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An Efficient Method for Isolating Individual Long-Chain Alkenones for Compound-Specific Hydrogen Isotope Analysis

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Hydrogen isotope ratios ($^2\text{H}/\text{H}$ or D/H) of long-chain unsaturated ketones (alkenones) preserved in lake and marine sediments hold great promise for paleoclimate studies. However, compound-specific hydrogen isotope analysis of individual alkenones has not been possible due to chromatographic coelution of alkenones with the same carbon chain length but different numbers of double bonds. Published studies have only reported the δD values of the mixture of coeluting alkenones. We developed an efficient procedure to isolate individual alkenones based on double-bond numbers using silica gel impregnated with silver nitrate. The chromatographic procedure is simple, inexpensive, and highly reproducible, offers 87–100% sample recovery, and allows for the first time hydrogen isotopic measurement on individual alkenones. δD values of specific di-, tri- and tetraunsaturated C_{37} alkenones produced by an *Emiliania huxleyi* culture, as well as those isolated from Greenland lake sediments, differ consecutively by 43–65 ‰. These findings suggest that alkenones with different numbers of carbon–carbon double bonds express significantly different δD values and that coelution of different alkenones may lead to erroneous source water δD reconstructions. Our alkenone isolation approach opens a new avenue for paleoclimate reconstructions using hydrogen isotope ratios of individual alkenones.

Long-chain (C_{37} – C_{39}) unsaturated methyl and ethyl ketones (alkenones) are an important class of lipid compounds for paleoclimate reconstruction.¹ These compounds are only produced by certain members of the algal class Prymnesiophyceae (reviewed by Conte et al.²) and contain varying numbers of double bonds. The relative proportion of di-, tri-, and tetraunsaturated C_{37} alkenones (expressed as the U_{37}^K index) produced by the algae depends on the algal growth temperature,^{1,3,4} and this relationship has been widely exploited for reconstructing past sea surface temperatures from alkenones preserved in ocean sediment cores.

Alkenones are also of distinctive value for reconstructing D/H ratios of their source water. Since these lipids are produced solely by aquatic algae, D/H ratios of alkenones should directly reflect that of the source water from which they were biosynthesized.^{5–9} Short-chain *n*-alkanoic acids (e.g., palmitic acid) have been used for this purpose;^{6,7,10} however, these compounds are produced by a whole host of aquatic and terrestrial organisms with potentially different water sources and isotope fractionation relative to that of the source water, limiting the fidelity of the proxy in certain systems. Algal sterols are also potential candidates for source water D/H reconstruction⁵ but again are not as biologically specific as alkenones.

The D/H ratio (like $^{18}\text{O}/^{16}\text{O}$) of ocean water is primarily a function of global ice volume and can also reflect the amount of fresh water input in coastal areas and excessive evaporation from warm oceans (e.g., the Red Sea¹¹). D/H of lake water is largely controlled by that of precipitation as well as the amount of precipitation relative to evaporation.^{12,13} Thus, the hydrogen isotopic compositions of ocean and lake waters are a direct consequence of climatic and environmental conditions.^{14,15} D/H reconstructions of ancient water bodies based on sedimentary alkenone D/H can thus offer valuable insight into past variability of oceanic and atmospheric systems.

