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BIOLOGICAL SOURCES FOR PHENYLALKANE HYDROCARBONS*

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Introduction

Linear alkylbenzenes (phenylalkanes) represent an important class of molecular compounds used widely in today's society as building blocks for detergent manufacture. Indeed, the widespread use and acceptance of detergents has been reflected by the fact that detergent chemical components can be found in and around almost every location around the globe where human settlements exist.¹ This being the case, detergent chemical components represent a well-recognized indicator of pollution in the lakes, rivers, and coastal water systems of the world. The identification of phenylalkane hydrocarbons in ancient sedimentary organic matter has been the subject of much controversy and concern, owing to the ubiquitous presence of phenylalkanes in today's society. The finding of these components in sediments and crude oils has been interpreted as evidence of "detergent contamination". New evidence, however, suggests that the identification of phenylalkanes in ancient geological materials may actually represent an input from ancient algae and/or bacteria which contributed to the organic biomass from which the sediment or crude oil was derived. Moreover, the finding also of phenylalkane hydrocarbons in the lipid extracts of thermophilic bacteria still living today represents the first evidence of a natural system producing these compounds.

Evidence is presented to support the proposition that phenylalkanes in some Australian crude oils and sediments are of geochemical origin rather than resulting from contamination from byproducts of the petrochemical synthesis of surfactants.² Evidence presented shows that: (1) an unexposed sediment core was found to contain phenylalkanes; (2) the molecular weight range of phenylalkanes in sediments and crude oils is usually wider than that found in surfactants, extending in some cases beyond C₃₅; and (3) phenylalkanes were found in the neutral lipid extract of extant *Thermoplasma* bacteria. *Thermoplasma acidophilum* is an obligate acidophilic (pH 2) and thermophilic (60°C), cell wall-less archaeobacterium originally isolated from self-heating coal refuse piles enriched in pyritic materials

Experimental

Solvents (Blanks) and materials. Due to the ubiquitous presence of phenylalkanes in laboratory detergents, additional care was employed to ensure all solvents and materials used were free from hydrocarbon contamination. All glassware was annealed prior to use, and solvent blanks were performed on all solvents and culture medium. No phenylalkane hydrocarbons were detected in any of the blanks.

Isolation of monoaromatic fractions. Sedimentary rock extract (Olive River Shale) and crude oil (Rough Range - Carnarvon Basin) were separated into alkane and aromatic fractions by chromatography on silica. The aromatic components were further separated into monoaromatic, diaromatic, and triaromatic fractions

using alumina thin layer chromatography with *n*-hexane as eluent. The monoaromatic, diaromatic, and triaromatic fractions were each obtained by scraping bands from the plate and extracting the alumina with dichloromethane.

Analysis of compounds in the monoaromatic fraction using GC-MS techniques. 2-Phenyldodecane was identified in the monoaromatic fraction by co-chromatography with the synthesized standard. In all cases, the compounds in the monoaromatic fractions assigned as phenylalkanes were identified using the synthetic mixture of phenylalkanes. Identifications were based on co-elution of peaks in two co-chromatography experiments using capillary columns coated with both DB-1 and DB-5 stationary phases. The mass spectra of the phenyldodecanes were also measured and in all cases matched those of the synthetic compounds and those reported in the literature.¹

*Analysis of *Thermoplasma* neutral lipids.* *Thermoplasma* was grown aerobically, essentially as previously described.³ Care was taken to avoid exposure of cells to any possible traces of detergent from any source in biomass preparation (e.g., glassware or equipment used), since exposure inhibits growth and can cause cellular lysis of the organism. Isolation of the neutral lipids from *Thermoplasma* was achieved via exhaustive repetitive extraction with dichloromethane/methanol (3:7) using ultrasound. Extracts were recombined, evaporated to dryness, and filtered through a column of alumina using pentane/diethyl ether (80:20) as eluent to remove polar compounds.

Results and Discussions

Phenylalkanes were identified by comparison of their GC retention times and their mass spectral characteristics¹ with those of synthetic reference compounds (Figure 1). Figure 2 shows partial summed mass chromatograms (m/z 91+105) of the monoaromatic fraction of a shale extract. The chromatogram shows that phenylalkanes with carbon numbers ranging from 16 to 21 are prominent components of the monoaromatic fraction from this sample. Isomers with the phenyl substituent located near the center of the alkyl chain have lower retention times than compounds with the phenyl substituent near the end of the chain. In the case of compounds with 16, 17 and 18 carbon atoms, all isomers were resolved. However, higher members with the phenyl substituent located near the center of the chain could not be resolved.

