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# Kerogen-bound glycerol dialkyl tetraether lipids released by hydropyrolysis of marine sediments: A bias against incorporation of sedimentary organisms?

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ABSTRACT

Over the past 10 years, the membrane lipids of archaea, particular glycerol dialkyl glycerol tetraethers (GDGTs) have been recognised as significant components of marine sediments and as potentially powerful tools in palaeoclimate reconstruction. However, relatively little is known about their preservation and, in particular, their incorporation into macromolecular organic matter fractions. Here, we examine abundances, distributions and stable carbon isotope ratio ($\delta^{13}C$) values of GDGTs and the diether archaeol in sediments from the Benguela Upwelling Region [Ocean Drilling Program (ODP) Site 1084] and a Mediterranean sapropel (S5). These are compared with distributions of biphytanes released from the kerogen (solvent-extracted residue) fraction using hydropyrolysis (Hypy). GDGTs, likely derived from pelagic crenarchaeota, are amongst the most abundant extractable components in both Benguela and S5 sediments, but are an order of magnitude more abundant in the former. Moreover, the Benguela sediments have far greater concentrations of the acyclic GDGT than expected; consistent with the presence of methane in core 1084, co-occurring archaeol and variable GDGT $\delta^{13}C$ values, the excess acyclic GDGT probably arises from a contribution from sedimentary methanogens. Hypy-released biphytanes have similar abundances in Benguela and sapropel sediments, and represent a significant proportion of the total tetraether lipid pool at the latter site; however, the distributions of Hypy-released biphytanes, especially in Benguela sediments, do not always match those of the free GDGTs. At both sites, Hypy-released biphytane $\delta^{13}C$ values are relatively invariant, consistent with a single source, again likely to be pelagic crenarchaeota. These observations suggest that GDGTs can be incorporated into sedimentary geomacromolecules relatively rapidly, with those derived from pelagic crenarchaeota more likely to be incorporated than those derived from sedimentary methanogens. It is likely that this bias arises from a lack of reactive substrates, i.e. oxygen radicals, in deeper anoxic sediments, consistent with an important role for oxidative cross linking in kerogen formation.

## 1. Introduction

Archaeal lipids are common in marine sediments, with the glycerol dialkyl diether lipid, archaeol, being widespread (e.g. Boot et al., 2006; van Dongen et al., 2006; Parkes et al., 2007) and glycerol dialkyl tetraether lipids

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(GDGTs) being apparently ubiquitous (e.g. Schouten et al., 2000). GDGTs tend to be the most abundant archaeal lipids in marine sediments and waters, with the largest contribution from either crenarchaeol or the acyclic glycerol dialkyl glycerol tetraether; other GDGTs tend to be present in much lower abundance (e.g. Schouten et al., 2000). Similarly, in the analysis of tetraether lipids as biphytanes generated by chemical cleavage of the ether bonds, the acyclic biphytane is normally the most abundant biphytane; monocyclic biphytane is the least abundant and there are typically approximately equal amounts of bicyclic and tricyclic biphytanes (e.g. King et al., 1998; DeLong et al., 1998; Schouten et al., 1998; Pearson et al., 2001).

Sedimentary archaeal lipids can derive from either archaea living in the water column or those living within the sediment. It now seems clear that the preponderance of GDGTs derive from the ubiquitous pelagic crenarchaeota (Hoefs et al., 1997; DeLong et al., 1998; Schouten et al., 2000; Sinninghe Damsté et al., 2002). In contrast, archaeol in marine sediments has been attributed to either anaerobic methanotrophs (Parkes et al., 2007) analogous to those found at cold seeps (e.g. Hinrichs et al., 1999; Pancost et al., 2001b) or methanogens (e.g. Boot et al., 2006). Potentially, archaeal lipids can be used as tracers for changes in past microbial communities (e.g. Kuypers et al., 2001), in a manner analogous to how methylhopanes have been used to study past changes in cyanobacterial populations (Brocks et al., 1999; Kuypers et al., 2004; Xie et al., 2005) and methanotrophic bacterial activity (Brocks et al., 2003). Perhaps even more useful is the application of GDGT distributions as a tool for studying past sea surface temperatures ($TEX_{86}$ ratio values; Schouten et al., 2002). Indeed, the $TEX_{86}$ SST (sea surface temperature) proxy has already been applied to sediments ranging in age from the Cretaceous (e.g. Jenkyns et al., 2004) to the Holocene (Huguet et al., 2006), making important contributions to palaeoceanographic investigations.

Despite the considerable interest in these compounds, there have been only a few studies providing insight into their preservation and incorporation into macromolecular sedimentary organic matter (Michaelis and Albrecht, 1979; Schouten et al., 2004; Kuypers et al, 2002). In the marine environment, diagenetic reactions between lipids and other compounds or inorganic species (e.g. $HS^-$ or free radicals) convert organic constituents into highly cross-linked, insoluble geopolymers (e.g. de Leeuw et al., 2006). The transformation is initially mediated by microbial activity in shallow sediments, with functional groups remaining on the residual OM reacting to form the polycondensed structures. Ultimately, insolublisation of the polycondensed material occurs, eliminating the hydrophilic functional groups and rendering the OM progressively more and more insoluble (Tissot and Welte, 1984; de Leeuw et al., 2006). Through these processes, much of the organic biomarker pool becomes chemically bound as macromolecular material and is not released by standard solvent-extraction procedures. Critically, the work of Michaelis and Albrecht (1979), Schouten et al. (2004) and Kuypers et al. (2002) has shown that pelagic archaeal lipids, including GDGTs, are not immune to this process and can be abundant components of sedimentary macromolecules.

Because solvent-extractable biomarkers are not necessarily representative of the total biomarker pool, especially in immature sediments, complete characterisation requires that biomarkers be chemically or thermally cleaved from the macromolecular material, using a method that results in minimal molecular alteration. Hydropyrolysis (Hypy) is a method that enables the release of covalently-bound biomarkers, in intact form with minimal alteration of structure or stereochemistry, from macromolecular organic material (Love et al., 1997; Maroto-Valer et al., 1997). It has been used to study the macromolecular structure of kerogen in petroleum source rocks (Maroto-Valer et al., 1997; Love et al., 1998) and to release kerogen-bound biomarker alkanes, particularly hopanes and steranes, for oil-source correlation purposes (Murray et al., 1998). Some investigations using the technique have shown that there are differences in the yields and distributions of biomarkers between the solvent-extractable and kerogen-bound fractions. For example, in the Göynük oil shale, $C_{29}$ to $C_{33}$ hopanes are between 3 and 10 times more abundant in the Hypy-released fraction than in the solvent-extractable fraction and retain the biologically inherited, thermodynamically unstable 17β,21β(H) configuration (Love et al., 1995, 1997); moreover, $C_{33}$ to $C_{35}$ hopanes were only detected in the Hypy-released fractions. The method has also been applied to more recent sediments, releasing hopanes from Priest Pot lake and Framvaren Fjord sediments, with the bound hopanoids representing 22–30% and 50–86%, respectively, of the total hopanoids (Farrimond et al., 2003).

However, it has not been applied to the investigation of macromolecularly-bound archaeal lipids. To explore how the abundances, distributions and carbon isotopic compositions of archaeal lipids differ between the bound and extractable fractions in marine sediments, we have performed Hypy on sediments from the Benguela upwelling system (ODP Site 1084) and the Mediterranean sapropel S5. These data are used to interpret potential sources of bound vs. free GDGTs, evaluate mechanisms of biomarker incorporation into kerogen and to explore the implications of such conclusions for the use of GDGTs in palaeoenvironmental investigations.