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Unfortunately, gas chromatographic (GC) techniques are unable to adequately resolve different alkenone compounds for compound-specific hydrogen isotopic measurement. Flame ionization detection (FID) is commonly used to quantify alkenone concentrations and to determine the U_{37}^K index and requires very small quantities for accurate measurements. Only ~ 1 ng of each alkenone compound needs to be injected onto the GC column for FID measurements; this amount is well below the capacity of typical GC columns. Each alkenone compound can therefore be fully resolved by GC-FID. However, isotope ratio mass spectrometry (IRMS) requires relatively large amounts of analyte gas (e.g., CO_2 for $^{13}\text{C}/^{12}\text{C}$, H_2 for D/H) for isotope ratio measurements, and hydrogen isotope analyses require up to 10 times the molar concentration of H than the C required for carbon isotope analyses.¹⁶ The IRMS requirements call for the injection of up to 70 ng of each alkenone compound for $^{13}\text{C}/^{12}\text{C}$ measurement and up to 400 ng of each desired alkenone for D/H measurement. These large quantities (particularly for D/H) exceed the capacity of typical GC columns and lead to chromatographic coelution of alkenones with the same carbon chain length but different numbers of double bonds. Due to incomplete compound resolution, D/H ratios of alkenones have previously been determined by measuring the D/H ratio of the mixture of coeluting alkenones of a given chain length.^{8,9} This approach is reasonable if the D/H ratios of the different alkenone compounds are identical. However, if there are significant differences among D/H ratios of the coeluting alkenones, the weighted-average D/H will be influenced by the relative proportions of the coeluting compounds, potentially obscuring attempts at source water D/H reconstruction.

Here, we report a silver nitrate-impregnated silica gel column chromatographic procedure for separating alkenones based on the number of double bonds. This procedure enables complete resolution of individual alkenone compounds for hydrogen isotope measurement by GC/IRMS. We also show that C_{37} alkenones with different degrees of unsaturation have very different D/H ratios; hence, future studies may require measurements of individual alkenones for paleoclimate reconstructions.

EXPERIMENTAL SECTION

Chemicals and Reagents. Silver nitrate-impregnated silica gel (10 wt %, 200+ mesh) was purchased from Sigma-Aldrich. HPLC grade dichloromethane and methanol were acquired from Burdick & Jackson, ethyl acetate was from Mallinckrodt, and toluene was from J. T. Baker. *n*-Heptatriacontane was purchased from Aldrich, and Vienna standard mean ocean water (VSMOW) was purchased from the National Institute of Standards and Technology. Isotope reference materials (*n*-alkanes) were provided by Arndt Schimmelmann at Indiana University.

Sample and Silver Nitrate–Silica Gel Column Preparation. Sediment samples were freeze-dried and homogenized using a mortar and pestle. They were extracted with dichloromethane (DCM) using an Accelerated Solvent Extractor ASE200 (Dionex). A sample size of 0.5–1.5 g of Greenland lake sediment¹⁷ was used for extraction. Extracts were separated into acid and neutral fractions using Supelco Supelclean LC- NH_2 solid-phase extraction (SPE) tubes.⁷ The neutral fraction was further separated into

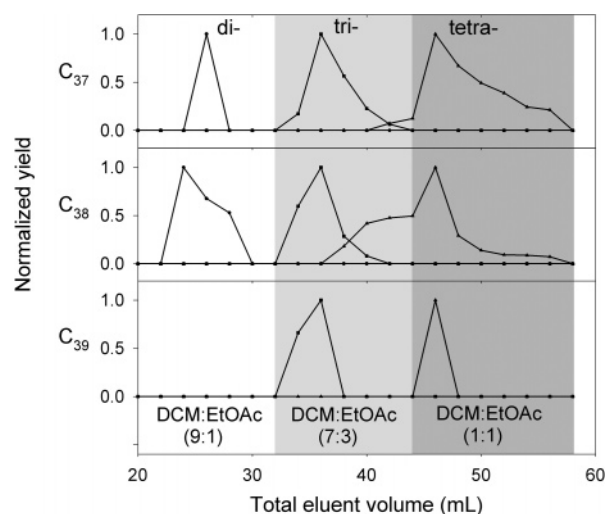


Figure 1. Normalized alkenone yield vs eluent volume for alkenone isolation by double-bond number using silver nitrate-impregnated silica gel liquid chromatography: top panel, C_{37} alkenones; middle panel, C_{38} alkenones; bottom panel, C_{39} alkenones. Shadings represent different solvents used as eluent. DCM = dichloromethane; EtOAc = ethyl acetate.

aliphatic hydrocarbon (hexane elution), ketone (DCM), and alcohol (ethyl acetate/hexane [1:3, v/v]) fractions using a flash silica gel column. The alkenones (in the ketone fraction) were quantified by gas chromatography–flame ionization detection (GC-FID) with *n*-heptatriacontane as internal standard.

