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The effect of hydrosulphurization on stable carbon isotopic compositions of free and sulphur-bound lipids

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Abstract—1-Decene was subjected to hydrosulphurization under phase-transfer conditions at room temperature. Compound-specific isotope analysis revealed that 1-decene becomes enriched in ^{13}C with increasing yields of sulphurization whilst the sulphur compounds formed are initially depleted in ^{13}C compared to the starting substrate. The average carbon isotopic fractionation effect of the reaction on the two carbon atoms involved is ca. -20% . Sedimentary sulphur compounds, however, do not show this fractionation effect indicating that either a different sulphurization reaction with different isotope effects has occurred or that functionalized lipids may have been fully sulphurized.

INTRODUCTION

Several studies have provided evidence that inorganic sulphur species react with functionalized lipids in the subsurface during early diagenesis (e.g., Sinninghe Damsté et al., 1989; Kohnen et al., 1990). Elemental sulphur, hydrogen sulphide, and polysulphides have been proposed as the species reacting with lipids (e.g., Schmid et al., 1987; Sinninghe Damsté et al., 1989; Kohnen et al., 1991; Fukushima et al., 1992; Rowland et al., 1993; Vairavamurthy and Moppe, 1987). Recently, the sulphurization of organic matter has been simulated in the laboratory by the addition of hydrogenpolysulphide ions to isolated double bonds and carbonyl-groups under mild conditions (i.e., 25°C ; de Graaf et al., 1992; Schouten et al., 1993). The type of products generated by this laboratory-simulated hydrosulphurization is similar to that found in immature organosulphur-rich sediments. Hence, it was concluded that these laboratory simulations mimic natural sulphurization occurring in surface sediments and, possibly, in anoxic water columns.

Stable carbon isotopic compositions of free and sulphur-bound hydrocarbons in sediments and crude oils can assist in determining the origin of these compounds (Hayes et al., 1987; Freeman et al., 1990; Kohnen et al., 1992; Schoell et al., 1992, 1994; Sinninghe Damsté et al., 1993; Kenig et al., 1994). Interpretations of ^{13}C -contents of sedimentary organic compounds are based on the assumption that these contents reflect the original stable carbon isotopic compositions of their precursor molecules. Indeed, several observations have indicated that ^{13}C -compositions of carbon skeletons remain virtually unchanged upon diagenetic demethylation and aromatization reactions (Hayes et al., 1990; Freeman et al., 1994). In most cases the number of carbon atoms involved in the reactive sites of the molecule is low compared to the number of nonreactive carbon atoms, so that any isotope effect is diluted. However, Hartgers et al. (1994) have shown that incorporation of isorenieratene into sedimentary macromolecules (partially through natural sulphurization) is associated

with a significant kinetic isotope effect. Although the average isotope effect on the reactive carbon atom was fairly small (-6%) the numerous reactive sites (eighteen out of forty carbon atoms) and the high yield of incorporation (99% of starting material) caused the residual free isorenieratene to be 8% enriched compared to the original ^{13}C -content of its precursor isorenieratene.

Although it has been assumed that sulphurization of lipids is not associated with any large fractionation effects, the data of Hartgers et al. (1994) seems to indicate that this is not always true. Because to the best of our knowledge, fractionation effects of (hydro)-sulphurization at low temperatures have never been reported, we performed laboratory hydrosulphurization experiments on 1-decene and measured the carbon isotope fractionation effects of this reaction.

EXPERIMENTAL

Hydrosulphurization Reactions

The conditions of the hydrosulphurization experiments performed are essentially identical to those previously described by de Graaf et al. (1992). Briefly, a two-phase system consisting of water and ethyl acetate was used as reaction medium. Tetrabutyl ammonium bromide was added as a phase transfer agent. The molar ratio of 1-decene, NaHS and S_8 was 1:100:5. In two experiments, the reaction mixture was stirred at 25°C for 7 weeks and in another experiment for 13 weeks at 25°C . In the latter case a sample of the reaction mixture was sampled after 3 and 13 weeks.

Gas Chromatography (GC)

GC was performed using a Carlo Erba 5300 instrument, equipped with an on-column injector. A fused silica capillary column (25 m \times 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. For detection a flame ionization detector (FID) was used. The samples (dissolved in ethyl acetate) were injected at 70°C and subsequently the oven was programmed to 320°C at $10^\circ\text{C}/\text{min}$ at which it was held for 10 min.

