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Concurrent purification of sterols, triterpenols and alkenones from sediments for hydrogen isotope analysis using high performance liquid chromatography

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ABSTRACT

Three methods are presented on how to purify acetylated sterols, acetylated triterpenols and individual alkenones for hydrogen isotope analysis from marine and lacustrine sediments using reverse-phase high performance liquid chromatography (RP-HPLC). The main advantages over previous HPLC methods are reduced operator time, increased automation and the ability to simultaneously purify multiple target compounds from a sample. These gains are achieved primarily by acetylating compounds prior to purification rather than after, and also by using a fraction collector with semi-preparatory rather than analytic configuration. The effectiveness of the method is demonstrated for (i) dinosterol and taraxerol in sediment from the brackish pond Poza del Diablo, Galápagos, (ii) for di- and tri-unsaturated C_{37} and C_{38} alkenones in cultured *Emiliania huxleyi*, (iii) for brassicasterol, and di-, tri- and tetra-unsaturated C_{37} alkenones in sediment from Manito Lake, Saskatchewan, Canada, and (iv) for brassicasterol, dinosterol and di-, tri- and tetra-unsaturated C_{37} alkenones in sediment from the Great Salt Lake, Utah. The purification process yields 80–90% recoveries and results in no measurable hydrogen isotope alteration.

1. Introduction

Selecting appropriate compounds for hydrogen isotope analysis of biomarkers in sediments for paleoenvironmental applications is often a balance among the biological source, its specificity and the relative ease of purification. The hydrogen isotopic composition of both terrigenous and aquatic lipid biomarkers are increasingly being used as hydrologic indicators. But their value is offset from the hydrogen isotopic composition of the environmental water due to a combination of biologically controlled isotopic fractionations, many of which are sensitive to secondary environmental parameters such as humidity, salinity or the growth rate of the organism (e.g. Schouten et al., 2006; Zhang and Sachs, 2007; Sachse and Sachs, 2008; Wolhowe et al., 2009; Zhang et al., 2009; Douglas et al., 2012). Many applications of hydrogen isotope measurements for paleoenvironmental investigation have attempted to work around these issues by targeting compounds that are produced by a wide range of organisms, such as leaf waxes, with the hope that the biological factors average out and that the isotope signal is reflective of the precipitation signal common to each compound-producing organism. Long chain *n*-alkanes and *n*-alkanoic acids are the most common leaf wax compounds measured. These are produced in high abundance by higher land plants, are relatively easy to purify from sedimentary lipid extracts, and many calibration studies have been performed in modern environments to demonstrate an empirical link between their hydrogen isotopic composition and that of local precipitation (Sachse et al., 2012). However, some calibration efforts have identified additional controls on the hydrogen isotopic composition of these compounds including humidity, salinity and shifting vegetation assemblages (Douglas et al., 2012; Ladd and Sachs, 2012; Nelson et al., 2013).

Although many of these shortcomings are not unique to leaf wax compounds, it is increasingly clear that the isotope signal recovered from any single molecule may not always be a reliable indicator of past hydrologic conditions. Nevertheless, the hydrogen isotopic composition of biomarkers remains a useful hydrologic indicator, provided that the complicating issues are understood and managed appropriately. Additional paleohydrologic proxy data may therefore be of value in providing supporting and complimentary information on which to interpret an isotope record from a single compound. One such strategy is to extend the investigation to include additional biomarkers since laboratories that are equipped to measure the hydrogen isotopic composition of one compound are well positioned to measure additional compounds. Lipids with higher degrees of source specificity and alternate sources are likely to be effective compliments.

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Sterols and triterpenols tend to be more source-specific than *n*-alkanes and *n*-alkanoic acids. Examples among these include dinosterol (4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol), which is produced primarily by dinoflagellates (Volkman et al., 1998; Volkman, 2003), and has been the target of previous hydrogen isotope calibration (Sachs and Schwab, 2011), and paleoclimate studies (Sachs et al., 2009; Smittenberg et al., 2011). Brassicasterol (24-methyl cholest-5,22-dien-3β-ol) has been applied as a biomarker in sediments to indicate the presence of diatoms, although it is also produced by other microalgae including haptophytes and cryptophytes, and can also be found in some higher plants (Volkman, 2003). However, the viability of the hydrogen isotopic composition of brassicasterol as a paleoenvironmental indicator has not been thoroughly evaluated. Taraxerol (3β-D-friedooleanan-14-en-3-ol) is a pentacyclic triterpenoid that is produced in high abundance by *Rhizophora* mangroves and has been shown to covary in sediments with *Rhizophora* pollen (Versteegh et al., 2004). This highly source specific compound presents an attractive target for evaluating the hydrogen isotope signal from higher plants in coastal tropical and subtropical environments, particularly given the recently demonstrated complexity associated with the hydrogen isotopic composition of leaf waxes from mangroves, and the potential for mixed mangrove and non-mangrove sources of *n*-alkanes and *n*-alkanoic acids (Ladd and Sachs, 2012). Alkenones (unsaturated C_{37-39} methyl and ethyl ketones), though not sterols or triterpenols, are specific to a relatively small number of haptophyte algae in both marine and lacustrine environments (Volkman et al., 1980; Conte et al., 2006; Theroux et al., 2010). Alkenones are the basis of the well established $U^{k'}_{37}$ sea surface temperature proxy (Brassell et al., 1986; Conte et al., 2006), have been the focus of previous hydrogen isotope investigations (Englebrecht and Sachs, 2005; Pahnke et al., 2007; van der Meer et al., 2007; Schwab and Sachs, 2011; Leduc et al., 2013) and are therefore also attractive targets for continued application. The potential for added perspective on paleoenvironmental conditions from δD measurements on algal biomarkers is already well documented, particularly when paired with δD measurements from higher plants, which together may reveal greater insight on paleoaridity than would be possible from either type of compound on its own (Sachse et al., 2004; Mügler et al., 2008).

