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Isotopic exchange of carbon-bound hydrogen over geologic timescales

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Abstract—The increasing popularity of compound-specific hydrogen isotope (D/H) analyses for investigating sedimentary organic matter raises numerous questions about the exchange of carbon-bound hydrogen over geologic timescales. Important questions include the rates of isotopic exchange, methods for diagnosing exchange in ancient samples, and the isotopic consequences of that exchange. This article provides a review of relevant literature data along with new data from several pilot studies to investigate such issues. Published experimental estimates of exchange rates between organic hydrogen and water indicate that at warm temperatures (50–100°C) exchange likely occurs on timescales of 10^4 to 10^8 yr. Incubation experiments using organic compounds and D-enriched water, combined with compound-specific D/H analyses, provide a new and highly sensitive method for measuring exchange at low temperatures. Comparison of δD values for isoprenoid and *n*-alkyl carbon skeletons in sedimentary organic matter provides no evidence for exchange in young (<1 Ma), cool sediments, but strong evidence for exchange in ancient (>350 Ma) rocks. Specific rates of exchange are probably influenced by the nature and abundance of organic matter, pore-water chemistry, the presence of catalytic mineral surfaces, and perhaps even enzymatic activity.

Estimates of equilibrium fractionation factors between organic H and water indicate that typical lipids will be depleted in D relative to water by ~75 to 140‰ at equilibrium (30°C). Thus large differences in δD between organic molecules and water cannot be unambiguously interpreted as evidence against hydrogen exchange. A better approach may be to use changes in stereochemistry as a proxy for hydrogen exchange. For example, estimated rates of H exchange in pristane are similar to predicted rates for stereochemical inversion in steranes and hopanes. The isotopic consequences of this exchange remain in question. Incubations of cholestene with D_2O indicate that the number of D atoms incorporated during structural rearrangements can be far less than the number of C-H bonds that are broken. Sample calculations indicate that, for steranes in immature sediments, the D/H ratio imparted by biosynthesis may be largely preserved in spite of significant structural changes. *Copyright © 2004 Elsevier Ltd*

1. INTRODUCTION

Hydrogen isotope ratios ($^2H/^1H$, or D/H) of individual organic compounds are being used to reconstruct D/H ratios of paleoenvironmental water and hence climatic records (Xie et al., 2000; Sauer et al., 2001; Huang et al., 2002). Whereas similar attempts using bulk isotopic analyses have had to contend both with complex mixtures of organic material and with the rapid exchange of O- and N-bound hydrogen (Krishnamurthy et al., 1995), the analysis of individual compounds such as *n*-alkanes and sterols potentially avoids both problems. With this opportunity come new questions about the isotopic fidelity of carbon-bound hydrogen over geologic timescales. Current evidence suggests that virtually all organic hydrogen will exchange on timescales less than the age of the Earth even at low temperatures (Koepp, 1978), but uncertainties about rates and catalytic effects still encompass many orders of magnitude.

In this report we 1) summarize information about rates of exchange of carbon-bound hydrogen; 2) evaluate new and existing methods for identifying and quantifying exchange in sedimentary organic molecules; and 3) examine relationships between hydrogen exchange and isotopic compositions. Specifically, we combine a review of published data with new, compound-specific D/H measurements from three pilot studies. In the first, we demonstrate that monitoring the incorporation of D_2O into organic molecules during incubation experiments can quantify exchange half-times of 10^5 yr in experiments lasting only a few months. Second, we extend the approach of Andersen et al. (2001) by comparing differences in the D/H ratio between polyisoprenoid and straight-chain lipids in rocks dating back to 1.6 Ga. Third, we examine incorporation of deuterium into isomerized and rearranged cholestene during incubation experiments and suggest that measurements of certain structural changes in molecules may be useful as a proxy for estimating hydrogen exchange. Convergence of the δD values of these molecules could provide evidence for hydrogen exchange over long timescales in sedimentary rocks.

2. EXPERIMENTAL

2.1. Incubation Experiments

The test compounds *n*-icosane and cholest-5-ene (Sigma Chemical Co., St. Louis, MO) were incubated with D_2O (99.5% D, Cambridge Isotope Laboratories, Andover, MA) on four different inorganic substrates:

2.1.1. Montmorillonite

The reference material SAz-1(Clay Minerals Society, University of Missouri), a homogenized Ca-montmorillonite clay, was cleaned by

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Table 1. Petroleum samples for D/H analyses.

Sample	Approx. age (Ma)	Formation/location	Description	Maturity	Reference
1810	1640	Barney Ck. Fm., McArthur Basin, NT, Aust.	Mesoproterozoic bitumen from calcareous shale GR-10	Immature	Summons et al. (1988)
306	1000	Nonesuch Fm., Midcontinent Rift, USA	Neoproterozoic oil seep from pyritic black shale	Mature	Pratt et al. (1991)
376	543	Athel Fm., Salt Basin, South Oman	Terminal Proterozoic Huqf oil	Mature	Grantham et al. (1988)
357	365	Nullara Limestone, Canning Basin, WA, Aust.	L. Devonian oil from Blina-1	Mature	Edwards et al. (1997)
364	340	Grant Fm., Canning Basin WA, Aust.	E. Carboniferous oil from West Terrace-1	Mature	Edwards et al. (1997)

three-fold ultrasonication in 9:1 dichloromethane(DCM)/methanol (MeOH) with centrifugation between extractions, followed by air-drying at 120°C for $>$ 2 d. This clay has a reported surface area of 97 m^2/g and a cation exchange capacity of 1.2 meq/g (Olphena and Fripiat, 1979). Total carbon content of the cleaned clay was 0.011 $\pm$ 0.004% on a dry-weight basis. The aqueous pH of this substrate, measured by adding 20 mL of distilled H_2O to 10 g of dry SAz-1, was 8.20.

2.1.2. Montmorillonite + XAD

A portion of cleaned SAz-1 was blended with 10% XAD-2 resin (a styrene-divinylbenzene copolymer, 2% crosslinked, 200–400 mesh pellets; Aldrich, Milwaukee, WI) by weight to simulate sediment containing abundant polymeric organic matter. The XAD resin was cleaned of extractable material by repeated ultrasonication in organic solvents. Blank extractions of both SAz-1 and clay + XAD yielded no detectable organic compounds by gas chromatography/mass spectrometry (GC/MS).

2.1.3. Silica

Powdered microcrystalline silica (Sigma Chemical Co.) was cleaned and dried as for montmorillonite. The reported grain size is 0.5–10 μm, with 80% between 1 and 5 μm. The total carbon content of the cleaned silica was $<$ 0.004%. Because the silica was found to catalyze substantial hydrogen exchange, trace and major element chemistry of the silica was analyzed by ICP-MS, which indicated that transition metals were present in the range 0.1 to 10 μg/g and platinum-group elements were present at 0.2 to 4 ng/g. Aqueous pH of the cleaned silica was 5.73.

2.1.4. Marine clay

Clay-rich, core-top sediments collected from the Gulf of Mexico (850 m water depth; 27.740°N, 90.771°W) were air-dried at 40°C over a period of weeks, then crushed to a fine powder and homogenized. They were further air-dried at 120°C for 2 d immediately before use. Total organic carbon content was 1.6 $\pm$ 0.7%. The sediment was treated with HCl to remove carbonate, but the measured value of $\delta^{13}C$ was $-$10.4‰, indicating that as much as half of the remaining C was probably inorganic. Carbonate content in the initial sample, determined by weight-loss after acidification to pH 1 with HCl then rinsing, was 2%. Aqueous pH of the sediment, measured after drying then rehydrating with distilled H_2O, was 7.57.

Samples were prepared in precombusted, 12-mm OD Pyrex tubes. Each tube contained 1 g dry substrate; 80 μL of a hexane solution containing 2.5 μg/μL each of icosane and cholest-5-ene; and 2 mL of D_2O. Concentrations of compounds added to the marine clay samples were $\sim$1000-fold higher than those of analogous compounds (*n*-C_{29}, cholesterol) native to the sediment. Tubes were evacuated to $\sim$1 mbar and sealed with a torch. Samples were incubated at 30° and 60°C in heated, insulated chambers, and at 7° in a laboratory refrigerator.

Incubations were stopped after 35 and 88 d. Samples were freeze-dried, then extracted by threefold ultrasonication with 9:1 methyl *t*-butyl ether (MTBE)/MeOH at room temperature. The entire procedure was conducted in the Pyrex sample tubes, specifically to minimize exposure to metal surfaces and high temperatures which might induce exchange of hydrogen with any residual D_2O. Extracts were treated with acetic anhydride/pyridine at 65°C to prepare the acetate derivative of androstanol, then analyzed by GC/MS on an Agilent 5973 MS with electron-impact ionization. Compound abundances were quantified by GC/FID by reference to a coinjected fatty acid methyl ester standard.