Gas Chromatography–Mass Spectrometry (GC–MS)

GC–MS was performed on a Hewlett-Packard 5890 gas chromatograph interfaced to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range m/z 40–800 and a cycle time of 1.8 s (resolution 1000). The gas chromatograph was equipped with

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a fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness = 0.2 μm). The carrier gas was helium. The samples were injected on-column at 50°C and subsequently the oven was programmed to 300°C at 10°C/min at which it was held for 10 min.

Isotope Ratio Monitoring–Gas Chromatography–Mass Spectrometry (Irm–GC–MS)

The MAT 252 irm–GC–MS–system used has been described previously (Hayes et al., 1990). The GC column was a 25 m Hewlett Packard Ultra 1 column (0.31 mm internal diameter; 0.17 μm film thickness). The carrier gas was helium at a flow rate of ca. 1.5 mL/min. The samples were injected via split injector (splitflow 20 mL/min) at 200°C. The oven was programmed from 50°C at 8°C/min to 320°C at which it was held for 1 min. The isotopic values were calculated by integrating the mass 44, 45, and 46 ion currents of the peaks produced by combustion of the chromatographically separated compounds and that of CO₂-spikes produced by admitting CO₂ with a known ¹³C-content at regular intervals via the dual inlet system into the mass spectrometer. All values reported were determined by two analyses and the results were averaged to obtain a mean value and to calculate the standard deviation. The stable carbon isotope compositions are reported in the delta notation relative to the PDB ¹³C standard.

RESULTS OF MODEL EXPERIMENTS

1-Decene reacted under phase-transfer conditions at room temperature (ca. 25°C) with hydrogenpolysulphides. The resulting products were predominantly 2-decanethiol, 2,2'-dicyldisulphide, and 2,2'-dicedyltrisulphide (Fig. 1) as was expected from previous sulphurization reactions with model compounds (Schouten et al., 1994). Yields and stable carbon isotopic compositions of the products were determined after reaction times of 3, 7, and 13 weeks (Table 1). The yield increased from 40% after 3 weeks to 100% after 13 weeks, indicating that even under these mild conditions the sulphurization reaction is relatively fast. The isotopic composition of the residual 1-decene becomes more enriched in ¹³C with increasing yields (Table 1). Following the mass balance, the sulphur compounds are depleted in ¹³C compared to the starting material. The experimental data allow for a calculation of the average isotopic fractionation effect per carbon atom involved in the hydrosulphurization reaction (defined as ε_{avg}) using the approximation derived by Mariotti et al. (1981):

$$\epsilon_{\text{avg}} = \frac{\Delta\delta}{\ln(1-f)}, \quad (1)$$

$$\Delta\delta = a * (\delta^{13}\text{C}_{\text{residue}} - \delta^{13}\text{C}_{\text{initial}}), \quad (2)$$

$$a = \frac{\text{number of total carbon atoms}}{\text{number of reactive carbon atoms}}, \quad (3)$$

where Δδ is the isotopic change of the reactive carbon atoms, δ¹³C_{initial} is the initial carbon isotopic composition of substrate, δ¹³C_{residue} is the isotopic composition of the unreacted substrate, and *f* is the fraction of reacted substrate. The fraction *f* can be determined either by integrating FID-traces or can be calculated from the isotopic mass balance:

$$\delta^{13}\text{C}_{\text{initial}} = f * \delta^{13}\text{C}_{\text{products}} + (1-f) * \delta^{13}\text{C}_{\text{residue}} \quad (4)$$

or

$$f = \frac{\delta^{13}\text{C}_{\text{initial}} - \delta^{13}\text{C}_{\text{residue}}}{\delta^{13}\text{C}_{\text{products}} - \delta^{13}\text{C}_{\text{residue}}}. \quad (5)$$

The δ¹³C_{products} was calculated by taking the weighted average of the δ¹³C-contents of the sulphur compounds. The yield after 3 weeks of reaction time calculated from this equation compares very well with the yield determined by integration of peak areas in the FID-traces (Table 1). The calculated yield after 7 weeks is somewhat higher (8%) than the yield determined by integration of the peak areas. This may be due to an underestimation of the latter yield since thermolabile products from the sulphurization reaction (i.e., dicycyltetrasulphide, dicycylpentasulphide, etc.) may dissociate on the GC-column and elute as a very broad ‘‘hump.’’ Thus, for calculation of ε_{avg} the calculated *f* was used.