The bottle of silver nitrate-impregnated silica gel (Ag + Si-gel) was wrapped in aluminum foil and stored in a drying oven at 50 °C to prevent photoreduction of the silver nitrate. Flash columns were prepared immediately prior to use with 2.1 g of Ag + Si-gel. Columns were tapped until compaction of the Ag + Si-gel ceased and wrapped in aluminum foil for the duration of the procedure to avoid photoreduction of the silver nitrate. The Ag + Si-gel was rinsed with at least four bed volumes of DCM and was kept saturated with DCM. After the elution procedure (see below) samples were run on GC-FID to assess the isolation of alkenones and to verify the complete removal of *n*-heptatriacontane standard. *n*-Heptatriacontane was then re-added to each fraction as an internal standard to quantify alkenone recovery.

Eluent Scheme. Samples were loaded onto a DCM-wetted Ag + Si-gel column and eluted with the following solvents: 20 mL of DCM, 12 mL of DCM/ethyl acetate (9:1, v/v), 12 mL of DCM/ethyl acetate (7:3, v/v), 14 mL of DCM/ethyl acetate (1:1, v/v), 20 mL of 100% ethyl acetate. Samples were collected in 2 mL increments to assess elution rates (Figure 1).

Instrumentation. Concentrations of individual compounds were evaluated using a Hewlett-Packard 6890 GC. A 30 m long, 0.25 mm \times 0.25 μm HP-1MS capillary column was used for GC-FID. The temperature program for the GC oven was held at 60 °C for 1 min, brought to 290 °C at 30 °C min^{-1} and held for 1 min, brought to 300 °C at 5 °C min^{-1} , then brought to 315 °C at 2 °C min^{-1} and held for 15 min. A Hewlett-Packard 6840+ GC–pyrolysis system interfaced to a Finnigan Delta+ XL stable isotope mass spectrometer through a high-temperature pyrolysis reactor was used for hydrogen isotopic analysis.⁷ Sessions¹⁶ provides an excellent review of isotope ratio detection for GC, including gas chromatography/thermochemistry–isotope ratio mass spec-

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Table 1. Columns Tested for Gas Chromatographic Optimization

column	length (m)	inner diameter (mm)	film thickness (μm)	width/height (s/mV)	theoretical plates ^a	resolution ^b
HP-1MS	60	0.32	0.50	0.040	91000	6.8
HP-1MS	60	0.32	0.25	0.028	194000	6.3
DB-1MS	60	0.32	0.10	0.026	105000	4.3
HP-1MS	60	0.25	0.25	0.028	85000	4.3
HP-1MS	30	0.25	0.25	0.019	73000	1.7
HP-5MS	30	0.25	0.25	0.076	20000	1.7
HP-1MS	15	0.25	0.25	0.058	17000	1.3

^a Theoretical plates (N) were calculated using the Gaussian 10% equation; $N = 18.42(\text{retention time/peak width at 10\% peak height})^2$.

^b Chromatographic resolution between $C_{37:3}$ and $C_{38:3}$ is defined as $2[(t_{R2} - t_{R1})/(W_1 + W_2)]$, where t_R is the retention time and W is the peak width at baseline.

trometry (GC/TC-IRMS), which was employed here. The precision (1σ) of the triplicate analyses was generally $<\pm 2\%$. Accuracy throughout the run was routinely checked by an injection of laboratory isotopic standards between every six injections, and IRMS accuracy is periodically assessed using n -alkane isotope references from Indiana University. Alkenones were identified by comparison of mass spectral data with previously reported standards and GC retention times.^{18,19}

Algal Cultures. We obtained a culture of the alkenone-producing alga *Emiliania huxleyi* (CCMP 374) from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton. Cultures were grown in f/2 medium²⁰ (using seawater from West Boothbay Harbor, Maine; $\delta D \sim 0\%$) at 20°C and were harvested during log-phase of growth. One liter of medium was filtered onto precombusted Whatman GF/F glass fiber filters (porosity of $0.7\ \mu\text{m}$). Filters were soaked once in methanol (MeOH) and twice in DCM/MeOH (1:1, v/v) to extract alkenones.

