

TOF-SIMS IMAGING OF BIOMARKERS IN MICROSCOPIC SECTIONS OF MICROBIAL MATS

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Time of Flight - Secondary Ion Mass Spectrometry (ToF-SIMS) is a technique that allows to simultaneously analyse inorganic and organic molecules on solid surfaces (Benninghoven et al., 1994, Belu et al., 2003). A most advantageous property of ToF-SIMS is its ability to generate images displaying the intensities of any detected ion in a given area of interest while achieving spatial resolution on a very small scale (sub- μm , e.g. Hagenhoff, 2000). To date, this is not possible with any of the extract-based techniques routinely used in biomarker studies, namely GC/MS and LC/MS (coupled gas chromatography/mass spectrometry, coupled liquid chromatography/mass spectrometry). In organic ToF-SIMS analyses, compound identification can be achieved through the precise mass determination of the molecular species, ideally in combination with GC/MS and/or LC/MS data.

We used ToF-SIMS with a Bi_3^+ cluster primary ion source for the study of lipid biomarkers in native microbial mats from methane seeps in the Black Sea (Michaelis et al., 2002), and from the subsurface biosphere of the Äspö tunnel in Sweden (Pedersen, 1997). On 10 μm -thick microscopic cryosections of a Black Sea microbial mat, for instance, we simultaneously analysed archaeal isopranyl core lipids, together with their intact diglycoside (gentiobiosyl-) derivatives. Utilizing the imaging capability of ToF-SIMS, the spatial distributions of these biomarkers were mapped at a lateral resolution of $< 5\mu\text{m}$ in 500 x 500 μm^2 areas on sections. Different biomarker 'provenances' within this area were distinguished by individual patterns of the isopranyl glycerol diethers archaeol and hydroxyarchaeol, glycerol dialkyl glycerol tetraethers (GDGT), and gentiobiosyl-GDGT (Fig. 1).

Because ToF-SIMS is quasi-non-destructive it was possible to examine the studied areas 'post-measurement' using conventional microscopy, which enabled to associate the individual lipid patterns with particular morphological traits. Hydroxyarchaeol was directly associated with the precipitation of irregular, methane-derived CaCO_3 crystallites, whereas

GDGT-rich regions of the section revealed fluorescent, filamentous microorganisms showing the morphology of known methanotrophic ANME-1 archaea.

ToF-SIMS molecular imaging, in conjunction with other techniques, reveals interesting perspectives for a wide range of geological, ecological, biological, and medical applications, which share the need to detect and localize organic and inorganic compounds at high resolution. Potential geobiological applications include the elucidation of microbially assisted mineral precipitation, the analysis of organic compounds in unculturable microorganisms, and the clear-cut assignment of biomarkers to their biological source.

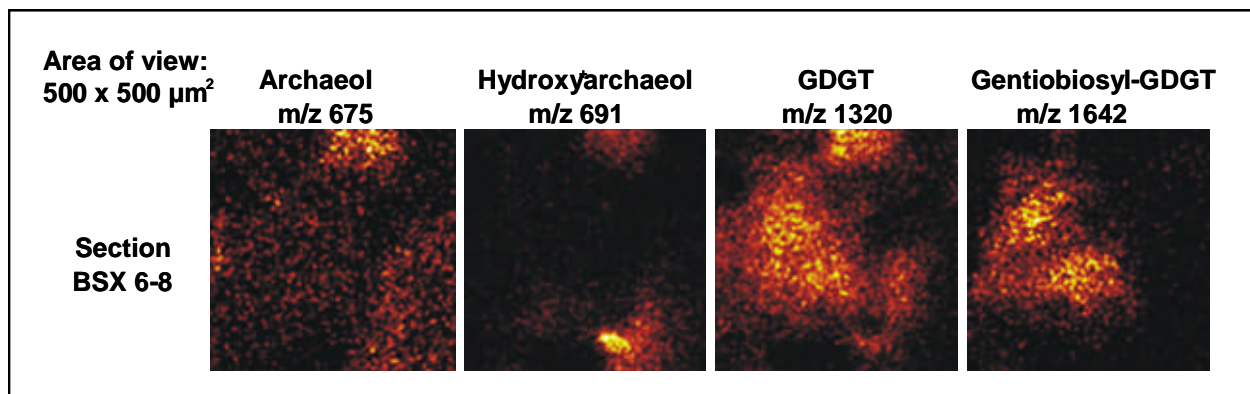


Figure 1. ToF-SIMS images of archaeal lipid biomarkers (as sodium adducts $[\text{M}+\text{Na}]^+$) on a microscopic section of a methanotrophic microbial mat from the Black Sea, revealing the lipid patterns of individual microbial colonies. Brightness corresponds to relative signal yield.

