

A SEARCH FOR MACROMOLECULAR SOURCES OF LONG CHAIN ALKYL DIOLS

S. Reiche^{1*}, E.C. Hopmans¹, J.S. Sinninghe Damsté^{1,2}, S. Schouten^{1,2}

¹ NIOZ Royal Netherlands Institute for Sea Research, and Utrecht University, The Netherlands

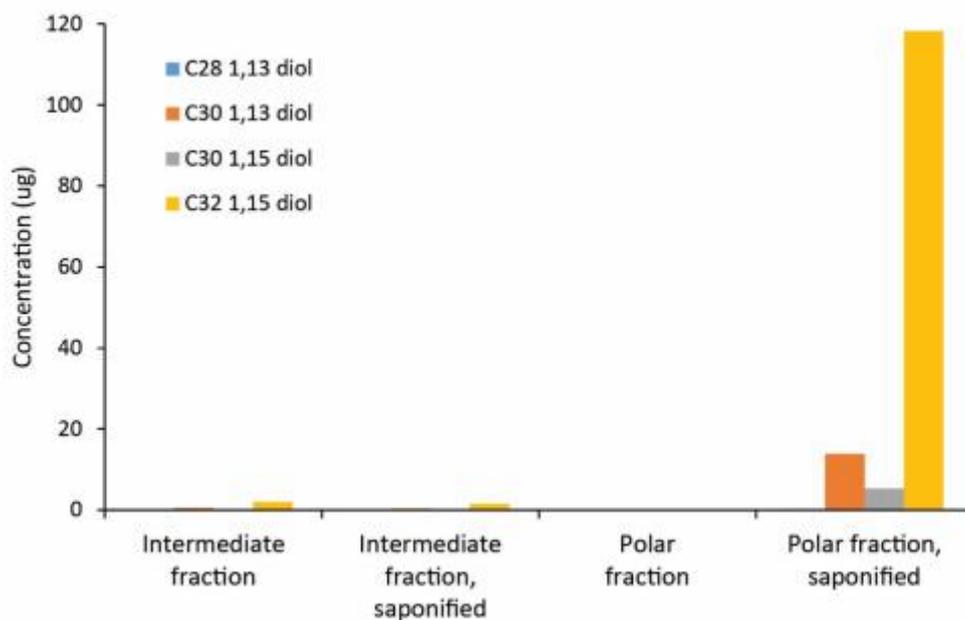
² Faculty of Geosciences, Utrecht University, The Netherlands

(*corresponding author: Sophie.reiche@nioz.nl)

Long chain alkyl diols are ubiquitously found in marine and lacustrine environments and are built up of a C₂₈ to C₃₂ alkyl chain with hydroxy groups at positions 1,13, 1,14 or 1,15 (Versteegh et al., 1997). They have been identified in *Proboscia* diatoms (Sinninghe Damsté et al., 2003), *Apedinella radians* (Rampen et al., 2011) and Eustigmatophyte alga (Gelin et al., 1997) and in the latter are thought to be among the building blocks of the biopolymer algaenan (Gelin et al., 1997). In recent years, indices like the long chain diol index (Rampen et al., 2012) and the diol index (Rampen et al., 2008; Willmott et al., 2010) have been established and are increasingly used in paleo-environmental reconstructions.

Nonetheless open questions about long chain alkyl diols remain. Analyses of diols in Eustigmatophyte algae show that they are mainly released after base saponification and occur as bound-extractable, ester- and amide-bound compounds (Gelin et al., 1997; Grossi et al., 2001). However, the nature of possible macromolecular precursors, from which these diols are released, is unknown. In a first attempt, identification of these macromolecules was tried by analysis of *Nannochloropsis oculata* biomass by Ultra High Performance Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS), scanning for MS² products with fragments potentially related to diols. This proved unsuccessful as no such fragmentation products could be identified which could be linked to long chain alkyl diols, probably due to the large abundances of intact polar lipids masking the signal.

In an alternative approach, we attempted to isolate the macromolecules containing the long chain alkyl diols, by using normal phase semi-preparative HPLC, following the method described by Bauersachs et al. (2009). Eluents were collected in 1 min fractions and aliquots of selected fractions were saponified in order to release diols from their precursors. In this first step, no bound-extractable diols could be identified in any of the collected fractions and it is hypothesized that precursors of long chain alkyl diols did not elute off the column using normal phase HPLC, possibly due to their high polarity. Taking these results into account, reverse phase semi-preparative HPLC was applied, following a method adapted from Zhu et al. (2013) to allow for elution of compounds with higher polarity. Furthermore, a cleaning step was employed by elution of biomass extract over an aluminum oxide column to remove free diols (Fig. 1). Following the isolation protocol as described above we aim to identify fractions that contain bound-extractable diols which will subsequently be reanalyzed by UHPLC-HRMS. Once these macromolecules are identified, we will develop an UHPLC-HRMS method to enable the environmental screening of these molecules.



*Fig.1: Yield of free and free+ bound-extractable diols, as assessed by saponification, by fractionation of *N. oculata* biomass over an aluminum oxide column. Free diols eluted in the intermediate fraction, while the polar fraction contained only bound-extractable diols. Therefore, the polar fraction was chosen for reverse phase semi-preparative HPLC analysis to identify macromolecular precursors.*

References:

- Bauersachs, T. et al., 2009 *Rapid Communications in Mass Spectrometry* 23, 1387-1394.
 Gelin, F. et al., 1997 *Org. Geochem* 26, 659-675.
 Grossi, V. et al., 2001 *Organic Geochemistry* 32, 795-808.
 Rampen, S.W. et al., 2008 *Earth and Planetary Science Letters* 276, 207-213.
 Rampen, S.W. et al., 2011 *Organic Geochemistry* 42, 572-574.
 Rampen, S.W. et al., 2012 *Geochimica et Cosmochimica Acta* 84, 204-216.
 Sinninghe Damsté, J.S. et al., 2003 *Geochimica et Cosmochimica Acta* 67, 1339-1348.
 Versteegh, G.J.M. et al., 1997 *Organic Geochemistry* 27, 1-13.
 Willmott, V. et al., 2010 *Antarctic Science* 22, 3-10.
 Zhu, C. et al., 2013 *Organic Geochemistry* 65, 53-62.