Terminology. We follow the standard delta notation ($\%$ VSMOW) for reporting hydrogen isotope values:

$$\delta D_{\text{sample}} = \frac{(R_{\text{sample}} - R_{\text{std}})}{R_{\text{std}}} \times 1000\ \text{‰} \quad (1)$$

where R is the absolute D/H ratio of a sample or the internationally accepted reference standard (VSMOW).

The fractionation factor (α) between reactant and product is defined as

$$\alpha_{\text{product-reactant}} = \frac{R_{\text{product}}}{R_{\text{reactant}}} \quad (2)$$

Isotopic enrichment (ϵ) between reactant and product is defined as

$$\epsilon_{\text{product-reactant}} = (\alpha_{\text{product-reactant}} - 1) \times 1000 \quad (3)$$

RESULTS AND DISCUSSION

Separation of Individual Alkenones. Alkenones can be effectively separated according to their degree of unsaturation

using Ag + Si-gel column chromatography (Figure 1). Saturated compounds elute more quickly than unsaturated compounds due to the affinity of the silver nitrate for electrons on the double bonds. Our internal standard, n -heptatriacontane, elutes with pure DCM and is completely separated from the alkenones. In the eluent scheme described above, diunsaturated alkenones ($C_{37:2}$, $C_{38:2}$, $C_{39:2}$) elute with DCM/ethyl acetate (9:1, v/v), triunsaturated alkenones ($C_{37:3}$, $C_{38:3}$, $C_{39:3}$) elute with DCM/ethyl acetate (7:3, v/v), and tetraunsaturated alkenones ($C_{37:4}$, $C_{38:4}$, $C_{39:4}$) elute with DCM/ethyl acetate (1:1, v/v). We tested various solvent elution schemes and found sequentially increasing solvent polarities afforded better isolation of target compounds than using a single solvent throughout. By collecting 12 mL of DCM/ethyl acetate (9:1, v/v) in one collection vial, 12 mL of DCM/ethyl acetate (7:3, v/v) in another vial, and 14 mL DCM/ethyl acetate (1:1, v/v) in a third vial, we routinely obtained fractions with cleanly separated C_{37} and C_{39} di-, tri-, and tetraunsaturated alkenones. C_{38} alkenones in marine and certain lacustrine sediments (including the Greenland lake sediments) comprise both methyl and ethyl ketones which apparently interfered with the complete Ag + Si-gel separation of $C_{38:4}$ and $C_{38:3}$ (Figure 1). However, while $C_{38:4}$ is abundant in the Greenland lake sediment, it is not present in most marine sediments, making this isolation step unnecessary. By performing GC-FID quantification before and after Ag + Si-gel column chromatography (with n -heptatriacontane as internal standard), alkenone recovery was determined to be 87–100%. We tested whether the incomplete recovery of alkenones from the Ag + Si-gel resulted in hydrogen isotope fractionation by eluting the same sample through a Ag + Si-gel column three times. The δD values of triunsaturated alkenones remained the same after each elution, indicating that the procedure does not result in hydrogen isotope fractionation.