To calculate ε_{avg} it is also necessary to define *a*. From the products of the hydrosulphurization reaction it is clear that two carbon atoms have reacted; carbon atom C-2 mainly with sulphur and carbon atom C-1 with hydrogen. Using fraction *f* from Eqn. 5, δ¹³C_{substrate} = −32.1‰ and *a* = 5 then the ε_{avg} can be calculated from the data obtained after 3 weeks and 7 weeks sulphurization. After 3 weeks ε_{avg} = −19.2‰ and after 7 weeks ε_{avg} = −20.1‰, indicating a relatively consistent fractionation effect. This means that for the two participating carbon atoms the ε_{avg} ≈ −20‰ for the hydrosulphurization reaction. The magnitude of the isotope effect on either carbon atom (C-1 or C-2) depends on the reaction mechanism. de Graaf et al. (1995) have suggested that the hydrosulphurization reaction mainly involves an irreversible concerted addition of sulphur and hydrogen to C-2 and C-1, respectively. If this is the case, then the step causing the isotopic fractionation is the addition of sulphur to carbon atom C-2 and thus ε_{C-2} ≈ −40‰ and ε_{C-1} = 0‰. However, if the reaction mechanism involves the reversible addition of sulphur followed by the irreversible addition of hydrogen, then the ε_{avg} is composed of an unknown ε_{C-2} (addition of sulphur) and of an unknown ε_{C-1} (addition of hydrogen).

By rearranging Eqns. 1 and 2 the theoretical change of carbon isotope values for the residual 1-decene with degree of sulphurization can be calculated:

$$\delta^{13}\text{C}_{\text{residue}} = \delta^{13}\text{C}_{\text{initial}} + \frac{\epsilon_{\text{avg}} * \ln(1-f)}{a}. \quad (6)$$

Using Eqns. 1 and 4 the change in isotopic composition of the sulphur compounds with the degree of sulphurization can be calculated:

$$\delta^{13}\text{C}_{\text{products}} = \delta^{13}\text{C}_{\text{initial}} - \frac{(1-f) * \epsilon_{\text{avg}} * \ln(1-f)}{f * a}. \quad (7)$$

Using Eqns. 6 and 7 the theoretical curves can be plotted (Fig. 2). This figure shows the change in carbon isotopic compositions of the residual 1-decene and the formed sulphur compounds with increasing yields based on an ε_{avg} of −20‰.

Preliminary experiments using another substrate and phase transfer catalyst (yielding primarily monosulphides) indicated an identical fractionation effect (−19.7‰; M. Kok et al., unpubl. data) thus demonstrating that the kinetic isotope effect is independent of substrate, phase transfer catalyst, and types of products.

GEOCHEMICAL IMPLICATIONS

de Graaf et al. (1992) and Schouten et al. (1993, 1994) have shown that the hydrosulphurization reaction is similar

