanogens

served in extant organisms. For example, the cyanobacterium *Nostoc carneum* contains ~22% lipids in its total dry cell mass (Kohlhase and Pohl, 1988) whereas the methanogen *Methanobacterium thermoautotrophicum* apparently has only 2.8% (Rowland, 1990).

2. A high *x* value also results when lipid material of *H* organisms is less prone to biologic reworking or other forms of degradation than lipids of *L* organisms. The highly resistant and lipid-rich algaenans from some extant algae are the best example of such a system (Tegelaar et al., 1989). However, pyrolysis experiments indicate that input of macromolecular lipids into late Archean kerogens was probably low (Brocks et al., 2003b) and this mechanism is therefore less likely than the following explanation.
3. Very high *x* values could occur when *L* organisms synthesize biodegradation-resistant non-lipid macromolecules. Such resistant biomacromolecules might become enriched during diagenesis by two to three orders of magnitude relative to other organic matter (Tegelaar et al., 1989). To explain the isotopically light Archean kerogens in combination with isotopically heavy bitumen, *L* organisms would have to preferentially generate non-degradable non-lipid organic matter such as recalcitrant cell wall material.
4. *x* might increase if organic matter from *H* organisms is exposed to oxidizing conditions for a longer period than organic matter from *L* organisms. Lipids usually become gradually enriched during biodegradation, as protein- and carbohydrate-derived organic matter is consumed first. Isotopically heavy lipids from phototrophic organisms might then become concentrated as their dead biomass sinks slowly through the upper oxygenated part of a stratified water column. The isotopically light dead biomass from microaerophilic *L* organisms, on the other hand, is less exposed to oxygen, travels shorter distances to its final sedimentary sink and experiences a lower degree of recycling. Thus, isotopically heavy lipids might become more enriched relative to total biomass than isotopically light lipids.

Although other mechanisms might have been responsible for the carbon isotopic disparities between the late Archean kerogens and bitumens, the simple model presented above demonstrates that a biologic explanation is plausible. It also shows that the isotopic data are in principle consistent with Hayes's (1983) hypothesis of global methanotrophy. The exact source of the isotopically light organic matter in the kerogens is as yet unresolved but significant information about Archean biology may reside in its solution. It might be possible to detect the ^{13}C depleted source by measuring carbon isotopic compositions of individual hopanes and steranes. However, this will only become feasible with the discovery of Archean bitumens with higher biomarker concentrations.

3. RECONSTRUCTION OF LATE ARCHEAN BIODIVERSITY

To reconstruct the late Archean biosphere, biomarker data should be viewed in the context of current paleontological knowledge. The following section reviews fossil and isotope evidence for the antiquity of the three domains of life—Bacteria, Archaea and Eucarya—and discusses new knowledge provided by Archean biomarkers (Table 1).

3.1. Domain Bacteria

Life has existed on Earth for probably > 3.5 Ga (Buick et al., 1981; Rosing, 1999). Thus, as the domain Bacteria forms a fundamental branch on the Tree of Life (Fig. 4) (Woese et al., 1990), an origin for the clade early in the Archean seems likely. However, the oldest unquestionable morphologic evidence for bacteria does not appear in the fossil record until 2.15 Ga (Hofmann, 1976). Accepting the syngeneity of the biomarkers discussed in this paper, the presence of hopanes is the first unequivocal testimony for the domain Bacteria in the Archean.

As bacteriohopanol biosynthesis occurs in a wide range of phylogenetically unrelated bacterial taxa, regular desmethylhopanes are not diagnostic below domain level (Ourisson et al., 1987). However, taxonomic information can be obtained from hopanes carrying an additional methyl group at ring-A. 2α-methylhopanes with an extended side chain are diagnostic