## 2. Sample sites

### 2.1. Benguela upwelling system

The Angola-Benguela current system is one of the world's five largest upwelling regions. The Benguela upwelling system (BUS) extends over the western margins of Congo, Angola, Namibia and South Africa, with upwelling being centred on the inner shelf and at the shelf edge. The upwelling forms filaments of cold, nutrient-rich water between the BCC and the Benguela oceanic current (BOC) that have been detected up to 600 km offshore. Remnants of these filaments form a zone of intermediate productivity via mixing with the low productivity ocean water (Lutjeharms and Stockton, 1987). The Benguela coastal upwelling is strongly seasonal (Shannon and Nelson, 1996), but seasonal effects differ between the northern Benguela region

(NBR) and the southern Benguela region (SBR), separated by the Lüderitz boundary (the site of maximum upwelling intensity; 26° to 27°S). Site 1084 (Leg 175) lies within the NBR, where a high rate of primary productivity is persistent (> 180 gC/m$^2$/yr; Wefer et al., 1998) and accumulation rates of phytoplankton-derived organic matter in the underlying sediments are high (Brown et al., 1991). Maximum total organic carbon (TOC) concentrations occur in the sediments of the inner shelf belt of diatomaceous ooze off Walvis Bay (Bremner, 1983; Rogers and Bremner, 1991), but the sediments from Core 1084A are still among the most organic-rich collected by the ODP (occasionally > 15% TOC; Shipboard Scientific Party, 1998b). Deeper sediments are characterised by well-expressed cycles of organic carbon (as well as diatoms and coccoliths) via cyclic variation in primary production (Little et al., 1997). Twelve samples from Site 1084 [Northern Cape Basin, off Lüderitz Bay (25°30.8345′S, 13°1.6668′E) were studied. These span two of the high and low productivity cycles, coming from two of the conspicuous decimetre thick intervals of dark, organic-rich, carbonate-poor, clay layers: five sediments from 123 to 127 m below sea floor (mbsf; 0.74–0.76 My: 14H5 65–67 cm and 142–144 cm; 14H6 99–101 cm; 14H7 9–11 and 34–36 cm) and seven from 211 to 219 mbsf (1.09–1.16 My: 25X1 85–87 cm; 25X3 84–86 cm; 25X4 20–22 and 125–127 cm; 25X5 20–22 cm; 25X6 20–22 and 85–87 cm), with TOC content ranging from 6% to 17% (Fig. 2; Robinson et al., 2002).

The organic matter in these Benguela sediments is mostly derived from marine organisms, with only minor contributions from terrestrial organic matter. Thus, pelagic crenarchaeota are likely sources of sedimentary GDGTs. However, there is also evidence for $CH_4$ cycling in Leg 175 cores. When the cores were collected, there were relatively high concentrations of headspace $CH_4$, $H_2S$ and $CO_2$ in the sub-bottom sediments of the majority of the Leg 175 boreholes. The high $CH_4/C_2H_6$ ($C_1/C_2$) values and absence of high molecular weight hydrocarbon gases indicate a biogenic origin for the $CH_4$ (Shipboard Scientific Party, 1998a,b). This likely occurs at shallow depth; within 5 m of the sediment-water interface, sulfate is completely consumed and $CH_4$ and $CO_2$ concentrations rapidly increase to high values, in the region of $10^5$ ppmv (Niewöhner et al., 1998; Shipboard Scientific Party, 1998b).

### 2.2. Mediterranean sapropel S5

The Mediterranean Sea contains a series of sapropelic sediments deposited during periods of elevated organic matter accumulation (Shipboard Scientific Party, 1996). They are generally TOC-rich (up to 30%), carbonate-poor (< 20%) and sulfur/pyrite-rich (up to 18% S; Struck et al., 2001; Bouloubassi et al., 1999). Sapropel S5 is an organic-rich, dark brown to black sediment laid down during the Eemian interglacial period (123–128 kya), marine isotope stage 5e (Verynaud-Grizzini et al., 1977; Shipboard Scientific Party, 1996). The samples are from borehole KS205 situated off the southern tip of Italy (38°11.86′N, 18°08.04′E) and collected as part of Vicomed. At this location, the sapropelic S5 sediments are situated between 4.82 and 5.06 mbsf and accumulated at an estimated rate of 4.7 cm/kyr (Rohling et al., 2002). TOC content ranges from 0.2% to 0.3% outside of the sapropel ('background' values) but from 1.5% to 6.2% within the sapropel, which is intercalated with organic-poor, yellowish brown to light grey foraminifera-rich nanofossil ooze.

## 3. Methods

### 3.1. Extraction and fractionation

Ca. 1.5 g of sediment was freeze dried and extracted (24 h) using a Soxhlet apparatus [2:1 dichloromethane (DCM):MeOH]. Varian or Phenomenex aminopropyl ($NH_2$) Bond Elut solid phase extraction columns (100 mg sorbent bed) were used to separate the total lipid extract into neutral, acid and polar/phospholipid fractions. The columns were conditioned with MeOH and 2:1 DCM:iso propanol, immediately prior to use. An aliquot (5 mg) of the extract was loaded onto the column in a small volume of 2:1 DCM:iso propanol and eluted sequentially using 12 ml of 2:1 DCM:iso propanol (neutrals), 12 ml of 2% (v/v) acetic acid in diethyl ether (acids), and 12 ml of methanol (polars). Subsequently, 100 μg of 1,2-di-*O*-hexadecyl-rac-glycerol were added as internal standard and the neutral fraction was separated into apolar (9:1 hexane:DCM) and polar (2:1 MeOH:DCM) fractions using flash column chromatography (0.75 g extracted alumina).

In some cases, urea adduction was used to separate straight chain hydrocarbons from branched and cyclic components. An aliquot was transferred to a 4 ml vial, to which 200 μl each of hexane, acetone and urea (10% in MeOH) were added. The sample was placed in a freezer for 30 min, until urea adduct crystals formed. Solvent was removed under a stream of $N_2$ and the urea extracted (×3) with *n*-hexane (branched/cyclic fraction). The crystals were then dissolved in 500 μl MeOH and 500 μl water and the adducted *n*-alkanes extracted (×3) with hexane. The adduction procedure was repeated on the adduct fraction twice more to ensure all non-adduct material was removed.

### 3.2. Ether bond cleavage

For subsequent carbon isotopic analysis, the neutral polar fraction was subjected to chemical cleavage with HI to release archaeal biphytanes from tetraether lipids. An aliquot in the minimum volume of MeOH was refluxed (120 °C, 4 h) in 2 ml HI. The mixture was extracted with hexane (3–4 ml) to obtain the alkyl iodides, with the subsequent fraction cleaned of HI by adding 3 ml $Na_2S_2O_3$ solution (5%). The sample was separated into polar and apolar fractions using an alumina column as described above. The alkyl iodides in the apolar fraction were reduced using $LiAlH_4$; the sample was dissolved in 3 ml 1,4-dioxane and 0.05 g $LiAlH_4$ added. The mixture was refluxed (120 °C, 1 h) and the $LiAlH_4$ quenched with 2 ml ethyl acetate. Water (5 ml) was added and the resulting solution extracted (×3) with DCM.