Icosane recovered from incubations on clean montmorillonite or silica was further purified by two-fold, and in some cases three-fold, urea adduction followed by silica-gel column chromatography to eliminate all D-enriched background components. D/H ratios were measured on a Finnigan-MAT 252 isotope-ratio mass spectrometer coupled to a Varian GC via a pyrolysis furnace operating at 1440°C (the system is described in detail by Sessions et al., 2001). Coinjected *n*-alkanes were used as isotopic reference peaks. Accuracy, estimated as the root-mean-square error of 15 *n*-alkanes in an external standard, averaged 5.8‰ during the period of these measurements. This relatively poor accuracy appears related to the presence of highly D-enriched background components in some of the samples (Sessions, 2001). To reduce H_3^+-related errors, peak heights for analytes and coinjected standards were matched to within $\sim$30%. The value of the H_3-factor used to correct data was determined by measuring a series of *n*-alkanes of varying peak height (Sessions et al., 2001).

2.2. Petroleum Samples

Five bitumen and oil samples from host rocks ranging in age from 340 to 1640 Ma were obtained from archives of the Australian Geological Survey Organization (now Geosciences Australia). Samples were chosen to represent old, organic-rich materials that have experienced as little thermal maturation as possible (Table 1). Samples were extracted and separated into silicalite adduct (containing *n*-alkanes and some monomethyl alkanes) and nonadduct fractions according to the procedures described by West et al. (1990).

D/H ratios were measured on a Finnigan Delta+XL isotope-ratio mass spectrometer (IRMS) coupled to an Agilent 6890 GC via the Finnigan TC interface held at 1440°C. For silicalite adduct samples, three fatty acid methyl esters (FAME's) were coinjected with the samples, and served both as isotopic reference peaks (C_{10} and C_{30}) and as an independent test of accuracy (C_{20} FAME). The accuracy of δD measurements was estimated on this basis as $\pm$3.8‰ for adduct fractions. Because chromatograms for the non-adduct samples were significantly more crowded, standards could not be coinjected and an external tank of H_2 was used as the isotopic reference. We estimate the precision of isotopic analyses for the nonadducted samples at $\pm$10.2‰, based on the pooled standard deviation of replicate analyses.

3. RESULTS

3.1. Incubations

Extracts of incubated samples yielded chromatograms with numerous peaks representing compounds present at very low concentrations but with very high D/H ratios (enrichments $>$10,000‰). Coelution of these components made it impossible to determine accurate values of δD for individual compounds in the extracts. Interferences from high-D products

Table 2. Measured changes in δD for icosane and calculated exchange half-times.

Sample[a]	Temp (°C)	Time (days)	ΔD (‰)[b]	σ_Δ (‰)[c]	$t_{1/2}$ (Kyrs)[d]
M7-35	7	35	5.3	6.4	>66.6
M7-88	7	88	11.8	7.2	90.9
M30-35	30	35	46.0	30.0	9.27
M30-88	30	88	22.8	7.2	47.0
M60-35	60	35	20.4	12.9	20.9
M60-88	60	88	53.7	23.0	20.0
S7-35	7	35	1.9	7.2	>59.2
S7-88	7	88	3.1	6.4	>168
S30-35	30	35	11.8	7.2	36.1
S30-88	30	88	−3.9	6.8	>158
S60-35	60	35	52.1	6.4	8.19
S60-88	60	88	88.0	9.5	12.2

[a] Sample numbers beginning with 'M' were incubated on montmorillonite, those beginning with 'S' were incubated on silica.
[b] Change in δD value, calculated as δD(final) − δD (initial), where δD(initial) = −54.6‰.
[c] Standard deviation of ΔD estimated from repeated measurements of samples and coinjected standards (Sessions, 2001).
[d] Exchange half-life, calculated using equation 2 and assuming F_e = 1.0. Numbers marked with '>' are minimum estimates for samples with no significant exchange.

could be eliminated only for the *n*-alkane fraction, which could be purified by urea adduction before isotopic analysis. Thus no IRMS data are reported for cholestene or its transformation products. Similarly, no IRMS data are reported for icosane incubated on the marine clay substrate because of the possibility of in-situ generation of strongly D-labeled icosane.

Hydrogen-isotopic compositions of icosane incubated on montmorillonite or silica appear in Table 2. Changes in δD values ranged from undetectable in most of the 7°C samples to a maximum of 88‰ in the 88-d sample incubated on silica. With one exception, the recovery of icosane from these experiments (before urea adduction) ranged from 47 to 100% and was generally above 65% (data not shown). Recovery of icosane in sample S30-35 was only 17%, and data from that sample are not included in subsequent calculations. In general, δD values increased systematically with increasing temperature and length of incubation, though several apparent reversals of this pattern were observed (e.g., compare M30-35 and M30-88 in Table 2). Such differences are considerably larger than analytical uncertainties.

3.2. Petroleum Samples

Values of δD for straight-chain and isoprenoid hydrocarbons extracted from oils and bitumens ranged from −57‰ to −145‰ (Table 3). Values of δD for homologous *n*-alkanes in each bitumen increased smoothly with chain length, as has been observed in *n*-alkanes from other reservoired petroleum samples (Li et al., 2001; Schimmelmann et al., unpublished data). The range of available isoprenoids is too narrow to discern whether a similar pattern exists for the isoprenoid hydrocarbons. Considering all samples, the mean difference in δD between each isoprenoid and the *n*-alkane of identical weight (e.g., phytane and *n*-C_{20}) was 15.8 ± 8.2‰, with the isoprenoids enriched in D relative to *n*-alkanes.

Table 3. Compound-specific δD values for petroleum samples.

Compound	1810[a]	306	376	357	364
			n-alkanes		
C_{14}	−142	−80	−110	−137	−106
C_{15}	−145	−84	−114	−140	−118
C_{16}	−143	−88	−115	−137	−119
C_{17}	−138	−86	−117	−134	−118
C_{18}	−137	−87	−107	−132	−117
C_{19}	−132	−85	−115	−132	−117
C_{20}	−128	−83	−107	−130	−112
C_{21}	−123	−83	−111	−132	−113
C_{22}	−121	−88	−105	−126	−112
C_{23}	−112	−82	−95	−122	−104
C_{24}	−115	−92	−99	−126	−114
C_{25}	−117	−95		−135	−116
C_{26}	−101	−80		−128	−108
C_{27}		−93		−131	−108
C_{28}		−85		−126	−109
C_{29}		−89		−132	−110
C_{30}		−64		−124	−110
C_{31}		−75		−129	−110
C_{32}		−57		−127	−112
			Regular isoprenoids		
C_{18} (norpristane)	−131	−71[b]	−99	−103	−100
C_{19} (pristane)	−113	−63[c]	−101	−107	−95
C_{20} (phytane)	−107		−108	−116	−103

[a] Sample numbers are explained in Table 1.
[b] Coeluting with *n*-C_{16}.
[c] Coeluting with *n*-C_{17}.

3.3. Incubations of Cholestene

Cholest-5-ene (the input) and three closely related compounds (cholest-4-ene, 20R-diacholestene, and 20*S*-diacholestene) were recovered from incubations on all four substrates. Relative abundances varied widely (Table 4). Under conditions of acid catalysis cholest-5-ene undergoes a number of reactions, including double-bond migration to give cholest-4-ene, backbone rearrangement to give diasterenes (5,14-methyl-10,13-nor-cholest-13(17)-ene), and inversion at C-20 (Table 4). Clean montmorillonite was the most efficient catalyst for these rearrangments, followed by silica then marine clay. Addition of XAD resin to montmorillonite substantially decreased the rate of rearrangement. Reaction rates were extremely fast, but yields did not vary as a function of temperature or time (for example compare yields of 20(R)diacholestene from montmorillonite samples in Table 4) and so appear not to be kinetically limited. The distribution of D in isomers of cholestene and diacholestene is described in detail elsewhere.

4. DISCUSSION

4.1. Taxonomy of Exchange Processes

The terms "exchangeable" and "nonexchangeable" hydrogen are commonly used to distinguish between hydrogen that exchanges with ambient water on a timescale of seconds to days (generally O- or N-bound H) from that which does not (generally carbon-bound hydrogen, but also potentially O- or N-bound H that is shielded from exchange by the tertiary structure of molecules). This usage arose from practical considerations during isotopic measurements of bulk organic hydrogen (Smith

Table 4. Recovery of steroid compounds from incubation experiments.[a]

Sample[b]	Cholest-5-ene[c]	Cholest-4-ene[c]	20R-dia-cholestene[d]	20S-dia-cholestene[d]	Total sterenes
M7-35	4	2	48	31	85
M7-88	13	5	59	35	112
M30-35	4	2	51	35	91
M30-88	30	6	37	18	92
M60-35	7	3	51	28	90
M60-88	9	4	43	20	76
X7-35	83	9	26	12	130
X7-88	29	0	8	0	36
X30-35	75	7	15	6	102
X30-88	85	10	19	6	121
X60-35	91	6	9	2	108
X60-88	61	6	6	0	74
S7-35	42	23	23	0	87
S7-88	68	22	43	5	138
S30-35	45	18	14	0	77
S30-88	48	17	29	0	94
S60-35	60	11	24	9	104
S60-88	52	8	19	7	87
G7-35	55	15	12	2	85
G7-88	55	13	12	2	83
G30-35	89	21	10	1	121
G30-88	72	24	10	0	105
G60-35	60	25	27	4	115
G60-88	38	15	16	3	73

[a] Values expressed as percentage of cholest-5-ene originally added, based on comparison of FID peak areas.