The possibility that this sediment sample was contaminated during laboratory processing is remote due to the extreme care taken to ensure the integrity of the sample (see Experimental). Also, the phenylalkanes obtained from commercial surfactant used in the laboratory (Figure 1) do not reflect those obtained from the sediment core sample (Figure 2). Contamination of the sample at the source via contact with drilling additives that may contain phenylalkane byproducts from surfactant formulations is also unlikely since an unexposed core was sampled. The sample was a solid piece of shale and is unlikely to have adsorbed and become completely saturated with surfactant and/or drilling additive products. Further experiments have shown that the surfactant does not permeate through shale.²

Figure 3 shows partial summed mass chromatograms (m/z 105 + 106) of the monoaromatic fraction from the Rough Range crude oil, which although complex with major peaks representing isomeric *n*-alkyltoluenes, shows the presence of 2-phenylalkanes extending from C₁₃ to C₃₅ and beyond. The mid-chain phenylalkane isomers were also identified up to approximately C₂₄, at which point identification of the higher homologues was not possible, again due to their low abundance and increasing number of possible isomers. This sample possesses a phenylalkane distribution far in excess of that normally found in petrochemical synthesis, further supporting the assertion that a natural source is responsible.

Langworthy et al.⁴ tentatively identified a series of branched alkylbenzenes in the lipid extracts from the thermoacidophilic bacteria, *Thermoplasma*. These were postulated to be branched alkylbenzenes with two methyl groups at varying positions in the alkyl chain. Analysis of the neutral lipids from cells harvested from a recently grown culture of *Thermoplasma*, however, revealed the 'branched alkylbenzenes' identified by Langworthy et al.⁴ were in fact phenylalkane hydrocarbons (Figure 4). The identification of phenylalkane hydrocarbons in the lipid components of *Thermoplasma* bacteria represents unequivocal evidence for a natural origin for these compounds.

Acknowledgments

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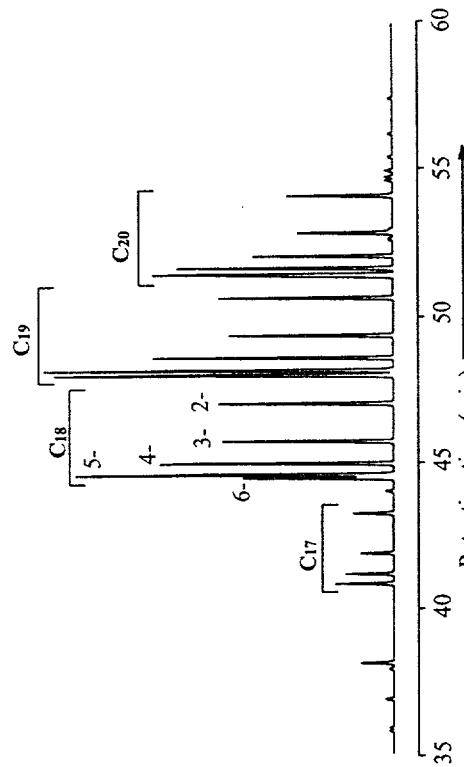


Figure 1. Partial summed mass chromatograms(m/z 91+105) of the phenylalkane hydrocarbons from detergent.

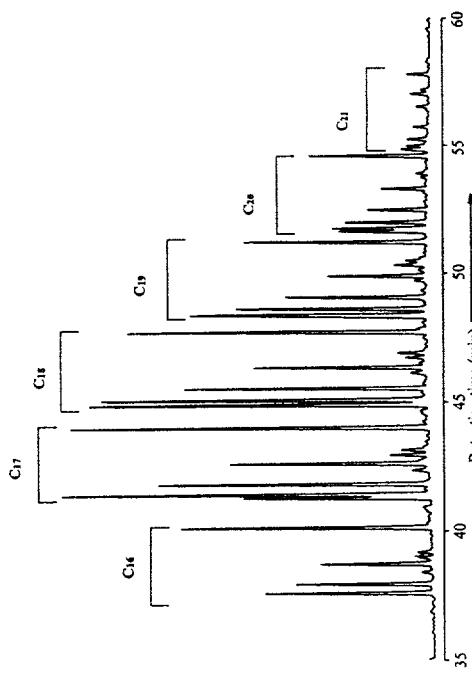


Figure 2. Partial summed mass chromatograms(m/z 91+105) of the phenylalkane hydrocarbons the monoaromatic fraction of the shale extract.