### 3.3. Hydropyrolysis (Hypy)

Hypy is pyrolysis assisted by high $H_2$ pressure, with a dispersed sulfided Mo catalyst (Love et al., 1995, 1997). It is an open-system method, in which a continuous flow of $H_2$ sweep gas removes products from the reactor bed, ensuring that generated volatile products have short residence time, in the order of seconds, in the heated reactor zone. A temperature programme controls reactor heating, so that the bound biomarkers are cleaved at the lowest possible reaction temperature (which depends on how strongly bound they are to the host matrix), thereby ensuring that the Hypy products are highly representative of actual bound biomarker assemblages. Resistive heating is achieved by passing a high electrical current between two electrical contacts on the reactor tube, providing a uniform temperature along the Hypy zone of the reactor tube. The temperature is monitored with a thermocouple in the sample bed. High pressure $H_2$ and a sulfided Mo catalyst serve as the reaction medium. The pyrolysate collects in a dry ice-cooled, silica-filled trap.

The samples were loaded with the catalyst [$(NH_4)_2MoO_2S_2$)], which rapidly decomposes to catalytically active oxysulfide Mo species at about 200 °C as the reactor heats up (Snape et al., 1989), being reduced further to $MoS_2$ at about 400 °C (Love et al., 1997). The catalyst was washed in DCM and *n*-hexane to remove any trace impurities and dissolved in a few ml aqueous MeOH by heating at 40–50 °C (5 min). Samples were impregnated with the aqueous MeOH solution of the catalyst to give a nominal molybdenum loading of 3 wt.% (i.e. catalyst weight = 0.03 × total sediment weight). The sediment, catalyst and 20–30 ml aqueous MeOH were added to a round-bottomed flask and agitated for 30 min. The aqueous MeOH solution was rotary evaporated, before freeze-drying the sediment and scraping it into a vial. Due to the fine nature of the solvent-extracted sediment residue, it was necessary to mix the sediment sample with an equal amount of coarse silica to create voids/adequate porosity and to prevent plugging of the reactor during HyPy.

The samples were pyrolysed with resistive heating from 50 to 250 °C at 300 °C $min^{-1}$ and from 250 to 500 °C at 8 °C $min^{-1}$, under a $H_2$ pressure of 15 MPa. A $H_2$ sweep gas flow of 5 l $min^{-1}$, measured at ambient temperature and pressure, ensured that the products were quickly removed from the reactor vessel. The pyrolysate was absorbed on to a column of silica held in a trap constructed from 0.635 cm 316 stainless steel (0.457 cm i.d.), designed to hold ca. 0.75 g silica (Meredith et al., 2004). To ensure that there was no contamination of the products, the silica used for trapping (35–70 mesh) was pre-extracted in a Soxhlet apparatus with *n*-hexane (24 h) and DCM:MeOH (93:7 v/v; 48 h) and heated in a muffle furnace at (600 °C 4 h). After Hypy, the pyrolysate was extracted from the trapping silica using a 3:1 azeotrope of DCM:MeOH.

### 3.4. Analysis

All gas chromatography (GC) based techniques were performed after appropriate derivatization of polar components. GC was performed with a Carlo Erba HRGC 5300 Mega Series instrument fitted with flame ionization detection (FID) and a fused silica column (50 m × 0.32 mm i.d.) coated with CP Sil-5CB (dimethylpolysiloxane equivalent) phase (0.12 µm film). Samples were injected on-column at 70 °C and eluted using $H_2$ as carrier gas; the temperature programme was: 70 °C (1 min) to 130 °C at 20 °C $min^{-1}$, 130–320 °C at 4 °C $min^{-1}$ (hold 20 min). For GDGT characterization and quantification, we adapted the high temperature gas chromatography (HT-GC) methodology of Nichols et al. (1993); this approach has yielded robust results for GDGT analysis of peat, where HT-GC and high performance liquid chromatography–mass spectrometry (LC–MS) gave similar results (Weijers et al., 2006a). As with standard GC analysis, neutral polar samples are derivatised with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Aliquots in ethyl acetate were analysed using a Hewlett Packard 5890 Series II gas chromatograph, equipped with FID, and using $H_2$ as carrier gas at a head pressure of 1.5 psi. An SGE HT5 (5% phenyl equivalent, polycarborane siloxane), 6 m × 0.53 mm i.d., aluminium clad column was used with the following temperature programme: 50 °C (1 min) to 140 °C at 20 °C/min and 140–420 °C (10 min) at 7 °C/min. The HT-GC system could not be coupled to a mass spectrometer due to the high flow rates; compounds were identified on the basis of comparison with a suite of samples with known GDGT distributions, as determined from LC–MS analysis (e.g. Hopmans et al., 2000).

GC–mass spectrometry (GC–MS) was performed using a Thermoquest Finnigan TRACE chromatograph interfaced to a Thermoquest Finnigan TRACE spectrometer operated with electron ionization at 70 eV and scanning a range of *m/z* 50–650 with a cycle time of 1.7 scans $s^{-1}$. The interface was set to 300 °C and the ion source at 200 °C. GC conditions were as for GC analysis, except that He was the carrier gas.

Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) was conducted using a Thermoquest Finnigan TRACE chromatograph connected to a Thermoquest Finnigan Delta PLUS XP spectrometer, or a Hewlett Packard 6890 chromatograph connected to a Thermoquest Finnigan Delta plus XL spectrometer, via a GC III combustion interface (comprising Cu, Pt and Ni wires within a fused alumina reactor at a constant temperature of 940 °C). GC conditions were as for GC and GC–MS. Duplicate or triplicate analyses were conducted for each sample, with values are reported in standard delta (‰) notation relative to Vienna Pee Dee Bee Belemnite (V-PDB); errors are < ±0.5‰. For derivatized samples, i.e. alcohols as trimethylsilyl ethers, standard mass balance correction procedures were used.

## 4. Results

### 4.1. The Benguela upwelling system

#### 4.1.1. Extractable lipids

The neutral fraction of all ODP Site 1084 sediments contains a range of algal, bacterial and archaeal biomarkers with subordinate abundances of higher plant biomarkers (Bouloubassi, Meyers and Pancost, unpublished data).

Phytoplankton biomarkers include phytol, cholesterol (mixed sources; Volkman, 1986), dinosterol (dinoflagellates; Withers, 1983), $C_{37}$, $C_{38}$ and a minor amount of $C_{39}$, alkenones (haptophyte algae; Marlowe et al., 1984), and $C_{32}$ 1,16-diol, $C_{30}$ 1,15-diol, $C_{28}$ 1,14-diol and $C_{26}$ 1,12 and 1,13-diols (from eustigmatophyte algae and diatoms; Volkman et al., 1999; Sinninghe Damsté et al., 2003). The algal lipids all have similar and relatively invariant carbon isotopic compositions. The $\delta^{13}C$ values of $C_{37:2}$ alkenone, cholesterol and dinosterol all are ca. −22‰. Probable sedimentary bacterial biomarkers include glycerol dipentadecyl diethers, which have been attributed to sulfate reducing bacteria (SRB; Pancost et al., 2001a). Compared to photoautotroph lipids, diethers of inferred SRB origin are relatively depleted in $^{13}C$, with an average $\delta^{13}C$ value of −32.6‰ (although values of −40‰ were obtained for 2.5 Ma sediments from Site 1083).