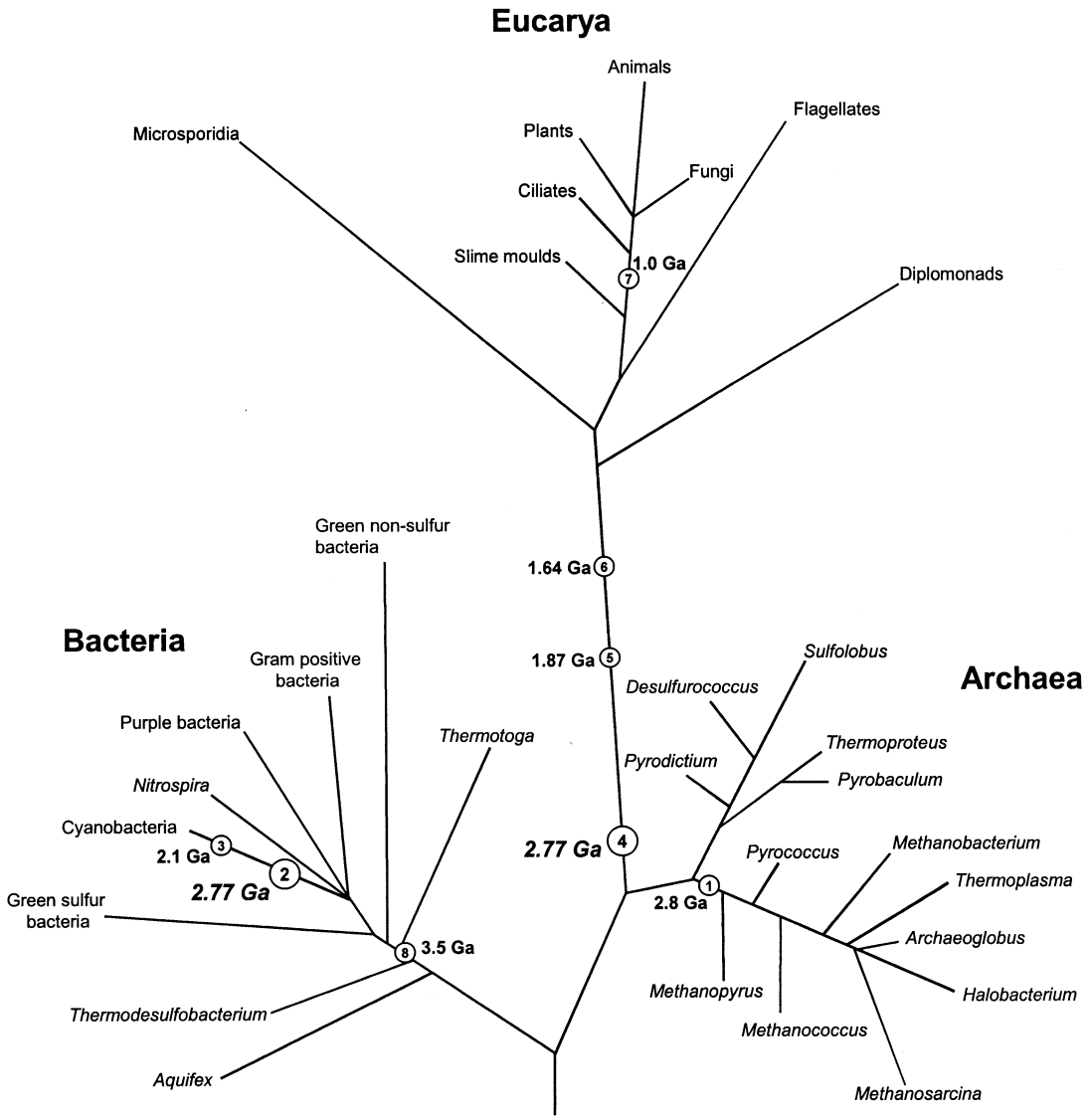

Fig. 4. The Tree of Life annotated with minimum ages of selected branches based on biogeochemical and paleontological data. New dates reported in this work are printed in italics. ① Indirect evidence for the activity of methanogenic Archaea derived from global carbon isotopic anomalies in the kerogen of ~2.8 to ~2.5 Ga sedimentary rocks (Hayes, 1983, 1994). ② Biomarker evidence (2α-methylhopanes) for cyanobacteria (Brocks et al., 1999). ③ Oldest known fossils with diagnostic cyanobacterial morphology from the 2.15-Ga Belcher Supergroup, Canada, (Hofmann, 1976). ④ Biomarker evidence (diverse steranes) for Eucarya (Brocks et al., 1999). ⑤ Oldest known fossils with possible eukaryotic morphology from the 1.87-Ga Negaunee Iron Formation, Michigan, (Han and Runnegar, 1992; Schneider et al., 2002). ⑥ Previous oldest sterane biomarkers from the ~1.64-Ga Barney Creek Formation, McArthur Basin, Northern Territory (Summons et al., 1988b). ⑦ Oldest known eukaryotic fossils assigned with confidence to an extant phylum (Rhodophyta) from the 1.26 to 0.95 Ga Hunting Formation, Somerset Island, Canada (Butterfield et al., 1990). ⑧ Sulfur-isotopic evidence for mesophilic sulfate-reducing Bacteria from North Pole, Pilbara Craton, Western Australia (Shen et al., 2001). Branch lengths and branching order are based on SSU rRNA modified from Woese et al. (1990), Shen et al. (2001), and Canfield and Raiswell (1999).