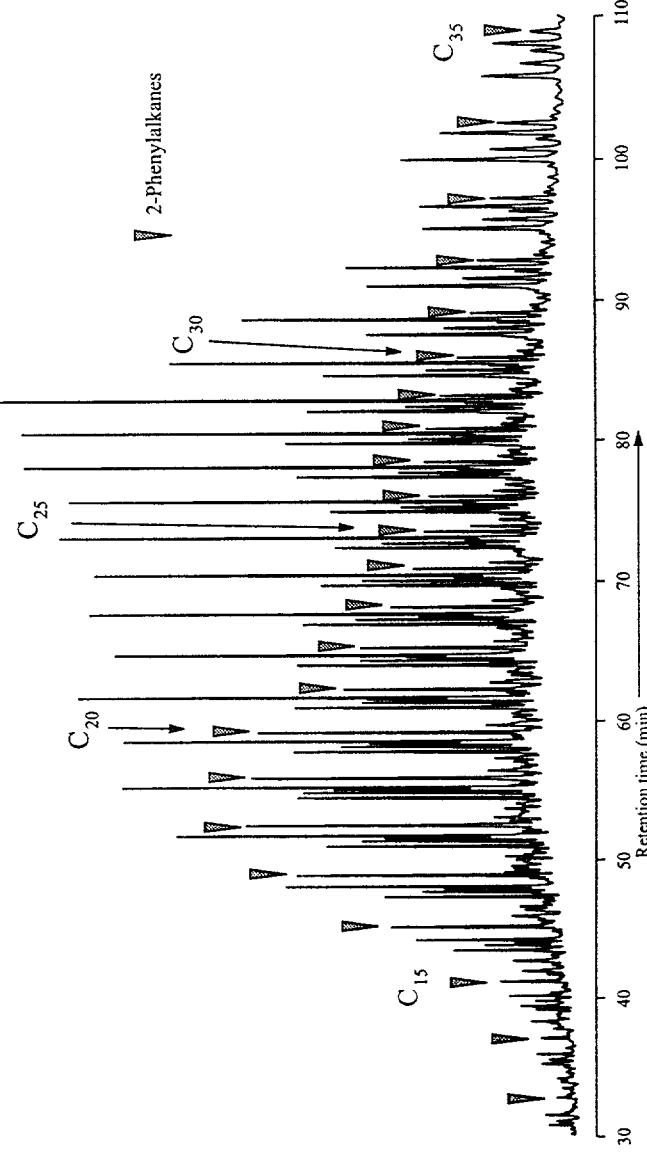


Figure 3. Partial summed mass chromatograms (m/z 105+106) of the monoaromatic fraction from Rough Range crude oil showing the distribution of 2-phenylalkanes extending from C13 to C35 and beyond.

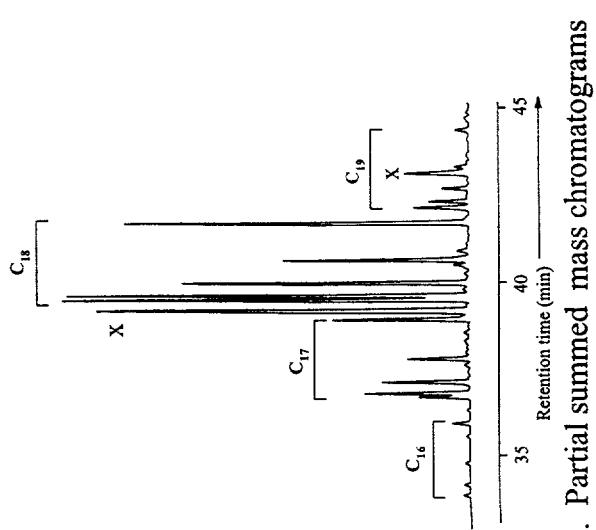


Figure 4. Partial summed mass chromatograms (m/z 91+105) of the phenylalkane hydrocarbons in the extracted neutral lipids from *Thermoplasma acidophilum*. X = not identified.