Also present are homologous series of *n*-alkanols, ranging from $C_{16}$ to $C_{32}$ with an even/odd predominance. These co-eluted with other compounds in the neutral lipid fraction; consequently, their carbon isotopic compositions were determined as *n*-alkanes, after conversion from alcohols during HI treatment. The $C_{28}$ and $C_{30}$ *n*-alkanols are depleted in $^{13}C$, consistent with a terrestrial higher plant origin (despite potential contributions from diols).

To characterise archaeal lipids, neutral lipid fractions were analysed using HT-GC (Fig. 1) and GC–MS. HT-GC analysis revealed that archaeal GDGT abundances are high, up to 98 μg/g sediment, and that these tetraether lipids are of comparable or greater abundances than the algal and terrestrial biomarkers. The acyclic GDGT concentrations range from 1.3 to 55 μg/g dry sediment; the 0:1 GDGT ranges from 0.1 to 8.3 μg/g dry sediment, the 1:1 GDGT from 0.05 to 2.5 μg/g dry sediment and crenarchaeol from 0.8 to 32 μg/g dry sediment. In all of the Benguela samples, the acyclic GDGT and crenarchaeol (2:3 GDGT) are the most and second most abundant lipids, respectively. Site 1084 sediments also contain archaeol (0.6–12.0 μg/g sediment), the most common archaeal diether, and acyclic to tricyclic biphytane diols. Soil bacterial GDGTs, characterised by methyl-branched alkyl rather than biphytanyl components (e.g. Hopmans et al., 2004; Weijers et al. 2006a), were not detected, consistent with the lack of significant terrigenous inputs.

The Site 1084 samples were subjected to HI cleavage and $LiAlH_4$ reduction to release the biphytane moieties from the tetraether structures, allowing GC-C-IRMS analysis. The average $\delta^{13}C$ values for the tricyclic and the acyclic biphytane are −21‰ and −23‰, respectively. Archaeal tricyclic biphytane $\delta^{13}C$ values exhibit little variation, ranging from −19.7‰ to −21.7‰, whereas acyclic biphytane $\delta^{13}C$ values range from −21‰ to −26‰ (Fig. 2).

### 4.1.2. Hypy products

The solvent-extracted sediments were subjected to Hypy, with the released hydrocarbons being separated using urea adduction into straight chain and branched/cyclic hydrocarbons. Under the optimised HyPy conditions used here, high conversions (typically >85 wt.% as soluble tar plus volatiles) of pre-oil window type I–III kerogens

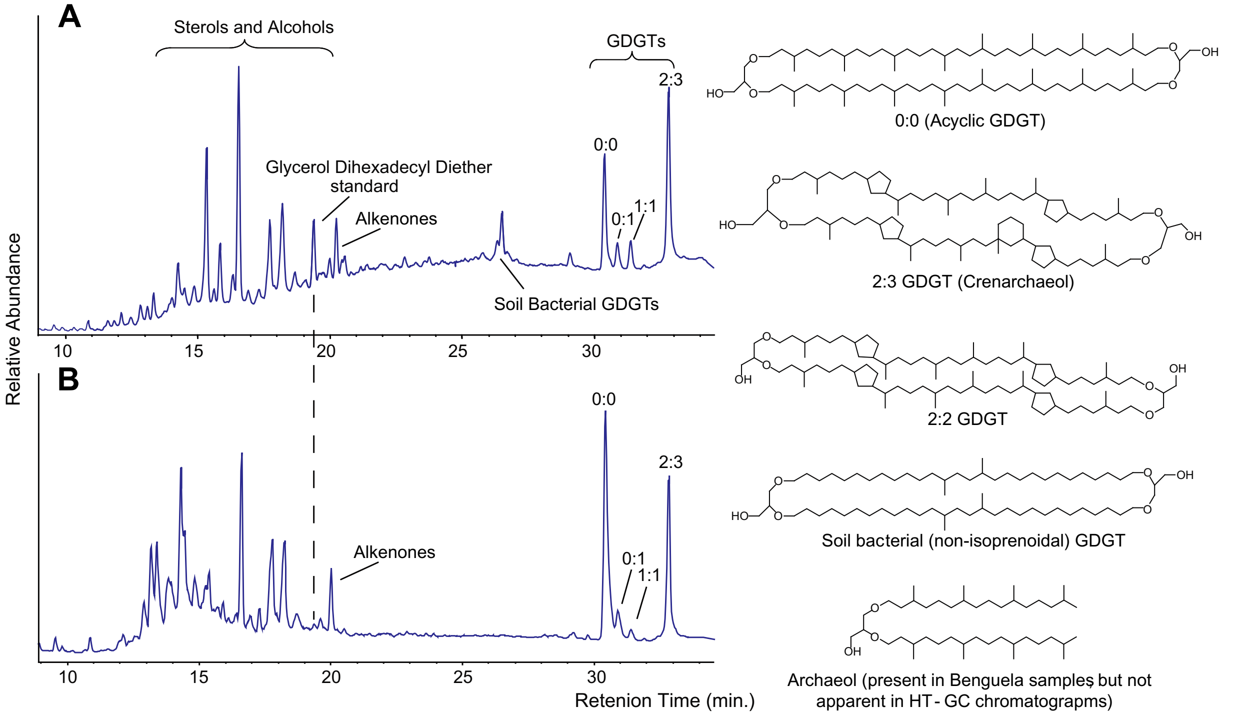

**Fig. 1.** Partial high-temperature gas chromatogram of typical (A) S5 sapropel and (B) Benguela (Site 1084) sediments, showing relatively high abundances of GDGTs. The numbers above the GDGTs refer to the number of cyclic moieties in the two biphytanyl components (i.e. the two biphytanyl components of the 2:3 GDGT have 2 and 3 rings).