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ORIGIN OF 4-DESMETHYL-DINOSTERANES IN SEDIMENTS AND OILS

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Dinosterol (4 α ,23,24-trimethyl-5 α -cholest-22E-en-3 β -ol) is a well-known sterol synthesized by dinoflagellates. The presence of dinosterol and its diagenetic products, dinosterane and triaromatic dinosteroids, in sediments is generally accepted as a marker for dinoflagellate productivity (e.g. Boon et al., 1979), although a few other sources are known. In addition to dinosterol, other sterols possessing side-chains methylated at positions C-23 and C-24 are assumed to be specific for dinoflagellates, even though 4-desmethyl-23,24-dimethylsterols have been reported in a number of other organisms (e.g. Volkman et al., 1980). Moldowan and Jacobson (2000) showed that their diagenetic products, triaromatic 4-desmethyl-dinosteranes (23,24-dimethylcholestanes), can be useful as age-diagnostic biomarkers as they almost exclusively occur in oils and marine source rocks from the Triassic and younger. Still, there are only a few reports on 4-desmethyl-dinosterane (e.g. Schouten et al. 1997).

We have analyzed >100 cultures of different diatom species and although dinosterol was not found, we identified relatively abundant 23,24-dimethylsterols in twenty-one diatoms belonging to six different orders, indicating that diatoms may be an important source for these sterols and their diagenetic products. For unambiguous identification, we isolated 23,24-dimethylcholest-22E-en-3 β -ol (4-desmethyl-dinosterol) from a 300L culture of *Ditylum brightwellii* (sterol composition shown in Fig. 1) and determined its structure using NMR. An aliquot of the purified 23,24-dimethylsterol was used to synthesize a 4-desmethyl-dinosterane. The mass spectrum of 4-desmethyl-dinosterane is very similar to that of 24-ethylcholestane, but 4-desmethyl-dinosterane has an enhanced m/z 98 ion fragment, caused by cleavage in the methylated side chain.

Partial mass chromatograms of m/z 98 from desulphurized samples of the Miocene Monterey Formation (Schouten et al., 1997) revealed four putative 4-desmethyl-dinosterane isomers, likely with varying C-5 and C-23 configurations. In the desulphurized fractions from the Miocene Monterey Formation, 23S,24R-dimethyl-5 α -cholestane is the most dominant isomer, followed by 23R,24R-dimethyl-5 α -cholestane; the latter co-elutes with 24-ethylcholestane, something also observed for dinosterane and 24-ethyl-4-methylcholestane

(Summons et al., 1987). This co-elution may cause an overestimation of 24-ethylcholestane concentrations, as the presence of 23,24-dimethylcholestane is easily overlooked.

Moldowan and Jacobson (2000) have shown that dinosterane and triaromatic dinosteroid concentrations quickly increased to maximum values in the Triassic, whereas the concentration of triaromatic 4-desmethyl dinosterane started to increase from the Triassic but reached its maximum value only during the Cretaceous. This difference in sterane concentration profiles may be caused by increasing 4-desmethyl dinosterol contributions of diatoms, which do not synthesize dinosterol, as diatoms evolved during this time. Thus, the occurrence of 23,24-dimethylcholestanes in sediments should not automatically be related to dinoflagellate input, but a diatomaceous origin should also be taken into consideration.

