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Geochemical implications of the lipid composition of *Thioploca* spp. from the Peru upwelling region—15°S

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Abstract—*Thioploca*, a genus of colorless, sulphur-oxidizing, filamentous bacteria, constitutes as much as 80% of the biomass in dysaerobic surface sediments ($[O_2] < 0.1$ ml/l bottom water) in the coastal upwelling regimes of Peru and Chile. The lipid composition of *Thioploca* collected from sediments from the oxygen minimum zone in the Peru upwelling region near 15°S is presented here, and we provide the first assessment of the influence of *Thioploca* on organic compound distributions in upwelling regime sediments. Since marine species of *Thioploca* have been found only in dysaerobic surface sediments of upwelling regimes, biomarkers for this organism may be useful in identifying similar depositional conditions in the sedimentary record. *Thioploca* (dry) was found to be ≈3.8–4.1 wt% lipid. Three fatty acids: *cis* 16:1Δ9, 16:0 and *cis* 18:1Δ11 accounted for 69–72% of this lipid. Hydroxy fatty acids, hopanoids and hydrocarbons were conspicuously absent from the *Thioploca*. The *Thioploca* was found to contain cyclolaudenol, a C_{31} sterol with an unusual structure; diagenetic alteration products of this sterol may serve as markers for *Thioploca* input to sedimentary organic matter, and hence as markers for paleo-upwelling depositional environments in the sedimentary record.

Key words—*Thioploca*, bacterial mats, Peru, surface sediments, C_{31} sterol, cyclolaudenol, fatty acids, hopanoids, stable isotopes, Monterey Formation

INTRODUCTION

Thioploca, a genus of colorless, sulphur-oxidizing bacteria, consists of bundles of filaments encased in a common, whitish-yellow mucilaginous sheath as much as several cm long and 500 μm wide (Maier and Gallardo, 1984). Marine species of *Thioploca* have been found only in dysaerobic surface sediments ($[O_2] < 0.1$ ml/l bottom water) of coastal upwelling regimes, including coastal Peru and Chile (Gallardo, 1977; Maier and Gallardo, 1984; Henrichs and Farrington, 1984; Gallardo, 1985), and Walvis Bay, Southwest Africa (Morita *et al.*, 1981). In the coastal upwelling regimes off Peru and Chile, where the low oxygen waters of the Peru–Chile Subsurface Countercurrent impinge on the surface sediments, *Thioploca* constitutes as much as 80% of the biomass (Gallardo, 1977; Rosenberg *et al.*, 1983; Maier and Gallardo, 1984; Gallardo, 1985) and, therefore, has a significant impact on the organic geochemistry of these sediments.

Some aspects of the lipid composition of *Thioploca* collected from the oxygen minimum zone in the Peru upwelling region near 15°S are presented here, and we provide the first assessment of the influence of *Thioploca* on organic compound distributions in upwelling regime sediments. These data are of interest for several reasons:

(1) The environmental specificity of *Thioploca* makes biomarkers for this organism potentially useful in identifying similar depositional conditions in the sedimentary record.
(2) Since some ancient sediments deposited in coastal upwelling regimes (such as the Miocene Monterey Formation of the California Borderland) are important petroleum source rocks (see, for instance, Hunt, 1979; Katz and Elrod, 1983), markers for *Thioploca* could aid in future studies of the relative importance of oxic vs. suboxic conditions during petroleum source rock deposition in upwelling environments.
(3) The occurrence of abundant fossilized bacterial mats in the Monterey Formation led Katz and Elrod (1983) to suggest *Thioploca* as a possible source for a diagenetic precursor to the unusual bisnorhopane [17α(H),18α(H),21β(H)-28,30-bisnorhopane] present in many Monterey oils and sediment bitumens (Philp, 1985; Katz and Elrod, 1983), but, as discussed below, our data indicate that this is unlikely.
(4) The abundant fossilized bacterial mats in the Monterey Formation have also led to the suggestion (Williams and Reimers, 1983; Williams, 1984) that sulphur-oxidizing bacterial mats in these environments may be an important kerogen precursor; our characterization of the lipid composition of these organisms is a necessary first step for future investigations of this possibility.