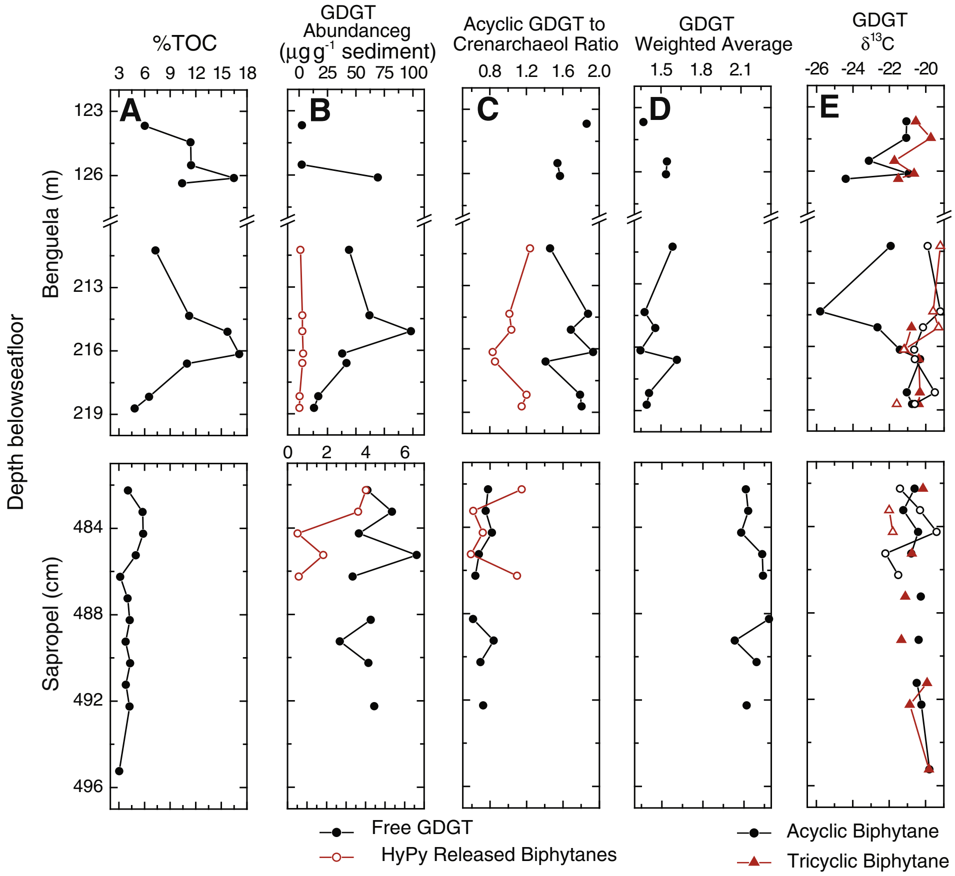

**Fig. 2.** Geochemical data and GDGT abundance, distribution and $\delta^{13}C$ parameters as depth profiles through Benguela Site 1084 and sapropel S5 sediments. In panel C, the acyclic GDGT to Crenarchaeol ratio in the Hypy-released component is the ratio of acyclic biphytane to the summed abundance of bicyclic and tricyclic biphytane. In panel E, $\delta^{13}C$ values of free GDGT components (determined after HI cleavage) are solid symbols, whereas those of Hypy-released components are shown as open symbols.

are routinely achieved (Love et al., 1995, 1997, 1998), with a significant proportion of the products (typically >60 wt.%) recoverable as DCM-soluble tar. It is highly unlikely then that a significant proportion of the hydrocarbons released by Hypy were simply derived from reductive conversion of any residual free lipids that escaped solvent extraction, since these would have been swamped by the much larger pool of hydrocarbon skeletons released from fragmentation of the kerogen. The straight chain hydrocarbons are dominated by a homologous series of *n*-alkanes ranging from $C_{18}$ to $C_{35}$ (data not shown). In general, there is a subtle even/odd predominance, with $C_{22}$ and $C_{24}$ dominant, and each *n*-alkane peak preceded by a corresponding minor *n*-alk-1-ene peak. A variety of steranes, sterenes and hopanes are present in the branched/cyclic hydrocarbon fraction, but the fraction is dominated by a large unresolved complex mixture (UCM; Fig. 3). Cholestane, cholestene, 24-methylcholestane, 24-ethylcholestane and 24-ethylcholestene are the most abundant of the steroids, but are still present in very low abundance. The hopanes are dominated by the $C_{29}$ to $C_{35}$ 17βH,21β isomers, followed by the $C_{30}$ to $C_{35}$ βα-hopanes. As with the steranes and sterenes, even the most abundant hopanes occur as only very small peaks amongst the total alkane products.

A range of biphytanes (acyclic, monocyclic, bicyclic and tricyclic) was also released by Hypy of the extracted residues. The acyclic biphytane is the most abundant of the biphytanes (0.24–1.55 µg/g dry sediment); tricyclic biphytane and bicyclic biphytane are the next most abundant, with the two compounds having similar abundances ranging from 0.10 to 0.96 µg/g dry sediment; monocyclic biphytane is consistently the least abundant biphytane, with concentration between 0.03 and 0.18 µg/g dry sediment. Not only are these abundances much greater than those of hopanes and steranes, they are comparable to those of pristane and phytane (and their unsaturated equivalents) and the *n*-alkanes. This predominance of archaeal lipids relative to lipids from other sources, and especially *n*-alkanes, is an unusual characteristic of Hypy analysis. All of the Hypy-released biphytanes have similar stable carbon isotopic composition, with total downcore variation being less than 3‰ (−19.1‰ to −21.7‰); in each sample, the acyclic and tricyclic $\delta^{13}C$ values are the same within error margins.

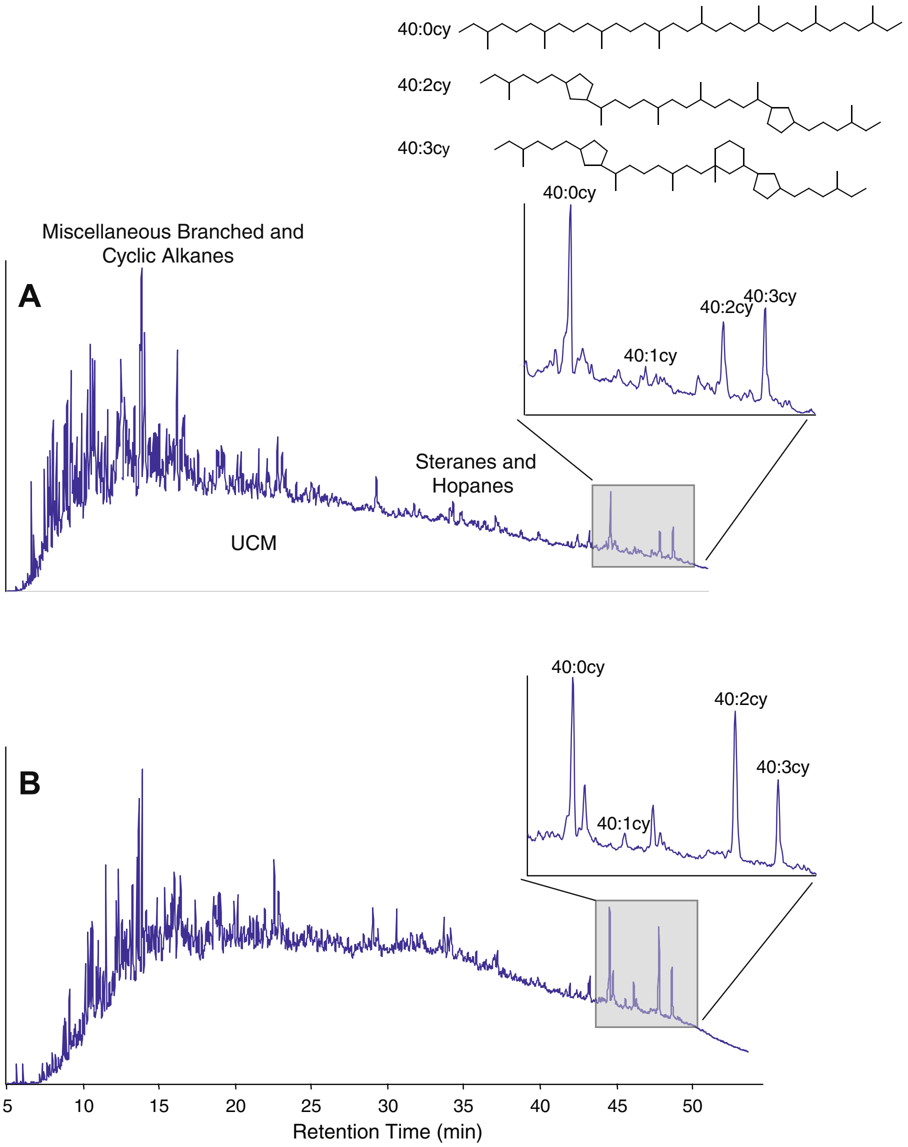

**Fig. 3.** Partial gas chromatograms showing Hypy-released hydrocarbons (urea non-adduct) released from typical (A) Benguela (Site 1084) and (B) S5 sapropel sediments.