biomarkers for cyanobacteria, and 3β-methylhopanes are consistent with methanotrophs, methylotrophs or acetic acid bacteria.

3.1.1. Cyanobacteria

Microfossils (Knoll, 1996), stromatolites (Walter, 1994) and geochemical evidence (Buick, 2001) all suggest that mat-forming and filamentous photosynthetic organisms existed in the Archean. However, unequivocal evidence that they were cyanobacteria has still to be found (Brasier et al., 2002). The most ancient microfossils with a complex morphology diagnostic of cyanobacteria are no older than 2.15 Ga (Node ③ in Fig. 4) (Hofmann, 1976). The high abundance of 2α-methylhopanes with an extended side chain in late Archean bitumens now confirms that cyanobacteria were indeed extant by 2.7 Ga (Node ② in Fig. 4) (Brocks et al., 1999; Summons et al., 1999).

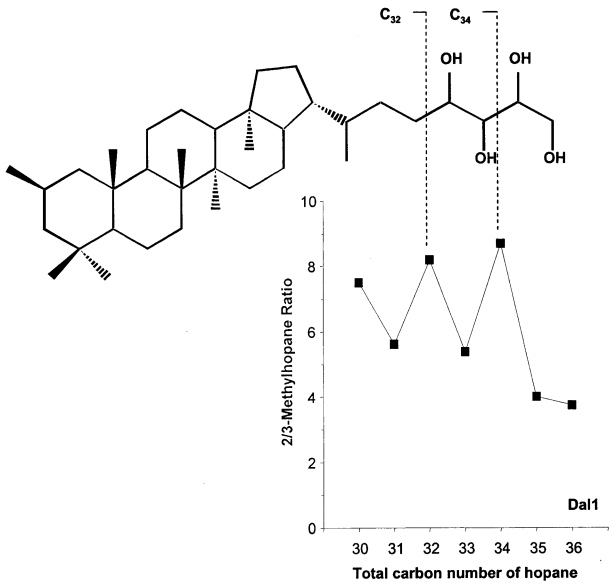

Fig. 5. Structure of 2β-methylbacteriohopanetetrol, and plot of 2/3-Methylhopane Ratios against carbon number (sample Dal1, Brockman Iron Formation, Hamersley Group). The Cn2/3-Methylhopane Ratio is defined as Cn2α-methyl-$\alpha\beta$-hopane/Cn3β-methyl-$\alpha\beta$-hopane.