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Table 1. Lipid data from solvent extracts of *Thioploca* (THIO # 1 and THIO # 2) and surface sediments (0–1 cm) of Soutar core SC7. Stable isotope data for whole *Thioploca*

	THIO # 1	THIO # 2	SC7(0–1 cm)
Sampling location Lat:	75°36.81′W	75°31.97′W	75°36.81′W
Lon:	14°54.20′S	15°04.27′S	14°56.62′S
Water depth	73–75 m	100 m	105–110 m
Sample size extracted (dry)[a]	19.2 mg	14.0 mg	1600 mg
Total lipids (% dry wt)	4.1	3.8	
$\delta^{13}C$ (relative to PDB)[a]	—	-21.8 ± 0.05‰	—
$\delta^{15}N$ (relative to air)[a]	—	$+4.8 \pm 0.1$‰	—
Cyclolaudenol (μg/gdw)	83.5	80.5	1.26
Total fatty acids (μg/gdw)	30800	27300	870
	Fatty acids (as % of total)		
14:1Δ9	0.015	0.036	0.247
14:0	0.627	0.635	7.78
iso 15:0	0.526	0.778	3.59
anteiso 15:0	0.370	0.605	4.14
15:0	0.270	0.195	2.01
iso 16:0	0.048	0.109	2.04
***cis*-16:1Δ9[b]**	**42.5**	**40.3**	**16.9**
16:0	**17.3**	**18.0**	**18.7**
anteiso 17:0	0.158	0.148	2.25
17:0	0.182	0.174	1.11
18:4	0.045	0.065	1.21
iso 18:0/18:3[c]	0.049	0.041	1.21
18:1Δ9/18:2[c]	0.468	0.303	8.42
***cis*-18:1Δ11[b]**	**36.0**	**37.8**	**15.2**
18:0	0.906	0.543	2.86
20:5	§	§	4.61
20:1Δ11	0.098	0.102	1.86
20:0	0.060	0.038	0.643
22:6	§	§	0.624
22:1Δ13	0.046	§	0.260
22:0	0.050	0.019	0.551
23:0	0.023	§	0.141
24:1Δ15	§	§	0.221
24:0	0.074	0.046	1.21
25:0	0.024	§	0.132
26:0	0.050	0.046	0.907
27:0	§	§	0.208
28:0	0.026	0.017	0.359
29:0	§	§	0.216
30:0	0.034	§	0.374

[a]Isotopic measurements of THIO # 2 were performed on a 1.3 mg (dry) whole (unextracted) *Thioploca* sample.
[b]Double bond geometry determined by GC–FTIR.
[c]Compounds separated by "/" coelute, and value presented is a sum of their concentrations.
§Concentrations <4.5 μg/gdw.

EXPERIMENTAL

Thioploca has never been successfully cultured, and therefore samples for lipid analyses had to be collected from the field. *Thioploca* was sieved from sediments collected from the oxygen minimum zone in the Peru upwelling region near 15°S, during July 1987, on leg 08 of the R/V *Moana Wave* cruise 87 (PUBS I), as part of a continuing study of early diagenesis of organic matter in the Peru upwelling region (Volkman *et al.*, 1983; Henrichs and Farrington, 1984; Farrington *et al.*, 1988). The relevant sampling locations are given in Table 1. Within minutes of sieving the sediments, individual strands of *Thioploca* were picked from the sieved material, rinsed in clean seawater, aggregated into groups of strands, and frozen for subsequent analyses. The samples of picked and rinsed *Thioploca* appeared by examination with a binocular stereoscope to be free of all other material, and as is discussed below, this is also suggested by the absence from the *Thioploca* samples of a suite of lipids characteristic of the surface sediments in the study area. Soutar-type box cores of sediment were collected from the oxygen minimum zone, and *Thioploca* was present in the surface sediments. These cores were immediately sectioned at 1 cm intervals and frozen for subsequent analyses.