## 4.2. Mediterranean sapropel S5

### 4.2.1. Extractable lipids

There have been a number of biomarker studies of sapropel sediments, including sapropel S5. These have shown that the organic matter is dominantly derived from marine sources, with terrestrial sources being more significant before and after sapropel events (Sutherland et al., 1984; Bouloubassi et al., 1999). The algal biomarkers in the KS205 S5 sediments include $C_{27}$ to $C_{30}$ sterols, $C_{28}$, $C_{30}$ and $C_{32}$ mid-chain diols and ketols, and long chain alkenones. *Iso* and *anteiso* fatty acids and β-hydroxy fatty acids represent the bacterial contribution. The relatively minor terrestrial signal includes higher plant wax derived *n*-alkanes and *n*-alcohols (Bouloubassi et al., 1999) but also non-isoprenoidal GDGTs derived from soil bacteria (Hopmans et al., 2004).

Archaeal GDGTs dominate the sapropel S5 sediment biomarker suite. Crenarchaeol is the most abundant lipid in the neutral fraction, ranging from 1.1 to 3.2 μg/g dry sediment, followed by the acyclic GDGT, which ranges in abundance from 0.9 to 2.2 μg/g dry sediment (Fig. 2). As in the Benguela sediments, other GDGTs are present but in much lower concentration, with the 1:1 GDGT being slightly more abundant (0.2–0.5 μg/g dry sediment) than the 0:1 GDGT. Archaeol is present in only trace abundances in the neutral polar fractions, but non-isoprenoidal soil bacterial GDGTs were observed (Fig 1A). For carbon isotopic determination, HI cleavage and $LiAlH_4$ reduction were used to cleave biphytanes from the archaeal tetraether lipids; all of the generated biphytanes had $\delta^{13}C$ values of ca. −20.5‰.

### 4.2.2. Hypy products

Five post-extraction sediment residues from sapropel S5 were subjected to Hypy and separated using urea adduction into straight chain and branched/cyclic fractions. The aliphatic hydrocarbon fraction contains numerous *n*-alkanes, with the $C_{17}$ to $C_{36}$ homologues predominant, but with no even/odd or odd/even predominance. A minor *n*-alk-1-ene peak precedes each *n*-alkane peak. The branched/cyclic hydrocarbon fraction contains steranes, sterenes, hopanes and numerous small aromatic compounds, but is dominated by a large UCM. The steroidal compounds include cholestane, cholestene, 24-methylcholestane, 24-ethylcholestane and 24-ethylcholestene, but these are minor. The hopanes are also present in very low abundance and include the $C_{27}$ and $C_{29}$ to $C_{35}$ ββ-hopanes, $C_{31}$ to $C_{35}$ βα-hopanes and $C_{31}$ to $C_{33}$ αβ-hopanes, with the $C_{31}$ to $C_{35}$ ββ-hopanes being predominant.

The most abundant Hypy biphytanes are the acyclic and bicyclic biphytanes (0.19–1.9 μg/g and 0.15–1.3 μg/g dry sediment, respectively). In all sediments, acyclic and bicyclic biphytanes are present in similar abundances. The tricyclic biphytane is the next most abundant of the biphytanes (0.08–0.72 μg/g dry sediment) and is always approximately half the concentration of the bicyclic biphytane. The monocyclic biphytane is the least abundant biphytane released by HyPy (0.08–0.42 μg/g dry sediment). Biphytane $\delta^{13}C$ values range from −19.4‰ to −22.4‰, ±1‰ (Fig. 2), with all biphytanes having similar values. Despite the presence of soil bacterial GDGTs in the free fractions, methyl-branched alkanes were not detected in the Hypy-released fractions.

## 5. Discussion

### 5.1. Predominance of archaeal biomarkers in Benguela and Sapropel sediments

Archaeal GDGTs are the most abundant biomarkers in the neutral lipid fractions in both the sapropel S5 and (especially) the Benguela sediments, despite the high production and preservation of algal biomarkers in these settings. Archaeal lipids are more abundant than algal and bacterial lipids in other marine sediments, such as Lido Rossello and Indian Ocean sediments (Hoefs et al., 1997; Schouten et al., 1998); in other sediments, such as the Monterey Formation, they vary substantially in abundance relative to algal lipids (Hoefs et al., 1997; Schouten et al., 1998).

Archaeal GDGTs are more abundant in the Benguela sediments (up to 54.5 μg/g dry sediment) than in the sapropel sediments (up to 3.2 μg/g dry sediment). This enhanced abundance of archaeal lipids in the Benguela sediments could be related to the elevated TOC content; however, even when TOC content is taken into account, archaeal tetraethers are still more abundant in the Benguela sediments, with TOC-normalised abundances being an order of magnitude greater than in the sapropel sediments (up to 0.4 mg/g TOC in Benguela sediments and 0.06 mg/g TOC in sapropel S5 sediments). This suggests that the archaeal biomarker input was greater in the Benguela upwelling system, perhaps indicating that either (i) archaeally-mediated nitrification (e.g. Wuchter et al., 2006) is an important component of nutrient cycling there or (ii) the higher productivity in the Benguela region fuels an archaeal community that is partially sustained by a heterotrophic ecology (e.g. Ingalls et al., 2006).

The Benguela and sapropel sediments also exhibit different GDGT distributions. In the ODP Site 1084 sediments, the acyclic GDGT is far more abundant than crenarchaeal, but the opposite is observed for the S5 sediments. The excess acyclic GDGT in the Benguela sediments could reflect colder temperatures at that site. Schouten et al. (2002) showed that the weighted average number of cyclopentyl moieties in GDGTs is related to sea surface temperature (SST), with more cyclopentyl moieties in the membrane lipids of crenarchaeota growing in warmer waters. As our study focusses on ancient sediments, we cannot compare our GDGT distributions to directly measured SSTs but we can compare them to SSTs generated by the alkenone-based $U_{37}^{K'}$ index (e.g. Brassell et al., 1986; Prahl and Wakeham, 1987). Such SSTs range from 15 to 22 °C for the BUS sediments and from 21 to 24 °C for the sapropel sediments. However, the lower BUS temperatures are not low enough to account for the observed high acyclic GDGT concentrations (Fig. 4A).

Alternatively, differences in distributions could reflect different sources of archaeal lipids in the sediments. For example, there could be additional contributions of the acyclic GDGT from either soils (Weijers et al., 2006b) or from pelagic euryarchaeota (Turich et al., 2007). However, neither explanation is satisfactory. The low abundance of terrigenous biomarkers in general and soil bacterial GDGTS in particular indicates that terrigenous inputs were low; and upwelling regions are thought to be characterised by relatively low rather than higher abundances of euryarchaeota compared to epipelagic settings (Turich et al., 2007).