Since the biosynthesis of hopanoids, including 2α-methylhopanoids, is confined to aerobes (Rohmer et al., 1984), a parsimonious interpretation would be that the late Archean cyanobacteria had developed a capability for oxygenic photosynthesis and were a source of free oxygen into the environment. Independently of this, the side-chain degradation pattern of the C_{30} to C_{36} 2α-methylhopane homologous series relative to the corresponding C_{30} to C_{36} 3β-methylhopanes (Fig. 5) suggests that late Archean cyanobacteria lived in an oxygenated micro-environment. The C_{30} to C_{36} 2α-methylhopane series has in all analyzed samples a characteristic even-over-odd carbon number predominance. The elevated abundance of the C_{32}-homologue relative to C_{31} and C_{33} indicates oxidative side-chain cleavage of a bacteriohopanetetrol to a C_{33} carboxylic acid and subsequent decarboxylation under non-reducing conditions (Peters and Moldowan, 1991). This process almost certainly required molecular oxygen (3β-methylhopanes, used as reference compounds for the methylhopane plot in Figure 5, are derived from organisms that preferentially inhabit suboxic conditions and are less affected by this type of oxidative side chain fragmentation). Therefore, the prevalence of the oxidative degradation pattern in 2α-methylhopanes together with the absolute abundance of these biomarkers is testimony for biogenic oxygen production at 2.7 Ga, ~700 Ma before significant oxygen accumulated in the atmosphere (Holland and Beukes, 1990). One major sink for the excreted oxygen was probably reduced iron, and cyanobacterial biomarkers are indeed abundant in shales interbedded with oxide-facies banded iron formations (BIF) of the Hamersley Group. So, although BIF could in principle have been formed by abiotic photochemical processes (Braterman et al., 1983) or by anoxygenic phototrophic bacteria (Widdel et al., 1993), those in the Hamersley Group probably formed as a result of photosynthetic oxygen production (Brocks et al., 1999). As bacteriochlorophyll biosynthesis in anoxygenic photosynthetic bacteria evidently evolved before chlorophyll biosynthesis in cyanobacteria (Xiong et al., 2000), the 2α-methylhopanes also provide indirect evidence that the four lineages of anoxygenic photosynthetic bacteria—heliobacteria, purple bacteria, green sulfur bacteria and green nonsulfur bacteria—were probably also extant by 2.7 Ga (Des Marais, 2000).

3.1.2. Methanotrophs

There is no visible fossil evidence of the existence of methanotrophic bacteria in the Archean or, indeed, in any other period. However, the global negative carbon isotopic anomaly of kerogen during the late Archean may be indirect evidence for the existence of methanotrophs by 2.8 Ga. According to Hayes (1983), isotopically light methane generated by methanogens was incorporated into the sedimentary record by methanotrophs. As isotopically light kerogens occurred globally and the excursion extended for $>$ 100 Ma, Hayes (1994) coined the term "Age of Methanotrophs" to describe the late Archean. Although the methanogen-methanotroph hypothesis is persuasive, the carbon isotopic excursion does not provide information about the taxonomic affinity of the methane recyclers. However, evidence that they were related to extant methanotrophic bacteria is now provided by the presence of the 3β-methylhopane series in the late Archean bitumens. However, 3-methylhopanoids have also been isolated from methylotrophic and acetic acid bacteria and may exist in other, as yet unknown, groups. Also, enhanced methane recycling by organisms related to extant methanotrophs should have left significant concentrations of isotopically light lipids. A continuing search for confirmation of methanotrophic activity should be focussed on the detection of ^{13}C-depleted hopanoids and other lipids.

3.2. Domain Archaea

Although fossils of Archaea should be morphologically indistinguishable from most Bacteria (Summons and Walter, 1990), the two domains generate substantially different biomarkers. Acyclic isoprenoids with between 20 and 40 carbon atoms and distinct modes of isoprenoid linking are diagnostic for the Archaea (Brassell et al., 1981; Summons, 1987; Grice et al., 1998; Vink et al., 1998). Shorter C_{14} to C_{20}-isoprenoids are also abundant in some halophiles and methanogens but they are frequently not distinguishable from degradation products of the phytyl side chain of chlorophylls (Volkman and Maxwell, 1986; Rowland, 1990). The late Archean bitumens only contain acyclic isoprenoids with $<$ 21 carbon atoms. So, although a contribution from Archaea cannot be excluded, these acyclic isoprenoids are more probably derived from phototrophic organisms (see section 2.4.). The only indirect geochemical evidence for Archaea in the Archean is the global occurrence of kerogens strongly depleted in ^{13}C at ~2.8 to ~2.5 Ga which, according to the 'Age of Methanotrophs' hypothesis (see section 3.1.2 and Hayes, 1994), should have ultimately resulted from Archaeal methanogenesis.