Optimization of GC Operation Conditions. One major benefit of making δD measurements on isolated alkenones rather than the mixture of coeluting alkenones is the improved accuracy associated with narrower peak baseline widths. Narrower peak width results in improved measurement accuracy through the incorporation of less background hydrogen into the measurement. We tested various GC operating conditions to optimize the peak shape of isolated alkenones. Seven different GC columns were tested (Table 1) allowing comparison of column length and film thickness, as well as a number of different temperature programs, inlet temperatures, and carrier gas flow rates (Figure 2). The optimization test results are reported in Figure 2 as both width/height (W/H) ratios and theoretical plates (N) using the Gaussian

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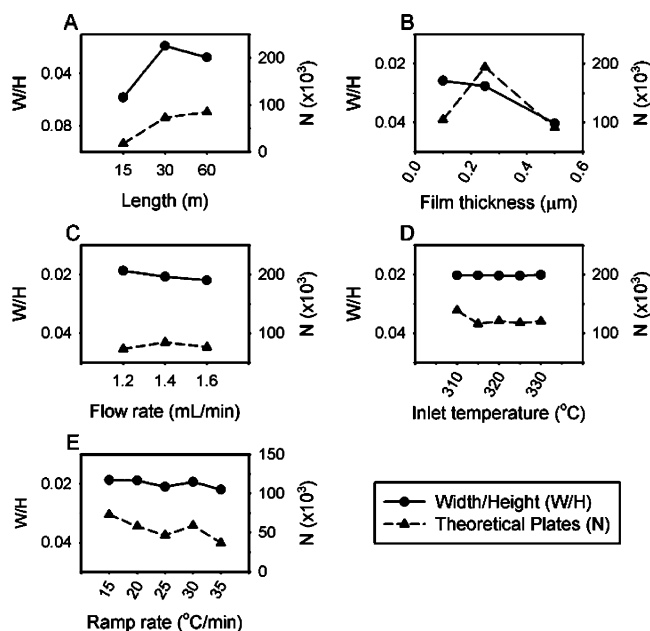


Figure 2. Optimization test results for shape (W/H = baseline width/maximum peak height [s/mV]) and efficiency (N = theoretical plates at 10% of peak height) of isolated $C_{37:3}$ alkenone peaks using GC/TC-IRMS: (A) GC column length, (B) film thickness, (C) carrier gas flow rate, (D) inlet temperature, and (E) rate at which the temperature was increased from 280 to 315 °C. Note the differences in the y-axis scaling among panels.

10% equation. All columns had chromatographic resolution (R) of the $C_{37:3}$ and $C_{38:3}$ peaks better than 1.5 (i.e., complete baseline separation), except for the 15 m HP-1MS column (Table 1).

The best peak shape (lowest W/H ratio) was achieved using the 30 m HP-1MS column, 315 °C inlet temperature, 1.2 mL/min flow rate, and the following temperature program: 40 °C for 1 min → 280 °C at 15 °C min⁻¹ → 315 °C at 2 °C min⁻¹ and hold for 20 min. Reducing the flow rate below 1.2 mL/min made negligible difference in W/H ratios.

GC/TC-IRMS traces of alkenone peaks prior to and following the Ag + Si-gel isolation are shown in Figure 3. Clearly, even with the best operating conditions, it was not possible to separate $C_{37:2}$, $C_{37:3}$, and $C_{37:4}$ by GC/TC-IRMS prior to Ag + Si-gel chromatography (upper panel, Figure 3).

δD Measurements of Specific Alkenones. δD measurements of Ag + Si-gel-separated alkenones reveal that the individual C_{37} alkenones have different hydrogen isotopic compositions (Tables 2 and 3). Triunsaturated C_{37} alkenones ($C_{37:3}$) isolated from the *E. huxleyi* culture (CCMP 374) are deuterium-depleted relative to diunsaturated alkenones ($C_{37:2}$) isolated from the same culture with a fractionation factor ($\alpha_{C_{37:3}-C_{37:2}}$) of 0.94 (Table 2). Tetraunsaturated C_{37} alkenones ($C_{37:4}$) extracted from three Greenland lake sediment samples are deuterium-depleted relative to $C_{37:3}$ isolated from the same samples. Furthermore, the fractionation factor between $C_{37:3}$ and $C_{37:4}$ ($\alpha_{C_{37:4}-C_{37:3}}$) in all three samples is very similar (0.94, 0.95, and 0.94) suggesting that this value may represent the biosynthetic hydrogen isotope fractionation that occurs as diunsaturated (and triunsaturated) alkenones bind to desaturase enzymes along the desaturation pathway toward triunsaturation (and tetraunsaturation).²¹