[b] Sample labels follow the pattern (Substrate) (Temp. °C) − (Time, days); M = montmorillonite, X = montmorillonite + XAD resin, S = microcrystalline silica, G = marine clay.

[c] All R configuration at C-20.

[d] Double bond position is Δ13(17).

and Epstein, 1970; Yapp and Epstein, 1982). However, such broad generalizations can be misleading, particularly in their implication that C-bound H is completely nonexchangeable. In addition, the term "hydrogen exchange" has sometimes been used by geochemists to encompass almost any process that leads to changes in organic D/H ratios over time. This broad usage obscures a great diversity of chemical mechanisms, and confuses the discussion and comparison of experimental results. To clarify the situation, we propose here a systematic and specific terminology for chemical mechanisms leading to the incorporation of exogenous H in organic molecules. While the examples provided here illustrate individual processes, many chemical and geochemical phenomena, such as the rearrangement of sterenes to diasterenes or the thermal maturation of kerogen, can exhibit characteristics of multiple processes.

Conceptually, chemical mechanisms capable of altering D/H ratios can be grouped into five categories, indicated schematically in Figure 1. Of these, three involve no change in the number of H atoms in the molecule and might commonly be described as *exchange* reactions. Differences among these processes are, however, very significant. The broad category of exchange is thus further subdivided into several more specific processes. The first of these is '*pure exchange*,' a term used here to describe a chemical reaction in which the reactants and products are chemically and structurally (but not isotopically) identical. Conceptually, pure exchange involves the replacement of a single hydrogen with no effect on the rest of the

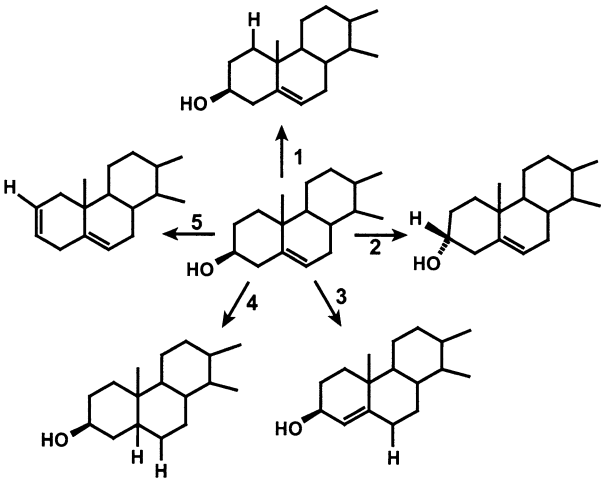

Fig. 1. Examples of chemical reactions potentially leading to changes in D/H ratios. (1) pure exchange, (2) exchange accompanying stereochemical inversion, (3) rearrangement, (4) hydrogen addition, (5) hydrogen loss. Hydrogen positions affected by each reaction are shown on the product.

molecule. For C-bound H, the process requires abstraction and replacement of a hydrogen with no accompanying isomerization (Fig. 1). Exchange at a chiral carbon atom, such as C-3 in cholesterol, may occur by the same mechanism and result in inversion of the chiral center. Since stereoisomers are commonly regarded as distinct structures, we distinguish this process as *stereochemical exchange* (Fig. 1).

A subtle question arises as to the identity of exchange at a tetrahedral carbon center bonded to four unique substituents, two of which are isotopes of the same element. For example, if C-2 in cholesterol contains both D and H as substituents it is distinct from its mirror image, and exchange of either hydrogen atom can lead to inversion of the tetrahedral center. Nevertheless, the distribution of isotopes is usually not considered when classifying a carbon atom as chiral, so we describe such a process as pure exchange while recognizing that most features of the reaction are identical to those in stereochemical exchange.

The final subcategory of exchange processes, *constitutional exchange*, includes all reactions in which the reactant and product are constitutional isomers (Moss, 1996). In such cases, the molecular structure changes but the chemical formula does not. Relevant reactions may involve double-bond migration (Fig. 1), methyl shifts, and carbon backbone rearrangements. These reactions fall outside the standard definition of isotope exchange reactions (Muller, 1994) but do lead to the incorporation of exogenous H, often at multiple positions.

A process is termed *addition* when the net inventory of hydrogen increases. Addition processes include the hydrogenation of double bonds (Fig. 1), as well as cleavage reactions such as decarboxylation of organic acids. Hydrogen *elimination*, such as in the dehydration of alcohols (Fig. 1), is frequently accompanied by a kinetic isotope effect that fractionates hydrogen isotopes in the product. Even in the absence of isotope effects, significant intramolecular isotopic ordering may exist, so that the loss of hydrogen at a specific position can change the average isotope ratio of the molecule.

4.2. Mathematics of Hydrogen Exchange

Exchange between an organic molecule and water can be represented by

$$RH + HDO \leftrightarrows RD + H_2O \quad (1)$$

Assuming that H_2O is present in excess, the rate of reaction 1 is proportional to the concentration of RH. The approach to equilibrium is described by

$$\frac{F_t - F_e}{F_i - F_e} = e^{-kt} \quad (2)$$

where F is the fractional abundance of D [= D/(D + H)] in the organic molecule *i*nitially, at time t, and at *e*quilibrium, and k is the reaction rate constant (Roberts and Urey, 1939; Wedeking and Hayes, 1983; Criss, 1999). Eqn. 2 is exact, but when working with organic samples and water containing a natural abundance of D ($F \approx 10^{-4}$), the isotope ratio (R) or δD value can be approximately substituted for F. Importantly for systems in nature, the value of the equilibrium fractionation factor (α_e) must be known to determine the value of F_e. In the special case of experiments involving exchange between organic hydrogen with a natural abundance of D and heavy water with $F \approx 1$, the equilibrium fractionation can generally be ignored and F_e assumed to be unity.

The left side of Eqn. 2 has a value of one initially, approaches zero at isotopic equilibrium, and can be evaluated for any t if k is known. Conceptually, that value represents the fraction of hydrogen atoms that remain unexchanged. By rearranging Eqn. 2, the isotopic composition of organic H can also be evaluated for any time. On geological time scales, it is frequently more convenient to think in terms of an exchange half-time ($t_{1/2} = (\ln 2)/k$) rather than a rate constant. After one half-time, the difference between δ_t and δ_e is half the initial value. After two half times it is one quarter, etc.

Two complications arise in regard to Eqn. 2. First, mineral surfaces frequently act as catalysts for exchange reactions in natural samples (Alexander et al., 1981). The overall rate constant for such reactions will generally be first order in both the reactant (organic substrate) and the availability of catalytic sites. The rate of exchange will also depend on competition by other species for catalytic sites. Extension of laboratory experiments to natural settings is thus difficult, both in predicting absolute rates of exchange and in extrapolating initial exchange rates to longer timescales.

