

## UNRAVELLING AN ANAMMOX BIOMARKER FOR PALEO-STUDIES: NMR ANALYSIS OF THE BACTERIOHOPANETETROL STEREOISOMER

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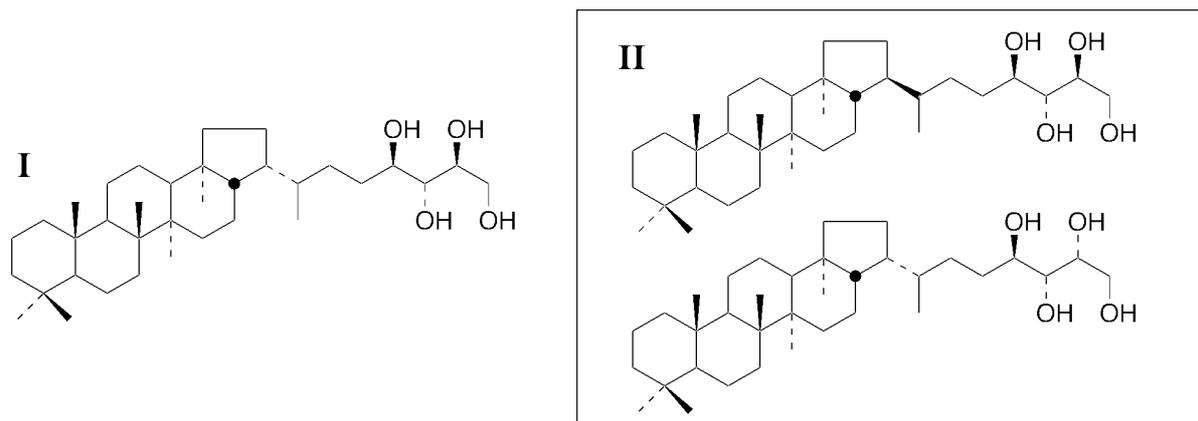
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Anaerobic ammonium oxidising (anammox) bacteria play a critical role in the marine nitrogen cycle. The anammox reaction removes bioavailable nitrogen by converting ammonium and nitrite into dinitrogen gas. By doing this, it provides a control on the availability of a limiting nutrient for primary productivity. As anammox occurs in anoxic or low-oxygen environments, studying the relationship between the density of marine oxygen minimum zones and anammox in paleoenvironments is particularly significant to understanding the impact of the increasing quantity of oxygen minimum zones resulting from ongoing anthropogenic climate change. Understanding anammox within the nitrogen cycle is key to understanding modern and paleo marine ecosystems, and to projecting the impacts of future climate change. Traditionally ladderane lipids have been used as biomarkers for anammox (Sinninghe Damsté et al., 2002). However, these lipids are not recalcitrant over extended periods of time, and the presence and magnitude of anammox in paleosediments cannot be evaluated until a more suitable biomarker has been identified. This biomarker must be produced by anammox bacteria, must be preserved over time and must have a known structure, distinct from biomarkers produced by other biological sources. The bacteriohopanetetrol stereoisomer (BHT isomer) is known to be produced at high abundances by marine anammox bacteria (Rush et al., 2014). The BHT isomer has the same elemental composition but distinct stereochemistry from the more ubiquitous BHT (Peiseler and Rohmer, 1992), making it a strong candidate as a biomarker for anammox bacteria. Similar bacteriohopanepolyols (BHPs) have been found to be preserved for up to 50 Ma (van Dongen et al., 2006), highlighting the paleoreconstruction potential of BHT isomer. However, the exact stereochemistry of the BHT isomer has not been established. Non-marine, non anammox bacteria (e.g. *Frankia* spp.; Rosa-Putra et al., 2001) are also known to produce BHT isomers, making it important to establish the exact stereochemistry of the BHT isomer produced by marine anammox bacteria.

Using preparatory liquid chromatography, we will isolate the BHT isomer from cell material of marine anammox (*Scalindua* sp.), known to synthesise BHT isomer (Rush et al., 2014). The pure compound will then be identified using high-resolution two dimensional nuclear magnetic resonance (2D-NMR) to establish the three-dimensional stereochemical structure (Figure 1). This knowledge will validate the application of the BHT isomer in studies of paleo-anammox. It also has implications for our understanding of the synthesis and role of BHPs in bacteria, as it demonstrates the stereochemistry of the type of BHP produced in oxygen-poor environments by anammox bacteria, which can be compared to the habitat and phylogeny of other bacteria that produce the BHT isomer. Complimentary work applies the BHT isomer biomarker in a variety of contexts including Mediterranean sapropels, sediments underlying oxygen minimum zones (e.g. from the Peru margin) and immature sediments from Cretaceous oceanic anoxic events. This allows us to provide the first records of anammox activity from sediments older than 145 ka, which is the previous oldest record of anammox activity.



**Figure 1** Chemical structures of (I) bacteriohopanetetrol (BHT), and (II) two possible structures of BHT stereoisomer (BHT isomer). Two-dimensional nuclear magnetic resonance experiments will determine the exact stereochemistry of BHT isomer.

## References

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