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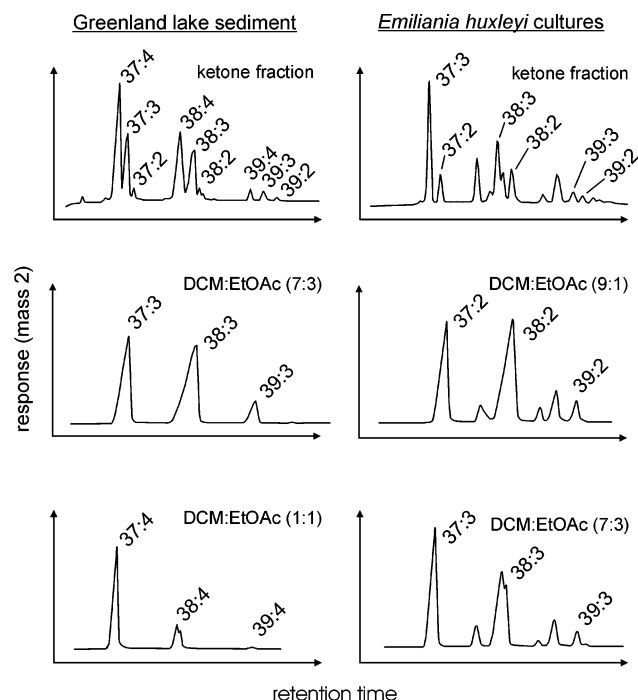


Figure 3. GC/TC-IRMS traces (mass 2) showing alkenones from Greenland lake sediment (left-hand panels) and *E. huxleyi* (right-hand panels). Upper panels show alkenones prior to separation by silver nitrate-impregnated silica gel liquid chromatography. The eluent used to isolate alkenones by double-bond number is indicated on each plot (DCM = dichloromethane; EtOAc = ethyl acetate). See text for a discussion of the optimized GC/TC-IRMS conditions.

Table 2. δD Values of Alkenones from *E. huxleyi* (CCMP 374)

sample ID	alkenone measd	$\alpha_{C_{37:3}-C_{37:2}}^a$	δD (‰ VSMOW)	1σ
<i>E. huxleyi</i>	$C_{37:2}$	{0.94}	-188.1	0.6
<i>E. huxleyi</i>	$C_{37:3}$		-232.8	1.7

$$^a \alpha_{C_{37:3}-C_{37:2}} = (\delta D_{C_{37:3}} + 1000) / (\delta D_{C_{37:2}} + 1000).$$

Table 3. δD Values of Alkenones from Greenland Lake Sediment

sample ID	alkenone measd	$\alpha_{C_{37:4}-C_{37:3}}^a$	δD (‰ VSMOW)	1σ
Limnæa Sø	$C_{37:3}$	{0.94}	-198.2	2.2
Limnæa Sø	$C_{37:4}$		-246.7	2.1
Lake E 80	$C_{37:3}$	{0.95}	-209.9	2.9
Lake E 80	$C_{37:4}$		-253.2	1.6
Lake E 80	$C_{37:2-4}^b$		-244.6	0.1
Lake E 101	$C_{37:3}$	{0.94}	-217.1	0.6
Lake E 101	$C_{37:4}$		-266.0	0.6
Lake E 101	$C_{37:2-4}^b$		-245.0	3.0

^a $\alpha_{C_{37:4}-C_{37:3}} = (\delta D_{C_{37:4}} + 1000) / (\delta D_{C_{37:3}} + 1000)$. ^b δD of the mixture of coeluting C_{37} alkenones (di-, tri-, and tetraunsaturated) measured prior to silver nitrate-silica gel separation.