Second, the variables in Eqn. 2 are specific to each hydrogen position For example, in *n*-alkanes the methyl and methylene hydrogens will exchange at significantly different rates, and will approach different equilibrium isotopic compositions. Moreover, each position—potentially including methylene positions that are otherwise equivalent—can start with a different isotopic composition as a result of intramolecular isotopic ordering (e.g., Monson and Hayes, 1980). Thus for virtually all organic molecules larger than ethane, it is strictly incorrect to apply Eqn. 2 to an entire molecule using average values of k, F_i, and F_e. Instead, the isotopic composition of each hydrogen position must be calculated independently as

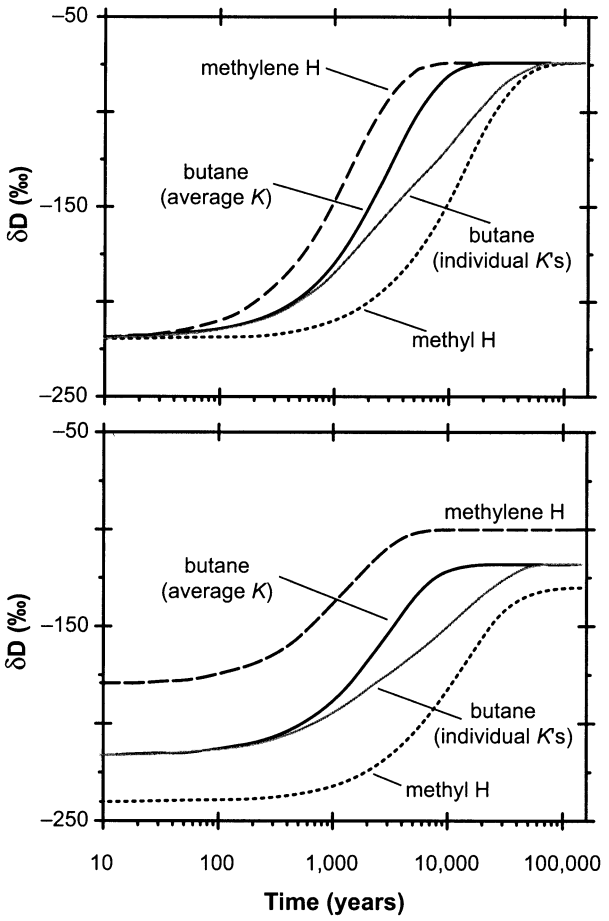

Fig. 2. The progress of hydrogen exchange for *n*-butane molecules with hypothetical exchange half-times of 1000 yr (four methylene hydrogens; dashed line) and 10,000 yr (six methyl hydrogens; dotted line). The gray solid line represents exchange of the bulk molecule calculated from Eqn. 4 using separate rate constants for each position, while the black solid line represents that calculated using Eqn. 2 with the weighted-average rate constant (i.e., 6400 yr). In the upper graph, the initial and equilibrium isotopic compositions of methyl and methylene hydrogens are the same; in the lower graph, they differ between the two groups.

$$^pF_t = (^pF_i - {}^pF_e)\exp(-{}^pkt) + {}^pF_e \quad (3)$$

where the left superscript denotes a variable that refers specifically to hydrogen position p in the molecule. The average isotopic composition of the molecule can then be calculated by summing over all individual hydrogen positions

$$\bar{F}_t = \frac{1}{n}\sum_{p=1}^{n}\left[(^pF_i - {}^pF_e)\exp(-{}^pkt) + {}^pF_e\right]$$

$$= \bar{F}_e + \frac{1}{n}\sum_{p=1}^{n}\left[(^pF_i - {}^pF_e)\exp(-{}^pkt)\right] \quad (4)$$

where n is the number of hydrogen atoms in the molecule.

A hypothetical example is provided in Figure 2 for *n*-butane, in which the hydrogens all belong to one of two groups of equivalent positions (i.e., methylene versus methyl), and as-

suming that the two positions undergo exchange with (again hypothetical) half-times of 1000 and 10,000 yr, respectively. In this example, δ values have been substituted for F in Eqns. 2 and 4. The top graph represents the case in which both methyl and methylene hydrogens have the same initial and equilibrium isotopic compositions. In the bottom, both δ_i and δ_e differ for the two positions. The regular sigmoidal curves represent exchange at individual methyl and methylene hydrogen positions and, between those extremes, a hypothetical molecule with all H positions having $t_{1/2}$ = 6400 yr, i.e., the weighted mean of the two rate constants. The distorted sigmoid displays the relationship between δD and t for the molecule calculated using Eqn. 4 and accounting for the different rate constants at each position.

As a practical matter, it is not generally possible to determine rate constants for every hydrogen position in even one compound, let alone in many different compounds. Since geochemists are often interested in identifying molecules that are *not* affected by exchange, Figure 2 suggests an expedient. Using average values of δ_i, δ_e, and k in Eqn. 2 will accurately predict the exchange behavior of the bulk molecule over short time scales, and will provide a conservative estimate regarding the onset of exchange. Conversely, values of k observed for the bulk molecule in short-time experiments, such as those presented in Table 2, will be estimates of weighted-average rate constants. On the other hand, using average values to estimate the time needed to reach equilibrium can result in errors of nearly an order of magnitude.

4.3. Equilibrium Isotope Effects

Exchange of hydrogen between an organic molecule and water will eventually lead to isotopic equilibrium. The D/H ratios of the products will then differ by an amount governed by equilibrium isotope effects. It is impossible to make quantitative assessments of hydrogen exchange based on δD values if the presumed end point of exchange is not known, an important point that is sometimes overlooked. Existing data regarding equilibrium fractionations are thus compiled here.

In principle, equilibrium isotope effects can either be directly measured or predicted from theoretical calculations. Laboratory experiments suffer from the fundamental difficulty of achieving hydrogen-isotopic equilibrium while simultaneously limiting other reactions of the organic molecule. Two experimental studies (Thomson, 1960; Meloche et al., 1977) used isomerase enzymes to catalyze exchange reactions at 35°C and thereby measure equilibrium fractionations (Table 5), but no data are available to extrapolate these results to other temperatures. Calibrations of equilibrium fractionations based on analyses of geologic samples have not been reported.

Theoretical calculations of equilibrium isotope effects have been based on spectroscopic data (Knyazev et al., 1992). That approach produces estimates of fractionation in the vapor phase which are described by

$$\ln\alpha_{A/B} = \ln\beta_{A^*/A} - \ln\beta_{B^*/B} \quad (5)$$

where α is the equilibrium fractionation factor defined as the ratio of isotopic ratios (R_A/R_B) for compounds A and B, and β is the ratio of reduced partition functions for each compound in its monodeuterated (denoted by an asterisk) versus undeuterated forms. To a first approximation, gas-phase fractionation will differ from that in the condensed phase by an amount equal to the difference in vapor pressure ratios for the isotopologues of each molecule

$$\ln\alpha_{A/B}(l) - \ln\alpha_{A/B}(g) = \ln P_{A/A^*} - \ln P_{B/B^*} \quad (6)$$

(see Knyazev et al., 1992, eqn. 6). Table 6 compiles values for $\alpha_{o/w}(l)$, the equilibrium fractionation factor between organic H and water in the liquid phase at 27°C, calculated from Eqns. 5 and 6 with data from sources noted in the footnotes to Table 6.

This approach ignores isotope effects on solubility and adsorption, which could be important for sedimentary organic matter. Nevertheless such uncertainties are likely small relative to those in calculated values of β for the organic compounds, which dominate the overall uncertainty in the reported fractionation factors. The uncertainties reported by Knyazev et al. (up to 100‰ 1σ) are quite large, and highlight the need for more accurate estimates. Comparisons of experimental (Table 5) and theoretical (Table 6) estimates agree quite well for methyl and methylene hydrocarbon positions, but very poorly for methyl alcohols. Assuming that the values for hydrocarbons are accurate, we can then predict that at equilibrium icosane will be depleted in D by ~70‰ relative to water (α = 0.837 for 6 methyl hydrogens and α=0.944 for 36 methylene hydrogens). Similar calculations indicate that cholestane would be depleted by ~160‰ and phytane would be depleted by ~110‰.

Table 5. Equilibrium fractionation factors measured experimentally between C-bound H and water.

Structure[a]	$\alpha_{o/w}$[b]	Source of Estimate
C-C**H**$_3$	0.84	Experiment, Meloche et al. (1977)
C-C**H**$_2$-C	0.93	Experiment, Thomson (1960)
C-C**H**$_2$OH	1.00	Calculation, Meloche et al. (1977)
C-C**H**OH-C	1.10	Calculation, Meloche et al. (1977)
C-C**H**(OH)$_2$	1.18	Calculation, Meloche et al. (1977)

[a] Fractionation factor is specific to the hydrogen position in bold type.
[b] Equilibrium fractionation factor, defined as R_o/R_w, at 35°C in the liquid phase.

4.4. Rates of Hydrogen Exchange

Many different techniques have been used to estimate rates of hydrogen exchange. In general, they involve measuring the incorporation or loss of isotopic labels (either deuterium or tritium) by scintillation counting, conventional and isotope-ratio mass spectrometry, and NMR. In this section we summarize available data—both from new experiments described above, and from literature reports—that are relevant to hydrogen exchange in a geologic context. Measurements of exchange in the presence of rare metal catalysts, strongly acidic conditions, organic solvents, etc. have not been included, nor have studies of exchange of oxygen- or nitrogen-bound hydrogen.

4.4.1. Deuterium label experiments

The use of isotope-ratio mass spectrometry to analyze molecules that have been chemically converted to H_2 provides a

Table 6. Calculated equilibrium fractionation factors between C-bound H and water.