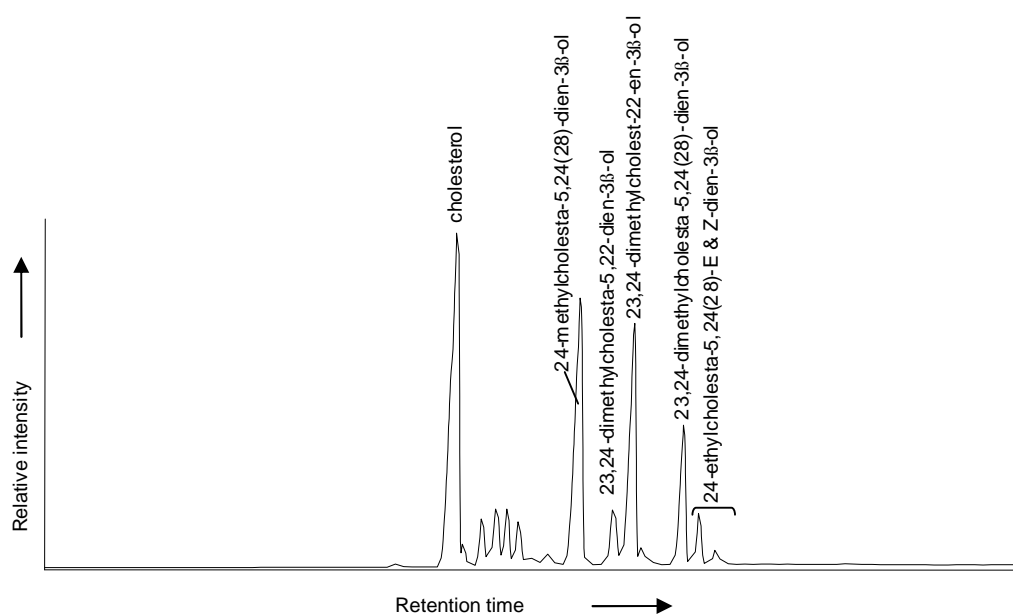


Figure 1. FID chromatogram of the sterol fraction of *Ditylum brightwellii*

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UNEXPECTED DEMETHYLATED HOPANES AND HOMOHO PANES IN OILS AND ROCK EXTRACTS FROM A BRAZILIAN MARGINAL BASIN

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The formation of 25-norhopanes by the microbial removal of the methyl group at C-10 in the hopanes nucleus during petroleum biodegradation is well documented in the literature (Peters *et al.*, 2005). On the other hand, the presence of 25-norhopanes in apparently non-biodegraded or slightly biodegraded oils is also observed. Is the presence of 25-norhopanes in this case suggesting an intense biodegradation event in the initial phases of reservoir filling? Or is there a possible source component of 25-norhopanes which was present since petroleum expulsion?

To investigate these hypotheses, quantitative analyses have been performed to evaluate the presence and abundance of the series of 25-norhopanes ranging from C₂₆ to C₃₄ in a set of oil samples with an increasing level of biodegradation, as well as in related immature source rocks from a Brazilian marginal basin.

The series of demethylated hopanes has been identified by MRM-GC-MS analyses in all oil samples, regardless of the aspect of the GC trace and the API gravity. Hence, even samples in which the GC trace showed no or slight biodegradation contained all the series of demethylated compounds, although the series from C₃₀-C₃₄ is present in very low amounts (< 5ppm in the saturated fraction). Two hypotheses can be advanced for the generalized presence of demethylated hopanes. On the one hand, it is possible that a certain background amount was present in the original oil since the onset of reservoir filling. In this case, the varying abundance of 25-norhopanes could be interpreted as the result of their relative enrichment due to different extents of biodegradation on a pristine oil already containing demethylated hopanes. On the other hand, reservoir filling can be envisaged as a continuous process in which alternate discrete events of charging and biodegradation follow in close succession. In the more intense biodegradation events, 25-norhopanes would be formed by demethylation of their hopane precursors. The formation of demethylated hopanes would be related only to bacterial activity on the hopanes compounds after the depletion of normal and branched alkanes.

Quantitative results of 25-norhopanes in extracts of potential source rocks in an immature stage demonstrate that in most extracts this compound series is present in very low concentrations. Hence, one can admit that a background amount of demethylated hopanes is

source-derived and inherited from the originally expelled oils, being present in oils submitted to no or slight biodegradation. Thereafter, with increasing biodegradation, their concentrations in the oil would relatively increase due to the greater susceptibility of other compounds to bacterial attack. However, our results indicate that the concentrations of 25-norhopanes increase by almost two orders of magnitude in the most biodegraded oils, and this increase cannot be explained solely by the enrichment related to the estimated maximum oil mass loss due to biodegradation, which is around 50-60% (Larter *et al.* 2005). Therefore, neoformation of 25-norhopanes by demethylation of their hopane precursors must be invoked to explain the increase of their concentrations in the studied oils. Our quantitative results established that there is an inverse relation between the hopane precursor (C₃₀ hopane) and its demethylated product (C₂₉ 25-norhopane) with increasing biodegradation. Furthermore, it is worth noticing that the decrease of C₃₀ hopane is not completely compensated by the formation of C₂₉ 25-norhopane, as also indicated by Bennett *et al.* (2005).

According to the new data obtained on Brazilian oils and their related source-rock extracts, the presence of the 25-norhopane series in oils can be interpreted as the combined result of processes previously considered under two independent hypotheses. The fact that apparently non-biodegraded oils contain 25-norhopanes can be explained by a source component of these compounds since petroleum expulsion. Therefore, the presence of these compounds in small amounts (< 5 ppm) cannot serve as a definitive indication that a paleobiodegradation event has occurred. From this point on, with the progress of biodegradation, source-derived 25-norhopanes will be relatively enriched in the oils. Concomitantly, as saturates are severely affected by biodegradation, neoformation of 25-norhopanes from their hopane precursors will occur. Nevertheless, not all hopanes are transformed into 25-norhopanes, and the extent of conversion of one into the other is quite variable. Hence, a unique scale relating extent of biodegradation and amounts of 25-norhopanes seems not to exist.

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