The lipid composition of two samples ("THIO # 1" and "THIO # 2") of *Thioploca* collected 17 km apart (Table 1) and the lipid composition of surface sediments were determined using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). The procedures and equipment used for lipid extraction, column chromatography, fatty acid derivitization, GC, and GC–MS of samples were the same as previously described (Farrington *et al.*, 1988) and can be briefly summarized as follows.

Frozen samples were thawed, internal recovery standards were added, and samples were sonic extracted successively with isopropanol, methanol–chloroform (1:1 v/v) and methanol-chloroform

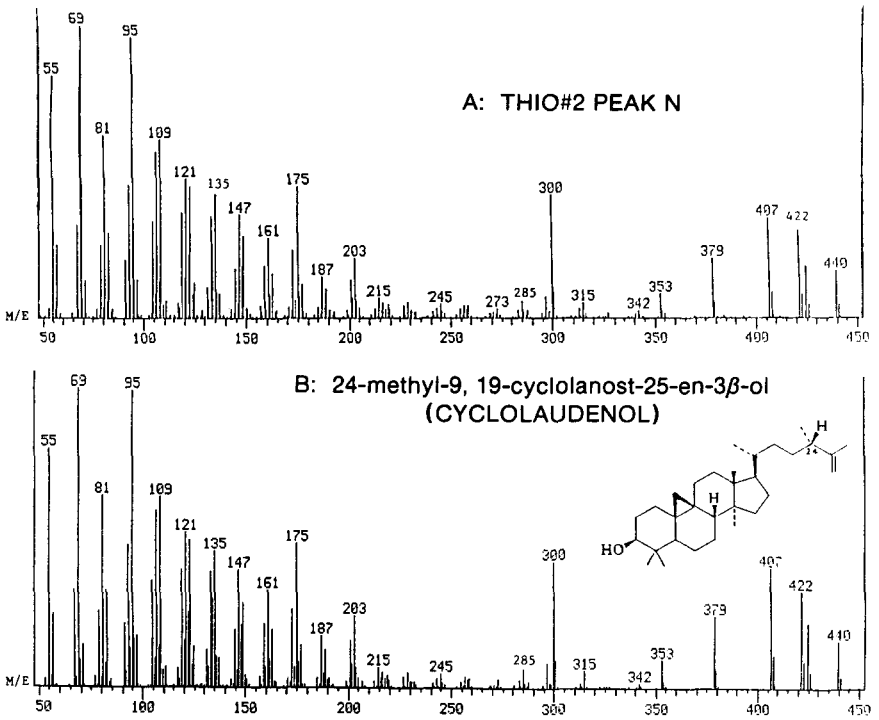

Fig. 1. (A) Electron impact mass spectrum of compound N in sample THIO # 2. (Finnigan 4500 quadropole mass spectrometer interfaced to a Carlo Erba 4160 gas chromatograph utilizing a column of the same type as that used in the GC analyses and helium as the carrier gas). GC–MS injections were on-column at 70°C and the GC was programmed at 3.5°C/min to 260°C and at 4°C/min to 310°C. GC–MS operating conditions were 50 eV ionization potential with the source at 100°C and electron multiplier voltage at 950 V with scanning rate from 50 to 650 amu per s. (B) Electron impact mass spectrum of authentic standard of cyclolaudenol; GC–MS conditions were same as above, except that the electron multiplier voltage was 1000 V. The mass spectra of compound N in THIO # 1 and THIO # 2 were the same.

(1:3 v/v); lipids were partitioned into isopropanol/chloroform by addition of $NaCl_{(aq)}$. One half of the total lipid extract (TLE) was separated into lipid classes by silica gel column chromatography. One fourth of the original TLE of each sample was treated with 0.5 N KOH in methanol to saponify the esterified fatty acids. Fatty acids were converted to fatty acid methyl esters (FAMEs) by reaction with 10% BCl_3–methanol and separated from the other lipids in the TLE using silica gel column chromatography. All sample fractions were analyzed by high resolution gas chromatography (GC) with a J & W Scientific Durabond DB-5 30 m fused silica capillary column and an on-column injector. Identification of individual FAMEs was based on a comparison of the GC retention times with authentic standards. Compound identifications were confirmed by electron impact GC–MS analyses, using conditions described in the legend of Fig. 1. The *cis* configuration of the double bond in 16:1Δ9 and 18:1Δ11 (Table 1) was determined by matrix isolation gas chromatography Fourier transform infrared spectroscopy (MI–GC–FTIR).