Structure*	ln $\beta_{R^*/R}$[a]	ln(P_{R/R^*})[b]	$\alpha_{O/W}$[c]	σ
		Alkanes		
CH_4	2.26 (0.02)	−0.0070[d]	0.692	0.042
C_2H_6	2.36 (0.07)	−0.0070[d]	0.765	0.099
CH_3-CH_2-CH_3	2.45 (0.06)	−0.0070[d]	0.837	0.078
CH_3-CH_2-CH_3	2.57 (0.09)	−0.0072[e]	0.944	0.102
C_6H_{12} (cyclohexane)	2.42 (NA)	−0.0072[e]	0.812	0.119
$(CH_3)_3$-CH	2.42 (0.06)	−0.0072[e]	0.812	0.080
		Alkenes		
CH_2=CH_2	2.33 (NA)	−0.0072[e]	0.743	0.130
C_6H_6 (benzene)	2.38 (0.06)	−0.0042[f]	0.783	0.083
CH_2=CH-CH=CH_2	2.09 (NA)	−0.0072[e]	0.584	0.165
		Ethers		
CH_3-CH_2-O-CH_3	2.38 (NA)	−0.0070[d]	0.781	0.124
CH_3-CH_2-O-CH_3	2.51 (NA)	−0.0072[e]	0.889	0.109
CH_3-CH_2-O-CH_3	2.45 (NA)	−0.0070[d]	0.837	0.115
		Carbonyl compounds, alcohols, amines		
CH_3-CHO	2.30 (0.07)	−0.0076[g]	0.721	0.105
CH_3-CO-CH_3	2.36 (0.08)	−0.0036[h]	0.768	0.112
CH_3-CO-O-CH_3	2.38 (0.13)	−0.0070[d]	0.781	0.180
CH_3-CO-O-CH_3	2.49 (0.13)	−0.0070[d]	0.872	0.161
CH_3-CO-OH	2.39 (NA)	−0.0070[d]	0.789	0.122
CH_3-OH	2.40 (.05)	−0.0070[d]	0.797	0.070
CH_3-NH_2	2.39 (NA)	−0.0070[d]	0.789	0.122
		Halogens		
CH_3Cl	2.36 (0.04)	0.0109[i]	0.779	0.059
H_2C=CCl_2	2.34 (0.07)	0.0109[i]	0.764	0.099
cis-HClC=CClH	2.31 (0.07)	0.0109[i]	0.741	0.102
trans-HClC=CClH	2.28 (0.07)	0.0109[i]	0.719	0.105

Fractionation factor is specific to the hydrogen position in bold type.

[a] Ratio of reduced partition functions for the organic compound, from Knyazev et al. (1992). Uncertainty is given in parantheses, and where not available a value of 0.09 is assumed for calculation of overall uncertainty.

[b] Vapor pressure isotope effect for the organic compound, sources of data are indicated by footnotes.

[c] Equilibrium fractionation factor for condensed phases at 27°C, calculated from Eqs. 5 and 6 using data in this table plus $\ln\beta_{HDO/H2O} = 2.55$ (Knyazev et al., 1992) and $\ln(P_{H2O/HDO}) = 0.0704$ (van Hook, 1968).

[d] Value for 2,2-dimethylpropane at 10°C reported by Hopfner (1969).

[e] Value for cyclohexane at 25°C reported by Kiss et al. (1972).

[f] Value for benzene at 20°C reported by Kiss et al. (1972).

[g] Value for toluene at 25°C reported by Kiss et al. (1972).

[h] Value for acetone at 20°C reported by Hopfner and Hostermann (1976).

[i] Value for chloromethane at −23°C reported by Hopfner (1969).

very high sensitivity to changes in isotopic composition. When organic matter with a natural abundance of D is equilibrated with D_2O, this translates into a very high sensitivity to hydrogen exchange. The natural abundance of deuterium is ~ 0.015% and the D/H ratio can be determined with a relative precision better than 1%. Incorporation of 1.5 ppm D is thus readily detectable and, if it occurred over the course of a month, the corresponding rate constant would be 1.8×10^{-5} yr^{-1} ($t_{1/2}$ = 38,000 yr). The price for this sensitivity is the inability to measure rates for specific hydrogen positions, because of the conversion to H_2 for analysis. Observing the loss of a D label could provide such information, but analysis of highly deuterium-enriched samples is problematic, particularly in the presence of a helium carrier gas.

Koepp (1978) first used offline combustion/reduction coupled with isotope-ratio mass spectrometry (IRMS) to measure the deuterium content of test compounds and petroleum fractions incubated with D_2O (Table 7). No catalysts were added to the experiments, and the observed exchange rates were very slow. Thus they probably represent a lower limit for what can be expected in natural environments. Extrapolation of the data in Table 7 to 100°C indicates exchange half-times of 10^7 and 10^5 yr for the saturated and aromatic fractions of petroleum (Koepp, 1978). These half-times are 3 to 4 orders of magnitude faster than for the model compounds *n*-hexane and toluene, possibly indicating the presence of more reactive compounds in the crude, saturated and aromatic fractions.

In contrast, exchange half-times determined for icosane on mineral substrates by our new, compound-specific analyses range from ~10,000 yr at 60°C to ~100,000 yr at 7°C (Table 7 and Fig. 3) Much of the increase in exchange rates relative to those reported by Koepp (1978) can likely be attributed to the presence of mineral catalysts. Still, these rates indicate that exchange in icosane should essentially be complete after a million years at any temperature above 0°C, a prediction which does not fit with available geochemical data (Andersen et al., 2001; Li et al., 2001). A possibility that remains to be investigated is whether catalytic mineral surfaces might be masked by the presence of polar organic material, thus explaining the lower apparent rates of exchange in natural sediments. Catalysis of hydrogen exchange by enzymes (e.g., Meloche et al., 1977) in natural sediments would serve to make the sterilized

Table 7. Exchange half-times derived from GC/MS and IRMS measurements of deuterium incorporation.

Analyte	Substrate	Temp (°C)	H Position	$t_{1/2}$ (yrs)	Reference[a]
methane				630,000	
n-hexane				87,000	
cyclohexane				140,000	
toluene	none[b]	200	all	350,000	1
saturates				7,700	
aromatics				200	
NSO compounds				0.99	
bulk oil				14	
methane				8,700	
n-hexane				1,700	
cyclohexane				8,700	
toluene	none[b]	240	all	23,000	1
saturates				870	
aromatics				28	
NSO compounds				0.22	
bulk oil				3.8	
		7[d]		>67,000	
		7[d]		91,000	
	mont.[c]	30	all	9,300	2
icosane		30		47,000	
		60		21,000	
		60		20,000	
		7		>59,000	
		7		>170,000	
icosane	silica	30	All	na	2
		30		>158,000	
		60		8,200	
		60		12,200	
pristane	mont.[e]	160	1°	2.1	3
			2°	1.5	
2,6,10-trimethylundecanoic acid			α-carbon	0.62	
			all	5.7	
3,7,11-trimethyldodecanoic acid	mont.	160	α-carbon	0.24	4
			all	2.3	
4,8,12-trimethyltridecanoic acid			α-carbon	0.21	
			all	2.3	

[a] (1) Koepp (1978); (2) this study; (3) Alexander et al. (1984); (4) Larcher et al. (1986).
[b] Samples were incubated with D_2O in Pyrex tubes, with no mineral substrate added.
[c] SAz-1 montmorillonite.
[d] Replicate temperatures represent 35 day and 88 day experiments.
[e] Montmorillonite, treated to give Al as sole interlayer cation, then saturated with D_2O.

laboratory exchange experiments anomalously slow, rather than fast, and so cannot explain this inconsistency.

The activation energy indicated by the data (proportional to the slope in Fig. 3) is lower than in all other experimental measurements of hydrogen exchange, including those examining aromatic hydrogen. A possible cause of anomalously fast exchange is that a small number of highly active catalytic sites exists in the mineral substrates, resulting in relatively quick exchange in a small subgroup of molecules. The "average" rate of exchange (i.e., the experimentally measured rate constant) early in the experiment will then be similar to that in the fastest-exchanging molecules, as illustrated in the example of Figure 2. Such uncertainties underscore the difficulty of using highly sensitive analytical techniques to measure very small increments of exchange.

Measurements of D-NMR or mass spectra for intact molecules (i.e., without conversion of the analytes to H_2) provide the ability to measure rates at specific molecular positions, but with greatly reduced sensitivity towards changes in deuterium content. Alexander et al. (1984) equilibrated pristane with Al-montmorillonite that had been vacuum-dried then rehydrated with D_2O. The incorporation of ~40% D into pristane was estimated via GC/MS, and proton NMR was used to assess relative deuterium abundances at different molecular positions. They estimate exchange half-times at 160°C of 2.1 and 1.5 yr for methyl and methylene positions of pristane, respectively (Table 7). These values are much slower than half-times determined for naphthalene under similar conditions (below), confirming that exchange of aromatic hydrogen is significantly faster than in aliphatic compounds.