Instead, it is likely that the excess acyclic GDGT in Bengulea sediments derives from methanogens (e.g. Koga et al., 1993); in fact, for this reason, Schouten et al. (2002) argued that the aforementioned weighted average of GDGT cyclopentyl moieties should not be used as a SST proxy and instead proposed the $TEX_{86}$ index, which excludes the acyclic GDGT. As discussed in the site descriptions, there is abundant evidence for methanogenesis in all of the Leg 175 BUS cores, including Site 1084. Moreover, archaeol, an archael lipid that has not been found in crenarchaeota, but which could derive from sedimentary methanogens, is abundant in Site 1084 sediments. Further evidence for a partial methanogen source for the acyclic GDGT comes from its stable carbon isotopic composition. In general, archaeal biphytane $\delta^{13}C$ values – either generated from GDGTs via $HI/LiAlH_4$ or from the solvent-extracted residue with Hypy – are similar, varying only from −19‰ to −22‰ across all samples and sites. These relatively high and invariant values are similar to those measured by previous workers (Hoefs et al., 1997; Schouten et al., 1998; Kuypers et al., 2001) and are consistent with evidence that pelagic crenarchaeota directly

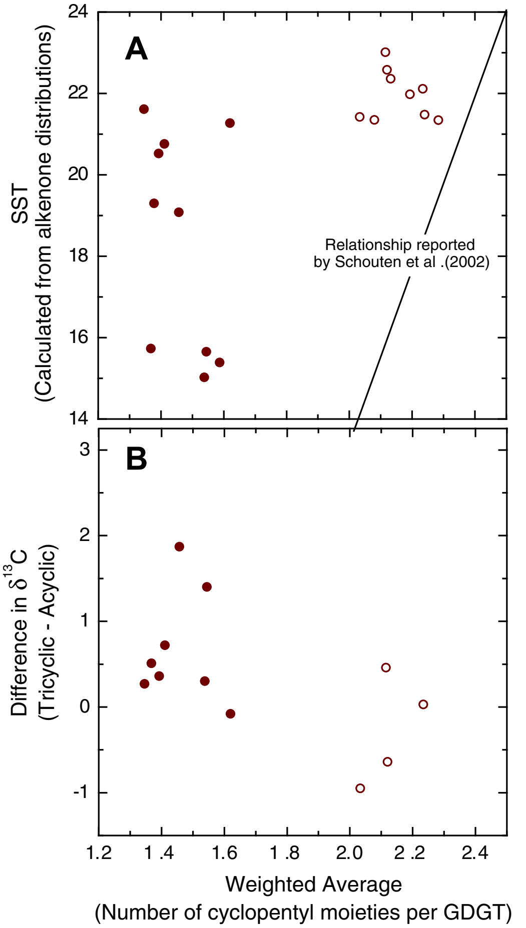

**Fig. 4.** Weighted average number of five-membered rings in Benguela (closed circles) and sapropel GDGTs (open circles) plotted vs. alkenone-derived SSTs (A) and the difference between acyclic and tricyclic biphytane $\delta^{13}C$ values (B).

assimilate dissolved inorganic carbon (Wuchter et al., 2003) using a carbon assimilation pathway other than the Calvin cycle (e.g. Menendez et al., 1999). In contrast, ODP Site 1084 acyclic GDGT $\delta^{13}C$ values have a larger and slightly more $^{13}C$-depleted range (−21‰ to −26‰). Archaeol $\delta^{13}C$ values in the Benguela sediments range from −30‰ to −32‰; thus, the wider range of acyclic GDGT $\delta^{13}C$ values is consistent with a partial and variable methanogen contribution of ca. −31‰. A similar interpretation was made by Schouten et al. (1998), who also observed that the biphytanes cleaved from acyclic GDGTs in a range of marine sediments are slightly depleted in $^{13}C$ relative to exclusively crenarchaeal-derived biphytanes, and the degree of depletion corresponds to the amount of excess acyclic GDGT (e.g. Fig. 4B).

### 5.2. Macromolecular organic matter characterised using Hypy

As expected, a range of *n*-alkanes was released from all solvent-extracted sediment residues of the Benguela upwelling system and sapropel S5. The *n*-alkane distributions differ significantly between the two sites, with an even/odd predominance in the BUS Hypy *n*-alkanes that is absent from the sapropel. The even/odd predominance could arise from a higher plant contribution to the macromolecular organic matter, possibly originally as carboxylic acids or alcohols incorporated via oxidative polymerisation mechanisms (e.g. de Leeuw et al., 2006; Gupta et al., 2006, 2007a,b). Particularly striking is the relatively large size of the UCM, perhaps also suggestive of early diagenetic polymerisation reactions. Hopanes and steranes were also detected in the Hypy-released fraction, but are of low abundance relative to total products.

#### 5.2.1. Archaeal lipids released using Hypy

This is the first study in which Hypy has been used to examine archaeal lipids; Hypy of both BUS and sapropel sediments generated acyclic, monocyclic, bicyclic and tricyclic archaeal biphytanes (Fig. 3). The TOC-normalised, Hypy-released biphytane abundances for Site 1084 sediments range from 0.001 to 0.013 g/g TOC, whereas the S5 sediments range from 0.001 to 0.047 g/g TOC. These concentrations are far greater than those of Hypy-released steranes and hopanes, which are present in only small amounts in the total ion current (TIC) traces. In fact, total biphytane abundances are comparable to those of the total recovered *n*-alkanes, with the ratio of biphytanes to *n*-alkanes ranging from 0.1 to 40. This suggests, like some of the early work by Michaelis and Albrecht (1979), that archaeal lipids can represent a significant component of some kerogens. Our Hypy analysis also reveals that the proportion of free to 'bound' archaeal lipids can be highly variable. In sapropel sediments, abundances of free GDGTs are comparable to those of biphytanes released with Hypy, whereas in Benguela sediments, free GDGTs are as much as fifty times more abundant than Hypy-released biphytanes. This is primarily due to the much larger quantities of free GDGTs in the Benguela samples, as Hypy-released biphytane abundances are comparable. This cannot reflect a greater degree of incorporation over time as the age of the sapropel sediments is nearly an order of magnitude younger than those examined from Benguela (ca. 100 kyr vs. 1 Myr).

Previous work has focussed on kerogen-bound archaeal lipids analysed using thermal and chemical degradation techniques. The first observation that archaeal lipids are incorporated into kerogen was based on HI cleavage of ether moieties in Messel shale kerogen (Michaelis and Albrecht, 1979), which released phytane as well as a range of biphytanes with zero, one or two cyclopentyl moieties. Inferred archaeal isoprenoid alkanes and alkenes were released from the kerogen of Albian OAE1b black shales (Kuypers et al., 2001, 2002) – flash pyrolysis released $C_8$ to $C_{15}$ isoprenoid alkanes/alkenes and $RuO_4$ treatment released 2,6,10,15,19-pentamethylicosane (PMI) and 2,6,15,19-tetramethylicosane (TMI). However, no biphytanes were released from the macromolecular component even though GDGTs were present in the solvent-extractable fractions. Archaeal lipids were also released from kerogen by HI/$LiAlH_4$ treatment of the Be'eri sulfur deposit (Burhan et al., 2002); phytane (perhaps related to archaeol), monocyclic biphytane, two isomers of bicyclic biphytane and a novel $C_{41}$ biphytane, apparently bearing two cyclopentyl groups, were identified after HI/$LiAlH_4$ treatment. Schouten et al. (2004) performed hydrous pyrolysis on sediment samples from the Italian Geososso-solfifera Formation to simulate artificial maturation and archaeal GDGTs were released. The increase in GDGT abundance between temperatures of 160 and 220 °C was attributed to release of GDGTs from the kerogen, as Koopmans et al. (1996) had previously released biphytane carbon skeletons using chemical degradation of kerogen from the Geososso-solfifera Formation. Our work is consistent with these studies, revealing not only that archaeal lipids have been incorporated into sedimentary macromolecules, but that archaeal lipids can be among the most abundant resolvable components of the macromolecular fraction and that incorporation can also happen relatively quickly (<100 kyr).