3.3. Domain Eucarya

Body fossils attributed to eukaryotes have never been recorded in rocks of Archean age. The oldest fossils proposed to

have eukaryotic affinity are 1.87 Ga old (Schneider et al., 2002) carbonaceous compressions of coiled filaments with a diameter of up to 30 mm (*Grypania*) from the Negaunee Iron Formation, Michigan (node ⑤ in Fig. 4) (Han and Runnegar, 1992; but see Samuelsson and Butterfield, 2001, questioning the eukaryotic nature of these structures) and spheromorphic acritarchs (Zhang, 1986) and large compressed disks and filaments (Hofmann and Chen, 1981) from the ~1.8 to 1.9 Ga Chuanlinggou Formation, China. Molecular evidence diagnostic of eukaryotes comes from sterane biomarkers in bitumen of the ~1.64-Ga Barney Creek Formation (node ⑥ in Fig. 4) (Summons et al., 1988b), and the oldest fossils that are demonstrably eukaryotic come from shales of the 1.49 to 1.43 Ga Roper Group, McArthur Basin, Australia (Javaux et al., 2001, 2003). In comparison to older fossils, Roper protists have a higher diversity and exhibit the features of processes, wall ornamentation and probable excystment structures. The oldest eukaryotic fossils that can be assigned with confidence to an extant phylum are rhodophyte algae from the 1.26 to 0.95 Ga Hunting Formation, Somerset Island, Canada (node ⑦ in Fig. 4 (Butterfield et al., 1990). However, the lack of fossils with diagnostic eukaryotic morphology in rocks older than ~1.9 Ga is not evidence for the absence of Eucarya in the Archean. Eukaryote microfossils are usually preserved in shales, which in Archean terrains are almost always metamorphosed and deformed, limiting the likelihood of their survival even if they were already extant. Furthermore, modern eukaryotes occupying lower branches on the phylogenetic tree lack degradation-resistant structures, making it reasonable to assume that any eukaryotes in the Archean would also have had a low potential for preservation (Knoll, 1992). Archean Eucarya also might have inhabited environments unfavorable for fossilization. Further, the probability that the characteristic ultrastructures of eukaryotic cells would be preserved in metamorphosed Archean rocks is extremely low, in which case fossils of small ancestral eukaryotes would be difficult to distinguish from prokaryotes. Indeed, small body-size may have been a common phenomenon in Archean and early Paleoproterozoic eukaryotes, as small diffusion distances can potentially compensate for a limited oxygen supply before ~1.9 Ga. So, the absence of Archean microfossils with familiar eukaryotic morphology should not be unexpected and cannot be used to rule out the early appearance of eukaryotes. However, indirect evidence for the existence of eukaryotes before 2.5 Ga has been drawn from the global carbon isotopic excursion of kerogens in the late Archean (Han and Runnegar, 1992). As previously mentioned, the isotopically light kerogens are best explained by widespread methanogenic activity and incorporation of isotopically depleted methane-derived carbon into the sedimentary record (Hayes, 1983, 1994). Large-scale methane generation points to the existence of methanogenic Archaea. As Archaea and Eucarya evidently shared a common ancestor (Woese et al., 1990), this would suggest that stem group eukaryotes existed by the late Archean (Han and Runnegar, 1992; for an alternative view see Lake, 1988).