Unfortunately, sterols and triterpenols that are specific to a particular organism or class of organisms such as those described above are not well resolved by gas chromatography–isotope ratio mass spectrometry (GC–IRMS) after a single extraction or compound class fractionation by aminopropyl or silica column chromatography. Existing methods developed for geochemical application using high performance liquid chromatography (HPLC) have proven effective for purifying dinosterol (Smittenberg and Sachs, 2007; Atwood and Sachs, 2012) and individual alkenones (Schwab and Sachs, 2009) from some sediment matrices, but these methods are all time consuming, require near constant operator attention and extensive post-purification handling procedures and include multiple steps where mishandling can induce isotopic fractionation. They are each also not well developed for purifying multiple target compounds from the same sample injection. Applications of HPLC based purification strategies for compound specific hydrogen isotope analysis by geochemists have been somewhat limited, but the approach has recently gained ground in the sports doping testing field (e.g. Piper et al., 2011, 2012, 2013). Literature reviews of other disciplines suggest that HPLC purification for compound specific hydrogen isotope analysis has not yet been implemented, although examples of HPLC purification for bulk isotope analysis exist, such as vanilla purification for food science applications (Lamprecht et al., 1994). HPLC based steroid purification techniques for purposes other than hydrogen isotope analysis have been applied more widely, including purification of steroids from coral (Jin et al., 2005) and mushrooms (Mattilaa et al., 2002), although many applications are analytical rather than semi-preparatory (e.g. McDonald et al., 2012).

Continued improvement of HPLC based compound purification procedures is important for eliminating the risk of compromising the sample and also for increasing sample throughput since paleoenvironmental applications require large numbers of analyses. Toward these ends we present a series of new HPLC based methods for purifying sterols, triterpenols and alkenones from complex sample matrices typical of lacustrine and marine sediment lipid extracts.

2. Methods

2.1. Samples and lipid extraction

Four samples were used to demonstrate the new HPLC methods that we present. A sediment sample from Poza del Diablo was collected in June 2008 using a Livingstone type coring device (Geocore, Columbus, OH). Surface sediment from Manito Lake, Saskatchewan, Canada, was collected in 2007 using a Van Veen sediment sampler (Bowman and Sachs, 2008), and surface sediments from the Great Salt Lake were collected as a part of the same field campaign. Excess material from an *Emiliania huxleyi* culturing experiment was collected after the conclusion of controlled conditions and passed through a 0.7 μm glass fiber filter (Whatman GF/F). This material was used only as a known source of readily available alkenones for method development purposes and is not representative of any known temperature or culture water δD value. All samples were freeze dried and extracted in a 9:1 mixture of dichloromethane (DCM) and methanol (MeOH) on an accelerated solvent extractor (ASE) Dionex 200 operated at 100 °C and 1500 psi with three 5-min static phases. Excess solvent was evaporated under N_2 from the total lipid extract (TLE) on a Turbo-vap system (Caliper, Hopkinton, MA, USA).

2.2. Pre-HPLC compound class separations

The sediment samples used to demonstrate the methods presented here were each a part of separate studies with independent goals, while the culture material was used only for HPLC method development. Due to the varied nature of the samples and their associated projects, pre-HPLC sample cleanup differed between the samples, but we note that no particular sample cleanup is preferred over another provided that the sample is soluble in the HPLC injection solvent when the target compounds are present at adequate concentration for hydrogen isotope analysis after purification. Galápagos, Great Salt Lake and *E. huxleyi* TLEs were eluted from glass solid phase extraction columns that were hand packed with 1 g of silica gel 60 (5% deactivated by weight; EMD chemicals, 35–75 μm). Hydrocarbon fractions were eluted with 10 ml of hexane, ketones with 6 ml DCM:hexane (1:1), alcohols with 8 ml ethyl acetate (EtOAc):hexane (1:4), followed by a polar fraction with 6 ml methanol. The Galápagos alcohol fraction, the *E. huxleyi* ketone fraction, and the combined ketone–alcohol fraction from the Great Salt Lake were purified further by HPLC. The Manito Lake TLE was separated into polar and non-polar fractions using a liquid–liquid extraction by dissolution in 2 ml of methanol, to which 2 ml of hexane was added. The mixture was then agitated and sonicated to ensure complete dissolution of the TLE. The solvents were allowed to separate, after which the hexane supernatant was removed by Pasteur pipette. This was repeated ten times with the supernatants combined. To assess recovery, 5% aliquots of the methanol and hexane fractions were removed and evaluated by gas chromatography–mass spectrometry (GC–MS), which revealed