Larcher et al. (1986) used GC/MS to measure the incorporation of D into isoprenoid acids at 160°C after incubation on D_2O-hydrated montmorillonite. They report the relative abundances of mono-, di-, and trideuterated molecules both for the

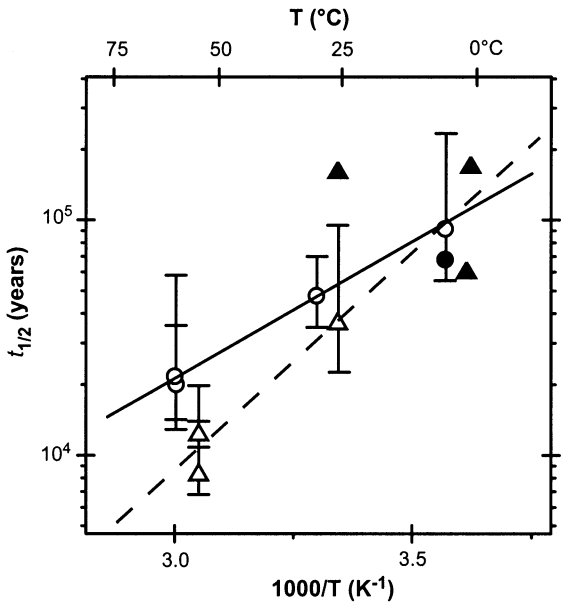

Fig. 3. Reaction half-times for hydrogen exchange in icosane incubated on clean montmorillonite (circles) or microcrystalline silica (triangles). Filled symbols indicate minimum estimates of exchange half-time based on no measured change in δD. Data for silica are offset slightly to the right for visual clarity.

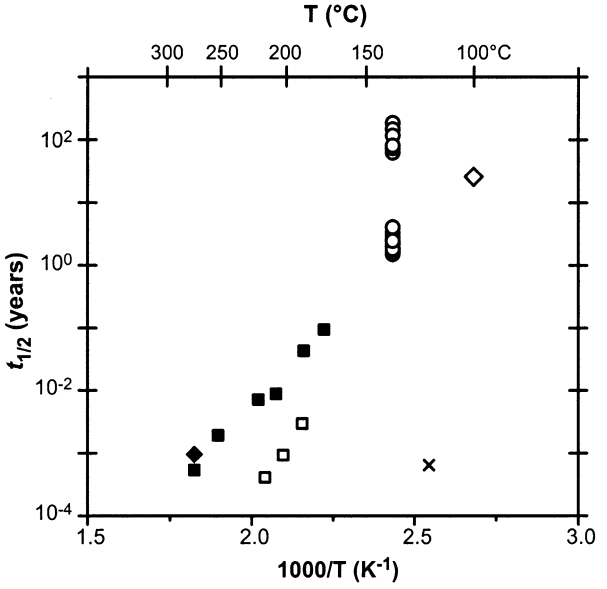

Fig. 4. Reaction half-times for hydrogen exchange in naphthalene incubated on various substrates. All data are from tritium label experiments by Alexander et al. (1981, 1982). Symbols represent: [1-^{3}H]naphthalene on Na-bentonite (solid squares), [2-^{3}H]naphthalene on Na-bentonite (solid diamond), [1-^{3}H]naphthalene on Al-bentonite (x), [3-^{3}H]2-methoxynaphthalene on Na-bentonite (open squares), [1-^{3}H]2-methoxynaphthalene on Na-bentonite (open diamond), and [1-^{3}H]2-methoxynaphthalene on powdered shale (open circles). Data for 2-methoxynaphthalene have been converted to naphthalene equivalents using the ratio of rate constants of 4800:1 measured by Alexander at al. (1982). Note that the y-axis is logarithmic.

molecular ions and for a rearranged fragment ion that includes the carboxyl group and α-carbon. From their data, we have calculated hydrogen exchange rate constants (Table 7) using Eqn. 2 by assuming $F_i = 0.000124$ (corresponding to δD = −200‰), $F_e = 1.0$, and that all of the deuterium in the rearranged ion is located on the α-carbon atom. As noted by Larcher et al., exchange at the α-carbon is very rapid, while exchange in the hydrophobic tail is even slower than for pristane incubated under identical conditions. They hypothesized that the presence of a polar carboxyl group preferentially orients the molecule on the highly polarized clay mineral surfaces, and thus effectively deactivates the aliphatic tail with respect to hydrogen exchange.

4.4.2. Tritium label experiments

Robert Alexander and coworkers pioneered the study of hydrogen exchange in hydrocarbons over 20 years ago by employing molecules labeled with tritium at specific molecular positions. The loss of tritium due to hydrogen exchange was monitored via scintillation counting. As for GC/MS measurements, the approach provides information about exchange at a specific molecular position but with relatively poor sensitivity. For typical tritium concentrations leading to count rates of ~10^3 dps, uncertainties in the measurement of specific activity can amount to half a percent or more. Accurate determination of rate constants thus requires experimental conditions that produce exchange over a period of days to weeks, not millennia. Such conditions include activated substrates, high temperatures, and potent catalysts.

The results of Alexander et al. (1981, 1982) are summarized in Figure 4. Tritiated naphthalene and 2-methoxynaphthalene were incubated on a variety of substrates, including powdered shales and bentonite clay. Where 2-methoxynaphthalene was studied, the resulting rate constants have been converted to equivalent values for naphthalene in Figure 4 (see figure caption) for ease of comparison with naphthalene data. The results show clearly that the rate of exchange in naphthalenes is affected by 1) temperature; 2) the surface acidity of mineral catalysts; and 3) the presence of electron-donating substituents on the aromatic ring system. For clay minerals, the surface acidity is primarily a function of mineralogy and the identity of interlayer cations, with Na producing the weakest acidity.

Replicate incubations of 2-methoxynaphthalene on a variety of shale samples at a constant temperature produced $t_{1/2}$ values ranging over 2 orders of magnitude (Fig. 4). Rates of exchange were not correlated with the concentration of organic carbon in the shale. Control samples lacking any mineral substrate produced no measurable exchange. These results demonstrate the important—and potentially variable—influence of mineralogy on exchange rates in natural samples.

Extrapolation of rates measured at temperatures of 177–275°C indicates exchange half-times for naphthalene of ~4500 yr at 50° and ~25 yr at 100°C on dry Na-bentonite (Alexander et al., 1982). These values are similar to the fastest (naphthalene-equivalent) rates for methoxynaphthalene incubated on dry, crushed shales. Alexander et al. (1982) further showed that the effective surface acidity of dry clay is several times that of wet clay. Their results for naphthalene therefore represent maximum rates of exchange that should be expected for aromatic compounds under natural conditions.

4.4.3. Measurements of sedimentary lipids

The ability to estimate hydrogen exchange in geologic samples has been limited, until very recently, by the ambiguity inherent in measuring D/H ratios of bulk organic matter. Two recent reports using compound-specific measurements are now available, in addition to our new data. Andersen et al. (2001) measured δD values of *n*-docosane and 5α-cholestane from organic-rich marls deposited in the Mediterranean Sea during the Messinian (~6 Ma). Hoping to select analytes minimally affected by exchange, they examined only compounds extracted from the sulfur-bound organic fraction. With one exception, they found that the difference in δD between those two compounds was consistently 55–90‰ (cholestane has more negative δD values). This offset is indistinguishable from that observed between *n*-alkanes and sterols in all modern plants observed thus far (Sessions et al., 1999), so Andersen et al. (2001) concluded that the differences in δD are consistent with little or no exchange having occurred over the last 6 million years.

Yang and Huang (2003) measured δD values of *n*-alkanes, *n*-alcohols, and *n*-acids preserved in fossil leaves and surrounding sediments from the Miocene (15–20 Ma) Clarkia Formation, as well as from modern relatives of the fossil trees. Values of δD were also measured for pore waters extracted from sediments surrounding the fossils, and for surface waters near the modern trees. The authors argue that hydrogen exchange in these samples was negligible based on the following lines of evidence: 1) large variability, up to 120‰, in δD values of individual lipids extracted from the same sample, and of lipids extracted from closely spaced samples, 2) large offsets in δD of ~100‰ between organic hydrogen and water, and 3) similar organic/water differences in δD for the fossil and modern analogs.

It appears likely that hydrogen exchange is indeed minimal in the compounds examined by both of these studies. However, basing such conclusions on the relative D/H ratios of organic molecules and water without considering the magnitude of equilibrium fractionations is ambiguous. For example, as discussed above icosane will be depleted relative to water by ~70‰ at equilibrium at 30°C. This value is close to the ~100‰ offset measured by Yang and Huang (2003). Similarly, δD values for docosane and cholestane, both in isotopic equilibrium with water having δD = 0‰, would be roughly −70‰ and −160‰, respectively. The difference between the two of ~90‰ is indistinguishable from those measured by Andersen et al. (2001). In both cases therefore, the large shifts in δD values between adjacent sedimentary layers (Andersen et al., 2001) and between homologous *n*-alkanes (Yang and Huang, 2003) provide much more robust evidence for the lack of exchange.