Accepting an Archean age for the bitumens studied here, the presence of steranes therein is the first direct evidence for the existence of eukaryotes so early in Earth history (node ④ in Fig. 4) (Brocks et al., 1999). Although a wide variety of sterane biomarkers are present, they do not provide any taxonomic information below domain level (see section 2.8.). Thus the phylogenetic position of the late Archean eukaryotes remains obscure. It is, for example, unclear whether late Archean Eucarya had acquired mitochondria and chloroplasts or even whether key attributes like a cytoskeleton or nucleus were present. However, one key characteristic, sterol biosynthesis, had evidently already developed.

3.4. Constraints on Late Archean Oxygen Levels

Atmospheric oxygen levels in the late Archean were much lower than at present (Kasting, 1993) and almost certainly $< 1\%$ PAL (present atmospheric level) (Rasmussen and Buick, 1999). However, the Archean bitumens contain evidence that the photic zone of the water column was at least weakly oxygenated:

1. The bitumens contain molecular fossils of bacteriohopanoids. Although hopanoid biosynthesis does not require oxygen, these lipids have never been isolated from strict anaerobes (Ourisson et al., 1987).
2. The Archean shales also contain high relative concentrations of cyanobacterial 2α-methylhopanes. These biomarkers are indirect evidence for oxygen release within the photic zone.
3. The side-chain degradation pattern of the 2α-methylhopane series (Fig. 5) indicates oxic conditions during earliest diagenesis of cyanobacterial organic matter.
4. The bitumens contain molecular fossils of sterols. Sterol biosynthesis in extant eukaryotes requires dissolved oxygen in concentrations equivalent to ~1% PAL (Jahnke and Klein, 1983).

The concentration of oxygen in Archean surface waters was thus probably high enough to allow aerobic respiration, as the Pasteur point at which aerobic respiration is favored over fermentation is ~1% PAL (Chapman and Schopf, 1983).

However, an O_2 level equivalent to ~1% PAL in the upper water column does not necessarily indicate an oxygenated atmosphere. Oxygen diffusing into the atmosphere would have been quickly scavenged by reduced biotic and volcanic gases, and oxygen mixed into the underlying anoxic water mass would have been consumed by reduced species of hydrothermal and detrital origin. But the loss of biogenic oxygen from surface waters to the atmosphere and to underlying anoxic water is a comparatively slow process. Depending on maximum rates of planktonic oxygen generation in the late Archean, up to 8% PAL oxygen might have accumulated locally in the photic zone in an otherwise anoxic environment (Kasting, 1992).

3.5. Reconstruction of a Late Archean Open Water Ecosystem

The late Archean sedimentary rocks studied in this work were mostly deposited in marine environments below storm wave base in oxygen-deficient water. The water column within the photic zone was evidently mildly oxygenated (~1% PAL) but oxygen concentrations in the atmosphere were probably well below this level. The photic zone was inhabited by oxygen-producing cyanobacteria, aerobic heterotrophic bacteria and eukaryotes of unknown physiology. The transition zone

between oxic and anoxic water was inhabited by microaerophilic heterotrophic bacteria, possibly including methanotrophs. Diagnostic biomarker evidence for organisms living under anoxic conditions was not obtained but the low ^{13}C contents of kerogen indirectly suggests activity of methanogenic Archaea.

Particulate organic matter was extensively reworked as it sank slowly through the water column. Because reworking conditions in the photic zone were oxidizing, straight-chain lipids from primary producers were extensively degraded and replaced by secondary isotopically enriched lipids, leading to an inverted carbon isotopic relationship between acyclic isoprenoids and *n*-alkanes. However, the presence of kerogen in the Archean rocks indicates that reduced organic carbon was evidently exported to the bottom sediment and, therefore, that excess oxygen was generated. The excess oxygen was subsequently consumed in sinks such as reduced volcanic gases, reduced hydrothermal metals and reduced terrigenous sedimentary detritus. Given the abundant occurrence of cyanobacterial biomarkers within banded iron formation, it appears that Fe oxidation constituted a substantial oxygen sink at this time.

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