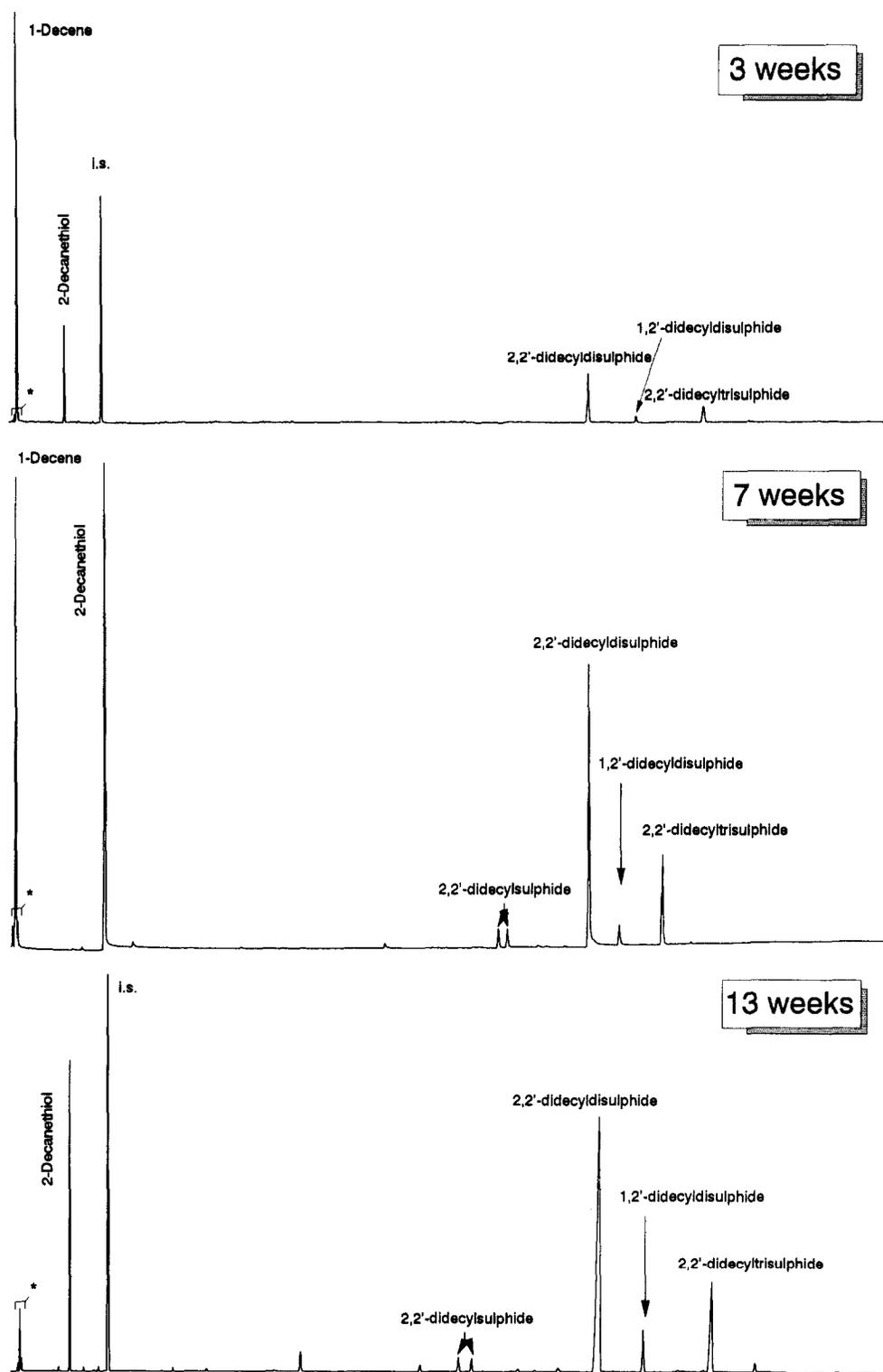


FIG. 1. Gas chromatograms of the reaction mixtures resulting from hydrosulphurization of 1-decene after 3, 7, and 13 weeks. i.s. = internal standard. * = impurities 1-decene.

to the natural sulphurization reaction taking place during early diagenesis. Though the products from the hydrosulphurization reaction and the natural sulphurization reaction can be somewhat different, the types of products and the

reaction mechanisms involved, seem to be comparable. This indicates that natural sulphurization of lipids may induce a carbon isotopic fractionation effect. Depending on the degree of sulphurization and the number of reactive

TABLE 1. Yields (sulphur compounds vs. 1-decene) of hydrosulphurization and molecular carbon isotopic compositions of 1-decene and sulphur compounds

Time	Yield (%)	Yield calc. (%)	$\delta^{13}\text{C}$ 1-decene (‰)	$\delta^{13}\text{C}$ 2-decanethiol (‰)	$\delta^{13}\text{C}$ didecyldisulphide(‰)	$\delta^{13}\text{C}$ didecyltrisulphide(‰)	weighted average $\delta^{13}\text{C}$ S-compounds (‰)
Start	0	-	-32.1 ± 0.1	-	-	-	-
3 weeks	40	39	-30.2 ± 0.1	-35.1 ± 0.1	-35.0 ± 0.2	-34.9 ± 0.3	-35.0 ± 0.3
7 weeks	70	78	-26.0 ± 0.1	-33.7 ± 0.2	-33.9 ± 0.3	-33.9 ± 0.5	-33.8 ± 0.4
13 weeks	100	-	-	-32.9 ± 0.4	-33.0 ± 0.3	-31.7 ± 0.2	-32.4 ± 0.7

sites vs. the number of unreactive sites, sulphurized lipids may be several permil depleted compared to their biolipid substrate. Conversely, the residual lipids may be several permil enriched in ^{13}C .