Thioploca and sediment dry weights were determined by drying an aliquot of each sample at 110°C for 24 h and then correcting the aliquot dry weight for salts (brine in the samples was assumed to have a salinity of 3.5 wt%). The weight of total lipids extracted from the *Thioploca* samples was determined by passing 1/4 of the original TLE through an activated copper column (to remove elemental sulphur). The resulting solvent–lipid mixture was evaporated to a small volume under an N_2 stream and then transferred to a 10 mg, tared, solvent-rinsed, aluminum CAHN balance pan, where the remaining solvent was evaporated and the residues weighed on a CAHN 25 Automatic Electrobalalance to $\pm 2\,\mu g$.

The solvent-extracted residue of one of the *Thioploca* samples (THIO # 2) was heated with refluxing 6N KOH in methanol/water (1:1 v/v) for 2 h to determine the quantitative importance of additional lipids bound to the solvent-extracted residues by ester linkages. The insoluble *Thioploca* residues from this KOH extraction were then heated with refluxing 6N HCl for 6 h under nitrogen to liberate any addition lipids that may have been bound to the *Thioploca* residues by amide or ether linkages.

Nitrogen and carbon stable isotope analysis of one *Thioploca* sample was performed for us (Dr Brian Fry's Laboratory; Marine Biological Laboratory,

Woods Hole) on a Finnigan 2540 magnetic deflection MS. Three *Thioploca* samples were analysed by Pyrolysis GC, and the pyrolysis products (peak P2) of one of these samples were analyzed by EI GC–MS. The pyrolysis GC–MS procedure was the same as that described in detail by Tarafa *et al.* (1987).

RESULTS AND DISCUSSION

Lipids liberated by solvent extraction

The total lipids from solvent extraction of the *Thioploca* samples were found to be 3.8–4.1 dry wt% of the samples (Table 1). The lipid extracts contained very low *n*-alkane concentrations (indistinguishable from the analytical blanks). Total fatty acids collectively constituted 2.7–3.1 dry wt% of the *Thioploca* (Table 1). Three fatty acids: *cis* 16:1Δ9, 16:0 and *cis* 18:1Δ11 accounted for 95–96% of the total fatty acids and 69–72% of the total lipid content of the *Thioploca* extracts (Table 1). In most species of bacteria (other than archaebacteria) fatty acids account for 2–8 dry wt% (Asselineau, 1966); therefore, the values determined for *Thioploca* are not unusual. The most commonly reported monounsaturated fatty acids in bacteria are, in fact, 16:1Δ9 and 18:1Δ11 (Perry *et al.*, 1979; Parkes and Taylor, 1983; Gillan and Johns, 1986). Given the high concentrations of these monoenoic straight-chain acids in *Thioploca*, the absence of large concentrations of branched acids (e.g. *i*15:0, *a*15:0, *i*17:0, *a*17:0) is not surprising, since bacteria with high concentrations of monoenoic acids typically do not produce large concentrations of branched acids (Gillan and Johns, 1986; Goossens *et al.*, 1986).

The fatty acids: 16:1Δ9, 16:0 and 18:1Δ11 are also the most abundant fatty acids in surface sediments from the study area (Table 1, and Farrington *et al.*, 1988). Since abundances of *Thioploca* ranging from $\approx$100–1000 g/m^2 (wet wt, concentrated near the sediment–water interface) are common in sediments from the oxygen minimum zone (OMZ) of the Peru–Chile upwelling regimes (Gallardo, 1977; Rosenburg *et al.*, 1983; Gallardo, 1985), the high concentrations of 16:1Δ9, 16:0 and 18:1Δ11 in *Thioploca* suggest that *Thioploca* may be an important source of these compounds in the sediments. However, a quantitative assessment of the importance of *Thioploca* as a source of these fatty acids in the sediments would require data on average rates of *Thioploca* organic matter synthesis in these sediments. The fatty acid distribution in surface sediments of core SC7 is shown in Table 1 and includes fatty acids from sources other than *Thioploca*. A detailed discussion of fatty acid biogeochemistry in sediments of this region is in preparation.