While demonstrating a lack of exchange can be quite difficult, demonstrating the occurrence of exchange is straightforward. Acyclic isoprenoid hydrocarbons extracted from several ancient bitumen and petroleum samples (Table 3) have δD values that are identical or slightly more positive than those of coexisting *n*-alkyl hydrocarbons. Assuming that biosynthetic processes have always produced isoprenoid and *n*-alkyl lipids with distinctly different values of δD (Estep and Hoering, 1980; Sessions et al., 1999, 2001; Chikaraishi and Naraoka, 2003) these data represent compelling evidence for isotopic exchange. An alternative explanation might be that isotopic fractionation (due to kinetic, not equilibrium isotope effects) of isoprenoids during thermal maturation leads to an increase in the D/H ratios of those compounds. But to produce virtually identical δD values in isoprenoid and *n*-alkyl molecules from every sample, regardless of age and maturity, would require extreme coincidence and is implausible.

Similar evidence for exchange is observed in reservoired oils from the Otway Basin of Australia (Schimmelmann et al., unpublished data) and from the Williston Basin of Canada (Li et al., 2001). Calculated equilibrium fractionation factors indicate that when phytane and icosane—representative examples of the isoprenoids and *n*-alkanes reported in Table 4—equilibrate with the same water at 30°C, phytane should become depleted in D by ~40‰ relative to icosane. The fact that this pattern is not observed might indicate that isotopic equilibrium was not reached, or that it was reached at a higher temperature where fractionations are smaller. It is doubtful, however, that differences of 40‰ are significant given the uncertainties reported in Table 6.

The mechanisms and timing for exchange in these ancient samples are still unknown. Schimmelmann et al. (2001) have shown unequivocally that water H is incorporated into the hydrocarbons generated during hydrous pyrolysis of kerogen, so catagenesis may be partially responsible. On the other hand, 75 samples from 4 separate Australian petroleum basins show no correlation between δD values of petroleum alkanes and formation waters (Schimmelmann et al., unpublished data). This suggests that wholesale exchange with environmental water is not occurring, although minor amounts of exchange cannot be excluded. A possible reconciliation of these results is that catagenesis involves extensive scrambling of hydrogen between organic compounds, but little exchange with water.

At the opposite end of the time spectrum, Hebting et al. (2003) have shown that the thiol analog of phytol, a possible intermediate in phytol diagenesis, readily participates in free-radical reactions with the potential for constitutional exchange of at least 11 out of 42 hydrogens. More generally, any process involving allylic thiols, which may be intermediates in the formation and decomposition of *S*-linked macromolecules, has a high likelihood of H exchange. Because of rapid isomerization around the tertiary carbon centers in isoprenoid compounds, this mechanism is likely to cause more extensive exchange in isoprenoids than in *n*-alkyl lipids. Such diagnetic reactions are conceivably responsible for some of the similarity in δD values between ancient isoprenoid and *n*-alkyl hydrocarbons, although the magnitude of the isotopic shift indicates they are not solely responsible.

4.5. Stereochemistry as a Proxy for Hydrogen Exchange

The preceding sections outline numerous difficulties in assessing the extent of hydrogen exchange in sedimentary organic compounds on the basis of isotopic data. Given the mechanistic similarities between stereochemical and pure hydrogen exchange, we consider here the possibility that stereochemical configuration can serve as a proxy for hydrogen exchange. Such an approach would be convenient because most natural products are synthesized with very specific and well-

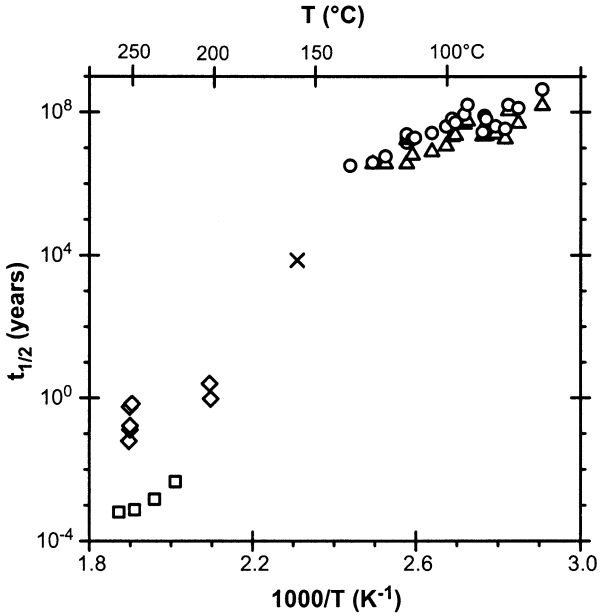

Fig. 5. Rates of stereochemical inversion at C-6 and C-10 in pristane incubated on shale plus elemental sulfur (squares; Abbott et al., 1985), at C-22 in hopanes incubated on shale (diamonds; MacKenzie et al., 1981), and in steranes (circles) and hopanes (triangles) extracted from North Sea and Pannonian Basin sediments (MacKenzie and McKenzie, 1983). The x represents the rate of bulk hydrogen exchange estimated for pristane incubated on shale (as described in the text).

known stereochemistry, and changes can be observed with great sensitivity via established GC/MS techniques.

Two laboratory studies of stereochemical inversion in pristane (Abbott et al., 1985) and hopanes (Mackenzie and McKenzie, 1983) are available. Abbott et al. (1985) incubated mesopristane with powdered shale plus elemental sulfur, which was added as a free-radical initiator and possible catalyst. Over the temperature range 224–261°C, they measured half-times for stereochemical inversion ranging from 5.6 to 40 h (Fig. 5). Mackenzie and McKenzie (1983) measured rates of stereochemical inversion for hopanes produced during laboratory maturation of shale at 203 and 253°C, and obtained results approximately 2 orders of magnitude slower than those of Abbott et al. (1985; Fig. 5). Mackenzie and McKenzie also measured stereochemical inversion at C-20 in steranes and C-22 in hopanes obtained from Pannonian Basin and North Sea shales. By reconstructing the thermal history of the sediments, they were able to calculate rates of stereochemical inversion over the temperature range 72–125°C (Fig. 5).

For comparison, we estimate the rate of hydrogen exchange in pristane under similar conditions as follows. From the data of Alexander et al. (1984), a weighted-average exchange rate for all pristane hydrogen of 4.5×10^{-5} h^{-1} on Al-montmorillonite was calculated. Comparison of Al-montmorillonite to shale was based on naphthalene incubated on Al-montmorillonite at 120°C ($k = 0.122$ h^{-1}; Alexander et al., 1982) and 2-methoxynaphthalene on shale at 138°C ($k = 0.15$ h^{-1}, the mean value from Alexander et al., 1981, converted to naphthalene equivalent of $k = 3.0 \times 10^{-5}$ h^{-1} using the ratio for methoxynaphthalene/naphthalene of 4800). The ratio of rates on Al-montmorillonite versus shale is then ~4070, leading to a predicted average exchange rate for pristane (on shale, 160°C) of 1.1×10^{-8} h^{-1} ($t_{1/2}$ = 7200 yr; Fig. 5).

The estimated rate of hydrogen exchange in pristane falls midway between experimental and empirical measurements of stereochemical inversion rates. The reasons for the very large discrepancies are not known. Possibilities include the importance of different reaction mechanisms at higher temperatures (and for hopanes versus pristane), the presence of catalysts in experimental measurements, and difficulties in integrating the thermal history of natural sediments. The results of Alexander et al. (1984) indicate that bulk exchange in pristane should be at least 10-fold faster than that at tertiary carbon centers alone (stereochemical inversion involves only tertiary positions), because exchange at positions adjacent to tertiary centers is more rapid than at the 3° carbon itself. This is consistent with the pattern observed for natural sediments but not for laboratory incubations. In summary, the possibility of using stereochemistry to gauge isotope exchange appears quite promising, but much more quantitative information on the relationship is needed.

4.6. Hydrogen Exchange during Structural Rearrangements

Changes in molecular structure, such as the migration of double bonds, provide an opportunity for constitutional exchange of hydrogen. They do not guarantee exchange. Thus useful isotopic information may be available even in molecules which do not retain the structure of their parent biomolecule. New data regarding the incorporation of D by cholest-4-ene and other isomerization products during incubations with D_2O allow us to assess this hypothesis directly.