Hartgers et al. (1994) reported a kinetic isotope effect ϵ_{avg} of -6‰ for the incorporation (partially by sulphurization) of isorenieratene into geomacromolecules. Although this ϵ_{avg} is substantially smaller than the fractionation observed for the hydrosulphurization reaction, it indicates that such kinetic isotope effects do take place in the natural environment. The difference in the magnitude of ϵ_{avg} may be attributed to different mechanisms of incorporation of isorenieratene (i.e., isorenieratene is not only bound by sulphur-linkages; Hartgers et al., 1994) which thus could dilute the shift in carbon isotopic composition of isorenieratene caused by hydrosulphurization.

Data reported for free and sulphurized sedimentary lipids by Kohnen et al. (1992) and Schouten et al. (1995) do not reveal any isotopic fractionation effects induced by sulphurization. For instance, free phytane has a value of -32.1‰ , whereas macromolecular sulphur-bound phytane is -30.5‰ in a Vena del Gesso sediment (Table 2). If the isotopic composition of free phytane is assumed to reflect that of the phytol (or derivatives thereof) left after the nat-

ural sulphurization reaction, then this free phytane would be expected to be substantially enriched in ^{13}C compared to sulphur-bound phytane. In contrast, however, free phytane is depleted in ^{13}C compared to the sulphur-bound phytane. Other $\delta^{13}\text{C}$ values reported for squalane in the Vena del Gesso sediment (Table 2; Kohnen et al., 1992) and for pristane, phytane, and the C_{25} HBI in the Monterey sediments (Table 2; Schouten et al., 1995) show either depleted values for the free lipids compared to their sulphur-bound counterpart or only a very small enrichment in ^{13}C . It is very unlikely that these depletions are caused by incomplete desulphurization since Kohnen et al. (1992) and Schouten et al. (1995) have shown that macromolecularly bound carbon skeletons have identical $\delta^{13}\text{C}$ values as their low-molecular-weight sulphurized counterparts. Clearly, the differences in ^{13}C contents between free and sulphur-bound lipids with identical carbon skeletons are due to source differences, as was proposed earlier by Kohnen et al. (1992), rather than to any diagenetic process.

There are several explanations for this apparent lack of kinetic isotope effects of hydrosulphurization in sediments as reported by Kohnen et al. (1992) and Schouten et al. (1995). The kinetic isotope effect of the natural sulphurization reaction may be, for some unknown reason, much smaller than that observed for the hydrosulphurization reaction in the laboratory. Alternatively, the functionalized lipids have been fully sulphurized in sediments. Any possible kinetic isotope effect left is then diluted by the differences in isotopic composition of free and sulphur-bound lipids induced by different sources as proposed previously (Kohnen et al., 1992). Investigations of more recent sed-

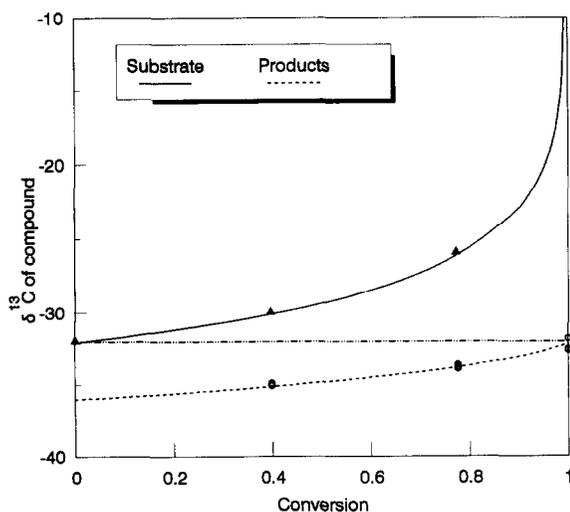


FIG. 2. Graph of ^{13}C content of sulphurized and residual decane carbon skeletons as a function of degree of substrate (f). Δ = 1-decene; \circ = sulphur compounds.

TABLE 2. Literature data on stable carbon isotopic composition of some free and sulphur-bound carbon skeletons

Compound	Sediment	$\delta^{13}\text{C}$ free (‰)	$\delta^{13}\text{C}$ sulphur-bound (‰)
Phytane	Vena del Gesso*	-32.8	-30.5
Squalane	Vena del Gesso*	-31.6	-33.2
Pristane	Monterey (KG-1)*	-29.7	-26.6
Phytane	Monterey (KG-1)*	-26.9	-25.2
C_{25} highly branched isoprenoid	Monterey (KG-3)*	-22.5	-20.5

* Kohnen et al. (1992)
 + Schouten et al. (1995)

iments where sulphurization is still an active process may shed more light on this subject.

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