Cyclolaudenol (24-methyl-9,19-cyclolanost-25-en-3β-ol), an unusual 4,4-dimethyl C_{31} sterol with a 9,19 cyclopropyl ring (Fig. 1B) was found in the *Thioploca* samples in concentrations $\approx$82 μg/gdw and accounted for >95% of the sterols in the *Thioploca* extracts ($\approx$0.21% of the total lipids). The cyclolaudenol was identified by coelution with an authentic standard, and by comparison of the mass spectrum of the compound (Fig. 1A) with the mass spectrum of the authentic standard (Fig. 1B). Although, prior to 1967, sterols had not been identified in procaryotes (Bird *et al.*, 1971), there have been several reports since that time of sterols in bacteria (see Kohl *et al.*, 1983 for a review). Bouvier *et al.* (1976) has reported significant concentrations of 4,4-dimethyl and 4α-methyl sterols in *Methylococcus capsulatus*, and has reported that sterol biosynthesis in this bacterium is blocked at the level of 4α-methyl sterols. *Thioploca* is another example of a bacterium that contains a 4,4-dimethyl sterol, but does not accumulate 4-desmethyl sterols. The lack of 4-desmethyl sterols, however, cannot be generalized to all bacteria, since Kohl *et al.* (1983) identified a substantial concentration of cholest-8(9)-en-3β-ol in *Nannocystis exedens*. Although 4-desmethyl sterols have also been found in several cyanobacteria (De Souza and Nes, 1968; Reitz and Hamilton, 1968), Ourisson *et al.* (1987) concluded that the low levels of the common 4-desmethyl sterols found in cyanobacteria were probably found as a result of sample contamination.

It is interesting to note that cycloartenol (9,19-cyclolanost-3β-ol), a sterol very similar to cyclolaudenol, has been reported in significant concentrations in sediments from the laminated cyanobacterial mat sequence in the Solar Lake adjacent to the Gulf of Aqaba (Boon, 1984; Edmunds and Eglinton, 1984), and in sediments from a cyanobacterial mat (Cardoso *et al.*, 1978) from the Persian Gulf coast of Abu Dhabi (United Arab Emirates). These studies did not identify the source of the cycloartenol in these sediments, but the presence of the similar sterol, cyclolaudenol, in *Thioploca* suggests that the cycloartenol in the cyanobacterial mat sediments may also have a bacterial source.

Ourisson *et al.* (1979) and others have noted that many hopanoids, or their diagenetic precursors, may serve as bacterial biomarkers. However, the hopanoids present in the Peru surface sediments (Volkman *et al.*, 1987; Farrington, unpublished data) are apparently not derived from the *Thioploca*, since hopanoids (with molecular wt <650 amu) were *not* found among the alkane, fatty acid (esterified or free), ketone, or alcohol fractions of the *Thioploca* extracts. In addition, the absence of hopanoids in the *Thioploca* suggests that, contrary to previous suggestions (Katz and Elrod, 1983), *Thioploca* is probably not a source for a diagenetic precursor to the unusual bisnorhopane [17α(H),18α(H),21β(H)-28,30-bisnorhopane] present in many Monterey oils and sediment bitumens. Steroids and hopanoids are biosynthesized by different pathways from the cyclization of squalene; Ourisson *et al.*, (1979) suggested that steroid biosyntheiss is evolutionarily more advanced than hopanoid biosynthesis, and they

noted that steroid-producing bacteria typically do not produce hopanoids. Therefore, given the presence of the cyclolaudenol in the *Thioploca*, the absence of hopanoids is perhaps not surprising.