While the biosynthesis of alkenones is still poorly understood, two possibilities may be considered with respect to the fractionation of hydrogen isotopes during alkenone biosynthesis. The first possibility is that there is a fixed isotopic enrichment (ϵ) between source water δD and the *weighted-average* alkenone δD (i.e.,

$\varepsilon_{C37\text{-water}}$ is constant). If this is the case, then weighted-average alkenone δD measurements can be used to reconstruct source water δD , regardless of temperature-induced changes in the relative concentrations of $C_{37:4}$, $C_{37:3}$, and $C_{37:2}$. It would be advisable in this case to isolate the different C_{37} alkenones for isotope measurement prior to calculating the weighted-average δD (rather than using the mixture of coeluting alkenones) in order to achieve optimal analytical accuracy. The second possibility, with respect to biosynthetic fractionation, is that there is a fixed isotopic enrichment between source water δD and the δD of each individual alkenone (i.e., $\varepsilon_{C37:2\text{-water}}$, $\varepsilon_{C37:3\text{-water}}$, and $\varepsilon_{C37:4\text{-water}}$ are each constant, and distinct from one another). If this is the case, then source water δD reconstructions will require δD measurements on individual alkenones, as temperature-induced changes in the relative concentrations of $C_{37:4}$, $C_{37:3}$, and $C_{37:2}$ through time would obscure weighted-average alkenone δD measurements. Preliminary results from our culture experiments with *E. huxleyi* indicate constant $\varepsilon_{C37:3\text{-water}}$ at different growth temperatures, suggesting that the latter scenario is more likely the case.

To illustrate the potential confounding effect of temperature-induced U_{37}^K changes on weighted-average alkenone δD reconstructions, consider the following example. A sea surface temperature increase of 5 °C, from 12 °C during the Last Glacial Maximum (LGM) to 17 °C during the Holocene, at a hypothetical ocean location would result in a change in the $C_{37:2}$ to $C_{37:3}$ ratio from 0.78 to 1.53 (based on the U_{37}^K calibration⁴). If we consider the δD values measured for $C_{37:2}$ and $C_{37:3}$ (−188 and −233 ‰, respectively) from *E. huxleyi* cultures in this study, and assume that their respective δD values do not vary as a function of the $C_{37:2}$ to $C_{37:3}$ ratio (i.e., the second of the two possibilities outlined above), we would measure weighted-average δD values (combining $C_{37:2}$ and $C_{37:3}$) of −213 ‰ for the LGM and −206‰ for the Holocene. This represents an apparent δD shift of +7 ‰ due solely to temperature-induced changes in relative concentrations of $C_{37:3}$ and $C_{37:2}$. The actual isotopic shift of ocean water δD due to ice volume changes between LGM and present is on the order of −7 ‰ (based on a $\delta^{18}O$ shift of −1.0‰^{22,23} due to ice volume changes and an estimated relationship of $\delta D = 7\delta^{18}O$ for ocean water²⁴). Therefore, in this proposed scenario the temperature-induced change in $C_{37:2}$ to $C_{37:3}$ ratio causes an apparent ocean water δD shift that cancels the actual δD shift of ocean water between LGM and present. It should be noted that CCMP374 was collected from the North Atlantic Ocean (Gulf of Maine) and is not necessarily representative of all *E. huxleyi* strains present in the world's oceans. Nonetheless, based on our findings we strongly recommend the use of individual alkenone compounds for reconstructing past ocean δD values.