However, the fact that comparable abundances of GDGTs are released from both Benguela and sapropel sediments, despite the free components being an order of magnitude more abundant at the former site, suggests that either Benguela GDGTs were less reactive or there is a finite capacity for the binding of GDGTs into kerogen. Archaeal lipids could be bound into the macromolecular fraction via a variety of mechanisms. However, because the GDGT core is relatively unreactive, it is likely that reactions occurred at the polar head groups of the original biological GDGT membrane lipids or the hydroxyl groups resulting from their removal. This is consistent with the fact that intact GDGTs were released in the hydrous pyrolysis experiments of Schouten et al. (2004). Lipids can become sulfur-bound during diagenesis by interaction of functional groups (carbonyls or double bonds) with inorganic sulfides (Sinninghe Damsté and de Leeuw, 1987). However, Kohnen et al. (1992) detected archaeal ether lipids in the free hydrocarbon fraction of five sulfur-rich immature sediments, but sulfur-bound archaeal lipids were not detected. Thus, we propose that archaeal lipids have been incorporated via condensation and polymerisation reactions analogous to those proposed for the selective preservation of leaf lipids (Gupta et al., 2007a,b), dinoflagellate casts (Versteegh et al., 2004) and the unusual archaea of OAE1b (Kuypers et al., 2001, 2002). If so, then depletion of reactive oxidizing substrates with depth will impose limits on the degree of GDGT incorporation into kerogen. Alternatively or additionally, Mollenhauer et al. (2007) have shown that there is considerable aging of crenarchaeol (and presumably other GDGTs of pelagic origin) during transport across the Namibian shelf; it is likely that, during transport, the reactive polar head group was lost, rendering Benguela GDGTs less reactive than those deposited in sapropels. Consistent with this, we could find no evidence for incorporation of terrestrially derived GDGTs into kerogen, although they are present in the extractable fraction of the S5 sapropel.

### 5.3. Comparison of bound and free GDGT distributions

Assuming that the Hypy-released bicyclic and tricyclic biphytanes both derive from crenarchaeol, and that the acyclic biphytane dominantly derives from the acyclic GDGT, then we can compare the GDGT distributions of the macromolecular and solvent-extractable fractions (Fig. 5). In the ODP Site 1084 sediments, the solvent-extractable fraction contains abundant GDGTs, with the acyclic GDGT being far more abundant than crenarchaeol (0:0 > 2:3). In contrast, the sum of the Hypy-released crenarchaeol biphytanes is greater than the Hypy-released acyclic biphytane ($C_{40:0} < C_{40:2} + C_{40:3}$), such that there is proportionally less of the acyclic GDGT in the macromolecular fraction than in the extractable fraction. This is not observed in the S5 sediments, in which extractable crenarchaeol is more abundant than the acyclic GDGT, and the sum of Hypy-released crenarchaeal biphytanes is correspondingly greater than that of acyclic biphytane.

As with the previously discussed differences between the Benguela and sapropel free GDGT distributions, the difference between Benguela free and bound GDGTs could reflect different sources for the two different pools. If the high abundances and low $\delta^{13}C$ values of acyclic GDGT are due to methanogen input, then the lack of these characteristics in the Hypy-released fractions suggests that incorporation into the macromolecular fraction is biased in favour of the pelagic crenarchaeota. This could indicate that polar head groups of pelagic crenarchaeal GDGTs are more reactive than those of methanogens; although this is possible, it is unlikely in Benguela sediments, where there is evidence that pelagic GDGTs are aged – with associated loss of polar head groups – as they are transported across the Namibian shelf (Mollenhauer et al., 2007). Instead, we suggest that the lack of methanogen GDGT incorporation reflects limitation of the inorganic substrates involved in condensation/polymerisation reactions in deeper sediments. Methanogens occur below the sulfate reduction zone and where oxygen is absent (currently ca. 5 m depth in Site 1084 sediments). Thus, incorporation of methanogen lipids via oxygen-mediated free radical mechanisms

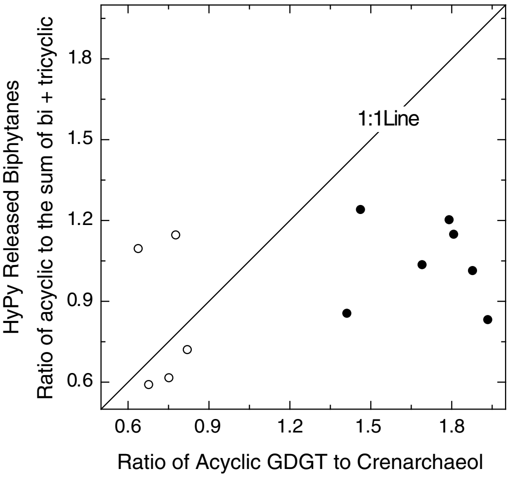

**Fig. 5.** Ratio of acyclic GDGT to crenarcheaol vs. the analogous ratio for Hypy-released biphytanes, the acyclic biphtyane/(bi + tricyclic biphytane) for Bengueal (closed circles) and S5 sapropel sediments (open circles). Note that for the latter ratio, acyclic biphytane could also derive from the 0:1 GDGT and the bicyclic biphytane could also derive from the 2:1 and 2:2 GDGTs; however, the dominant sources of these components will be the acyclic GDGT and crenarchaeol, respectively.

(Versteegh et al., 2004; Gupta et al., 2007b; de Leeuw et al., 2006) would be limited. This is consistent with the mechanism invoked to explain why the ratio of macromolecular to free GDGTs is so much lower in BUS sediments. Intriguingly, this observation could offer critical new insight into the timing and mechanism of such reactions, suggesting that they occur at relatively shallow depth, consistent with relatively rapid rates of hopanoid incorporation into kerogen (Farrimond et al., 2003).

## 6. Conclusions

Archaeal GDGT lipids are the most abundant solvent-extractable biomarkers in sediments from both the Benguela upwelling system and sapropel S5, dominating over algal, bacterial and higher plant lipids. GDGT carbon isotopic compositions and distributions suggest that they predominantly derive from pelagic crenarchaeota, but there is also evidence for a secondary methanogen source of acyclic GDGT in the Benguela sediments. Free GDGTs have been compared, with their distributions and abundances in the macromolecular fraction characterised for the first time using Hypy. Acyclic, monocyclic, bicyclic and tricyclic archaeal biphytanes were released from the macromolecular fraction and are among the most abundant hydrocarbons generated. For the sapropels, Hypy-released biphytanes have abundances and distributions similar to those of free GDGTs. However, for BUS sediments, Hypy-released biphytanes are less abundant than free GDGTs, with proportionally less of the acyclic component. Both these observations suggest that the mechanism by which GDGTs are incorporated into the macromolecular fraction was dependent on substrates not available in deeper (ca. >5 m) sediments, such that only limited incorporation of pelagic crenarchaeal lipids, and effectively no incorporation of methanogen lipids, occurred. Regardless of the mechanism, our results indicate that archaeal biomass could comprise a significant component of the macromolecular organic pool, but that this contribution could derive from archaeal assemblages different from those represented by the free GDGT distribution.

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