It may be possible to use cyclolaudenol and/or the products of its diagenetic alteration as markers for *Thioploca* input to sedimentary organic matter. The potential of cyclolaudenol and its diagenetic alteration products as *Thioploca*-specific biomarkers is enhanced by the limited reported terrestrial sources of this sterol [reported by Akihisa *et al.* (1986) in *Musa sapientum* (Banana peel); in the latex and stem of *Euphorbia caudicifolia* (Govardhan *et al.*, 1984); in maize (Goodwin, 1977); and in the rhizomes of a fern, *Polypodium vulgare* (Ghisalberti *et al.*, 1969)]. To the best of our knowledge, cyclolaudenol has not been reported previously in marine or lacustrine organisms or sediments. Volkman *et al.* (1987) did not report cyclolaudenol among the sterols in a Peru sediment core, "BC7", taken shoreward of the oxygen minimum zone and hence outside the zone of *Thioploca* occurrence. Smith *et al.* (1983) did not report cyclolaudenol among the sterols in Peru surface sediments from further north at 12°S; Smith *et al.* did not mention the presence of *Thioploca*, and therefore their core may have been taken from a less oxygen-depleted area outside the zone of *Thioploca* occurrence. Alternatively, since these studies were not looking for cyclolaudenol, its occurrence may have been overlooked. Thus far, our data for the sedimentary distribution of cyclolaudenol is preliminary, since under our analytical conditions partial coelution of cyclolaudenol with another, more abundant steroid in the sediments requires quantitation of cyclolaudenol by GC–MS. Our analyses of sediments from 0–1 cm, 4–5 cm and 8–9 cm of Soutar core 7 (SC7) indicate that the cyclolaudenol concentration in this core decreases rapidly with depth. Analysis of sediment from 45–51 cm in SC6, a core from the oxygen minimum zone at 15°S recovered in 1978 (Henrichs and Farrington, 1984) indicates the presence of cyclolaudenol, albeit in trace quantities. We cannot distinguish at this time whether or not the decrease in cyclolaudenol concentration with depth in SC7 is due to early diagenetic transformation (the hypothesis that we favor) or is due to variations in the abundance of *Thioploca* at the time of deposition. If cyclolaudenol (rather than its transformation products) is to be used as a biomarker for *Thioploca*, it may be necessary to use mass-selective detection to identify the compound among the other more abundant steroids in sediments. However, many potential diagenetic alterations of cyclolaudenol, such as loss of the alcohol function (by formation of the sterone or 2–3 sterene), saturation of the 25-ene, or opening of the 9–19 cyclopropyl ring, would lead to unique products which may also have significant potential as markers for *Thioploca*.

Thioploca has never been successfully cultured, but analyses of naturally grown samples, such as those used in this study, would be necessary for a study of the impact of *Thioploca* on sedimentary geochemistry even if cultured samples were available, since the lipid composition of cultured bacteria can be a function of the culturing conditions (Oliver and Colwell, 1973). It is unlikely that the cyclolaudenol identified in our *Thioploca* samples represents contamination of the samples by sedimentary organic matter. The careful sampling procedure has already been discussed. In addition, if contamination by sedimentary organic matter were the source of the cyclolaudenol, then the lipids in Table 2, which are examples of lipids far more abundant than cyclolaudenol in the sediments from the study area (this work, and Farrington *et al.*, 1988), should have also been detected in the *Thioploca* extracts, *but were not*. The absence of these lipids from the *Thioploca* suggests that the lipid composition of the *Thioploca* samples reflects that of the *Thioploca* itself.

Table 2. Examples of lipids (μg/gdw) more abundant than cyclolaudenol in sediments from the OMZ (core SC7) but not detected in *Thioploca* (THIO # 1 and THIO # 2): evidence that cyclolaudenol in the *Thioploca* is not from contamination by sediments

	THIO # 1	THIO # 2	SC7 (0–1 cm)[a]
Cyclolaudenol	83.5	80.5	1.26
Cholesterol	§	§	29.2
Dinosterol	§	§	22.2
$C_{37:2}$ Alkenone	§	§	5.41
$C_{37:3}$ Alkenone	§	§	3.09
Branched $C_{20:1}$[b]	§	§	2.84

[a]Farrington *et al.* (1988) report similar concentrations for the alkenones and branched $C_{20:1}$ compound in surface sediments from this region.