In addition to improving the accuracy of alkenone δD measurements though improved GC resolution, as discussed above, measurement of individual alkenones also appears to improve the precision of replicate measurements. Englebrecht and Sachs⁸ reported a precision (1σ) of 2.9 ‰ for the replicate δD analysis of a single alkenone sample ($n = 34$), with 1σ of weighted-average alkenone δD measurements from *E. huxleyi* cultures up to 6 ‰ ($n = 4$). Schouten et al.⁹ reported 1σ of weighted-average alkenone

δD measurements from *E. huxleyi* cultures up to 7 ‰. In this study, isolated alkenone δD measurements from *E. huxleyi* cultures had a precision of 1.7 and 0.6 ‰ for $C_{37:3}$ and $C_{37:2}$, respectively (Table 2).

Potential Applications. One potential application for seawater δD reconstruction is for dating marine sediment records. Marine sediment cores are routinely correlated chronologically using $\delta^{18}O$ measurements from benthic foraminiferal calcite tests found throughout the cores,^{25–27} which reflect the changing $\delta^{18}O$ values of the world's oceans through time. This global $\delta^{18}O$ signal allows temporal correlation of geographically distinct cores. Sediment cores lacking foraminiferal tests, such as those from regions with poor carbonate preservation (e.g., large portions of the Southern Ocean and certain upwelling regions with high organic content), cannot be aligned with global $\delta^{18}O$ records, limiting paleoceanographic and paleoclimatic reconstructions. Due to the fact that δD and $\delta^{18}O$ vary linearly in hydrologic systems,^{24,28} alkenone δD measurements could be useful for correlating sediment records to the global foraminiferal $\delta^{18}O$ time series.

Alkenone δD values have been used to assess the degree of lateral transport at ocean sites (i.e., drift sites).⁸ In order to determine the provenance of alkenones in cores taken from such sites, alkenone δD can be used in conjunction with the temperature-dependent unsaturation ratios (U_{37}^K).⁸ The use of isolated alkenones for δD measurement, rather than an integration of all C_{37} alkenones, may improve the effectiveness of this approach.

Alkenones continue to be discovered in lacustrine sediments worldwide.^{17,29–37} Alkenone-based δD reconstructions from these archives are valuable for reconstructing lake water isotopic variations. For example, Lake Qinghai, China offers the opportunity for long-term (millions of years) alkenone-based climate reconstruction.³⁸ The alkenone-bearing lakes of the Søndre Strømfjord in southwestern Greenland¹⁷ offer the possibility for paleohydrologic as well as temperature reconstructions from a region sensitive to the North Atlantic Oscillation.^{39,40} While sedimentary organic compounds such as sterols⁵ and alkanolic acids^{6,7,10} have been shown to record lake water δD , these compounds are produced by both aquatic and terrestrial sources, potentially

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complicating reconstructions. Alkenones derive solely from haptophyte algae and thus provide more robust lake water δD reconstructions. When present, accurate δD measurements of alkenones from lake sediments arguably offer the best opportunity for hydrogen isotope-based terrestrial paleoclimate reconstruction.

CONCLUSIONS

The silver nitrate-impregnated silica gel chromatographic procedure described here allows the isolation of individual alkenones for compound-specific hydrogen isotope analysis. Alkenone recovery using this procedure is $\geq 87\%$, and there is no associated hydrogen isotope fractionation.

δD values of di- and triunsaturated C_{37} alkenones isolated from a culture of *E. huxleyi* are very different, with $\alpha_{C_{37:3}-C_{37:2}}$ of 0.94. δD values of tri- and tetraunsaturated C_{37} alkenones isolated from Greenland lake sediments are also different, with $\alpha_{C_{37:4}-C_{37:3}}$ of 0.94–0.95 in three distinct samples, suggesting that this value may represent the biosynthetic isotope fractionation that occurs as alkenones bind to enzymes along the desaturation pathway.

There are numerous applications for alkenone δD measurements to paleoclimate studies. We recommend measuring δD of isolated alkenones rather than from mixtures of coeluting alkenones, as the latter may be significantly influenced by the relative proportions of different C_{37} alkenones (i.e., water temperature) and the former can provide improved accuracy and analytical precision.

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