[b]2,6,10-Trimethyl-7-(3-methyl-butyl)-dodecene.

§Not detected. Concentrations of 3 μg/gdw would be detectable for the *Thioploca* samples.

Lipids liberated by base extraction and acid extraction

The solvent-extracted residues of one of the *Thioploca* samples (THIO # 2) were treated with refluxing 6N KOH in methanol/water (1:1 v/v) for 2 h to determine the quantitative importance of additional lipids bound to the solvent-extracted residues by ester functional groups. The only additional lipids liberated by this technique were a small amount of fatty acids (14:0, 15:0, 16:1, 16:0, 18:1 and 18:0); the total additional fatty acids liberated were $\approx$400 μg/gdw, or $\approx$1.6% of the total fatty acids liberated by solvent extraction alone.

The insoluble *Thioploca* residues from this KOH extraction were then heated with refluxing 6N HCl for 6 h under nitrogen to liberate any additional lipids (frequently referred to as "tightly bound" lipids) that may have been bound to the *Thioploca* residues by amide or ether linkages. Goossens *et al.* (1986) and Mendoza *et al.* (1987) note the abundance of hydroxy fatty acids in many bacteria and report that hydroxy fatty acids are efficiently extracted from bacterial and sedimentary organic matter only by strong acid treatment or heating at high temperatures with base (Kawamura and Ishiwatari, 1984). Goossens *et al.*

propose that the tightly bound nature of the hydroxy fatty acids results from these compounds being bound by amide linkages to the insoluble biopolymeric residues. Our acid treatment of the *Thioploca* residue released no measurable quantities of hydroxy fatty acids (4 μg/gdw would have been detectable). The fact that these compounds would have been recovered by our procedure was confirmed by the good recoveries (>85%) of hydroxy fatty acid standards ($C_{16}\alpha$-OH FAME and $C_{14}\alpha$-OH FAME) refluxed with acid under similar conditions as the *Thioploca* acid extract. The acid extract of the *Thioploca* residues did contain small amounts of 14:0, 15:0, 16:1, 16:0, 18:1 and 18:0 fatty acids; the total additional fatty acids liberated were $\approx$400 μg/gdw, or $\approx$1.5% of the total fatty acids liberated by solvent extraction alone.

Thioploca pyrolysis GC

Pyrolysis GC of three *Thioploca* samples and pyrolysis GC–MS of one *Thioploca* sample were performed both to provide information on lipid moieties which may not have been detected by our extraction/analytical procedures and to determine the kinds of lipid moieties that thermal degradation of *Thioploca*-synthesized organic matter could potentially generate. Compounds in the low temperature pyrolysis peak, P1 ($\approx$6.5 mg/g ash wt of *Thioploca*), are largely the products of thermal distillation, and were not analyzed by GC–MS. Only the C_7–C_{24} pyrolysis products in the high temperature, or P2 peak (total P2 $\approx$ 20.0 mg/g ash wt of *Thioploca*; P2 temperature range $\approx$360–500°C; $T_{max} \approx$ 470°C) were analyzed by GC–MS.

Most of the compounds found in P2, including alkyl benzenes, phenols, indoles and other nitrogen containing compounds, were probably the products of carbohydrate and protein pyrolysis (Boon, 1984). This agrees with the low lipid concentration (<5 dry wt%) found in the *Thioploca* extracts discussed above (Table 1). Lipids in P2 included hexadecanoic acid (16:0 $\approx$ 7.6 μg/g ash wt of *Thioploca*), which was probably derived from esters, since esters can pyrolyze to the acid + a 1-alkene. The much lower abundance of 16:0 in the pyrolysate compared to the *Thioploca* solvent extracts may partially reflect the lower abundance in the *Thioploca* of bound 16:0 relative to unbound 16:0 illustrated by a comparison of the results of the solvent and base extractions discussed above. Low concentrations of several branched and unbranched alkanes were also found in P2 (total alkanes $\approx$0.1 mg/g ash wt of *Thioploca*). These alkanes eluted from the GC between nC$_{11}$ and nC$_{18}$, but the similarity of EI mass spectra of many branched acyclic alkanes prevents us from precisely identifying these compounds from GC–MS data alone. Alkanes are generally not subject to rearrangement during pyrolysis; therefore, the origin of the branched alkanes is not clear, given the lack of branched compounds (other than a small amount of *iso* and *anteiso* fatty acids) in the lipid extracts. Sulphur-containing compounds (including thiophenes) were also identified in the pyrolysate; however, interpretation of the presence of these compounds is complicated by the abundant intracellular elemental sulphur in *Thioploca* (several wt.%), since Schmid (1986) has demonstrated that elemental sulphur can react with organic matter when heated to form compounds such as thiophenes.

The carbon and nitrogen isotopic composition of Thioploca

Since *Beggiotoa*, a genus of sulphur-oxidizing bacteria which is a member of the same family (Beggiatoaceae) as *Thioploca*, has been found to fix isotopically light N_2 (Nelson *et al.*, 1982), it has been suggested that *Thioploca* may also fix nitrogen (Libes, 1983) with a light isotopic signature that could potentially be used to trace the input of *Thioploca*-synthesized organic matter to sediments. Although Maier and Gallardo (1984) disputed their findings, Morita *et al.* (1981) suggested that *Thioploca* was a methylotroph; the possibility of *Thioploca* existing as a methylotroph suggested to us that the carbon isotopic signature of *Thioploca* might also be sufficiently light as to have utility in quantifying the contribution of *Thioploca* to sedimentary organic matter. However, our determination of the carbon and nitrogen isotopic composition of a *Thioploca* sample (Table 1) suggested that the isotopic composition of the *Thioploca* is not sufficiently different from the average values for marine phytoplankton (Libes, 1983) to contribute to a quantification of the contribution of *Thioploca* to sedimentary organic matter.

CONCLUSIONS

—*Thioploca* (dry) was found to be $\approx$3.8–4.1 wt% lipid. Three fatty acids: *cis* 16:1Δ9, 16:0 and *cis* 18:1Δ11 accounted for 69–72% of this lipid.

—The absence of hopanoids (with molecular wt <650 amu) in the *Thioploca* suggests that, contrary to previous suggestions, *Thioploca* is probably not a source for a diagenetic precursor to the unusual bisnorhopane [17α(H), 18α(H), 21β(H)-23,30-bisnorhopane] present in many Monterey oils and sediment bitumens.

—Cyclolaudenol and/or its degradation products may be usable as markers for input of *Thioploca*-synthesized organic matter to the sedimentary record. Markers for *Thioploca* could aid in paleoenvironmental reconstruction, and could aid in characterizing the relative importance of oxic vs. suboxic conditions during petroleum source rock deposition in upwelling environments.

—Hydroxy fatty acids in the C_{12} + molecular weight range and n-alkanes in the C_{14}-C_{37} molecular weight range were not present in the

solvent, base or acid extracts of the *Thioploca*.

—Pyrolysis GC–MS of *Thioploca* (peak P2) revealed primarily carbohydrate and protein pyrolysis products. Lipid moieties included low concentrations of hexadecanoic acid and several branched and unbranched alkanes. Sulphur-containing pyrolysis products were found, but their significance is difficult to interpret, because of the unknown effects during pyrolysis of the large elemental sulphur content of *Thioploca*.

—The carbon and nitrogen isotopic composition of a *Thioploca* sample ($\delta^{13}C = -21.8 \pm 0.05‰$; $\delta^{15}N = +4.8 \pm 0.1‰$) suggests that in *Thioploca* these parameters are insufficiently different from the average isotopic composition of marine phytoplankton to contribute to a quantification of the input of *Thioploca* to sedimentary organic matter.

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