Comparison of the deuterium labeling pattern for cholest-4-ene (Δ^4) and cholest-5-ene (Δ^5) reveals that the Δ^4 compound, in which the double bond had migrated, incorporated ~0.5 deuterium atoms per molecule (Fig. 6).This distribution cannot easily be reconciled with a simple ionic mechanism for the isomerization of Δ^5 to Δ^4, which should result in exactly one D on every molecule of Δ^4. We hypothesize that hydrogen from C-4 is, with high probability, transferred to C-6 during double-bond migration. Observed differences in D incorporation during isomerization on various substrates can then be rationalized as due to differences in the efficiency of this transfer by different catalysts. For example, silica consistently produced less Δ^4 than did montmorillonite, yet Δ^4 produced on silica contains more D than that produced on montmorillonite. The addition of XAD resin to montmorillonite dramatically decreased both the amount of Δ^4 produced, as well as the amount of deuterium in the Δ^4 product.

Diacholestenes contained between 0 and ~12 D atoms per molecule (Fig. 7).This is less than half the level of enrichment observed for the same reaction occurring in deuterated acetic acid with toluene-*p*-sulfonic acid by Akporiaye et al. (1981). As with cholestene, montmorillonite produced a higher abundance of diasterenes than did silica, but diasterenes produced on silica incorporated more deuterium. Up to 50% of diasterene molecules produced on montmorillonite + XAD contained no measurable deuterium (Fig. 7), a stunning result considering the extent of rearrangement involved. An important question is whether the presence of XAD inhibits exchange by altering the

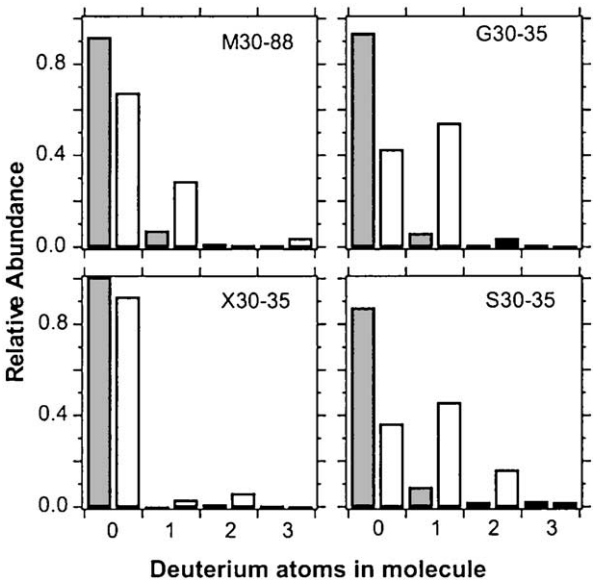

Fig. 6. Relative distribution of deuterium in cholest-5-ene (gray) and cholest-4-ene (white) in four representative samples. Abundances are normalized to give 100% for the sum of all isotopologues, and are calculated from the *m*/*z* 370 fragment. Sample numbers are explained in Table 4.

reaction mechanism, or whether organic hydrogen from the polymer replaces water as the source of hydrogen during geometric exchange. These different modes of action cannot currently be resolved, though their implications for preservation of D/H ratios are quite different.

Incorporation of deuterium into the 20R and 20S diasterene isomers was very similar, with the exception of those incubated on silica (Fig. 7). In silica samples, the 20S isomer contains ~1 extra deuterium relative to the 20R isomer. Since the first product of sterene rearrangement is the 20R diasterene (Kirk and Shaw, 1975; Peakman et al., 1988), subsequent chiral inversion should lead to the 20S isomer with one additional D. Two alternative explanations are that 1) inversion at C-20 is accompanied by exchange only on silica, or 2) exchange at C-20 occurs during rearrangement on substrates other than silica, hence no additional labeling is observed during subsequent inversion.

Our results for cholestenes and diacholestenes indicate that for double-bond migration, carbon-skeletal rearrangement, and possibly for stereochemical inversion, the number of hydrogens which exchange during rearrangement is significantly less than the number of C-H bonds that are broken. This exchange 'efficiency' ranged from ~50% for clean silica, the most effective catalyst for hydrogen exchange, to <10% for montmorillonite + XAD resin. Our results also show that conditions which produced substantial amounts of diasterenes resulted in very little exchange (<1 atom) in the remaining parent cholest-5-ene. These results lead to a number of predictions. First, the *absence* of rearranged diasterenes in sediments is probably a good indicator that little hydrogen exchange has occurred. Even when diasteranes are present, exchange in primary sterols or sterenes may be limited. Finally, it is conceivable that under certain conditions even highly rearranged diasterenes could preserve the hydrogen-isotopic ratio of the parent biomolecule.

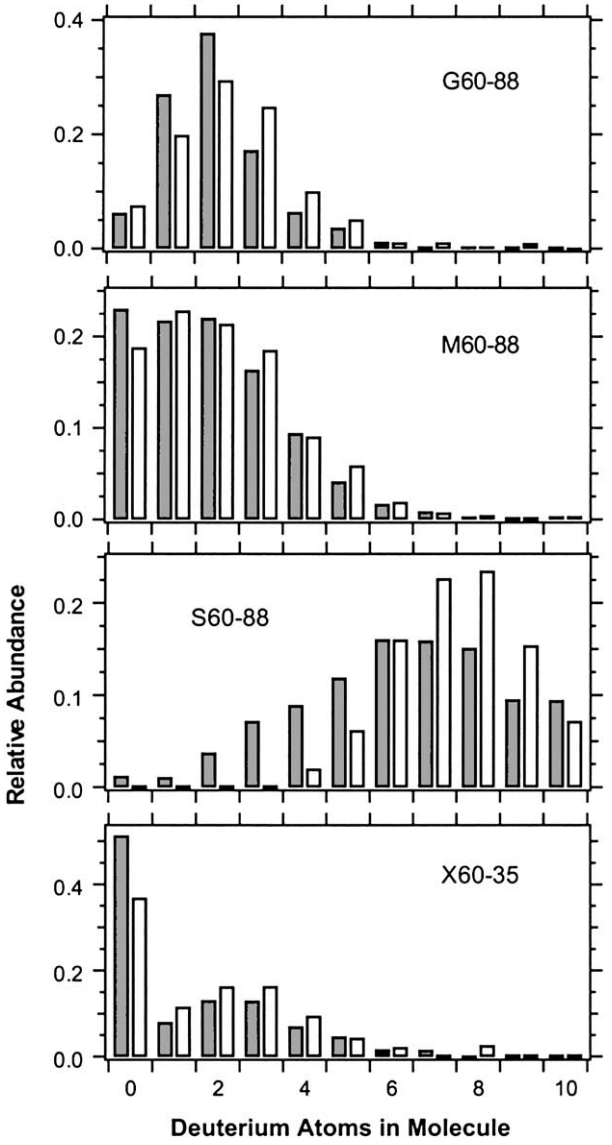

Fig. 7. Distribution of deuterium in 20R- (gray) and 20S-diacholestene (white) in four representative samples. Abundances are normalized to give 100% for the sum of all isotopologues, and are calculated from the *m*/*z* 355 fragment.

4.7. Hydrogen Isotope Ratios during the Diagenesis of Sterols

A wide array of processes can lead to hydrogen exchange in organic molecules. An important question concerns the net effect of these processes on the D/H ratio of a molecule. As an example, we have estimated changes in the δD value of cholesterol through a series of hypothetical diagenetic reactions (Fig. 8). These calculations are intended not as an accurate prediction of isotopic compositions but as an illustration of the types of changes that might be observed. The results also highlight several opportunities that might be exploited. Sterols were chosen as an example both because of the variety of processes affecting them, and because of the source specificity that can make sterol-based records so useful. The example also

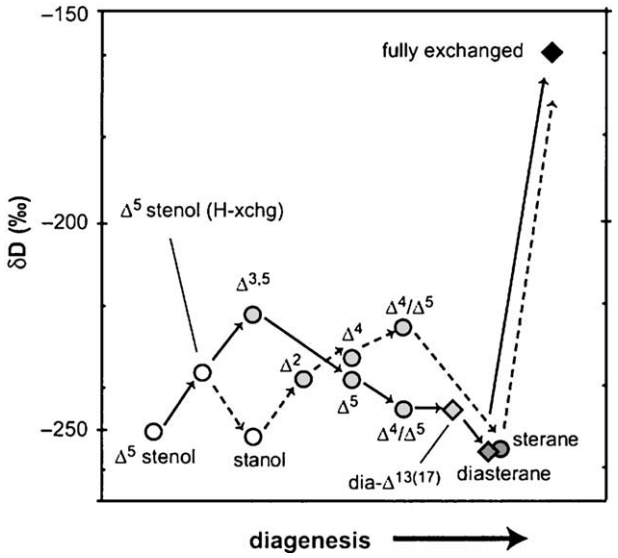

Fig. 8. Hypothetical example of changes in δD of products derived from cholesterol through a sequence of typical diagenetic reactions. White symbols are alcohols, gray symbols are alkenes, and black symbols are alkanes. Circles are molecules with the 10,13-dimethyl configuration of sterenes; diamonds have the 5,14-dimethyl configuration of diasterenes.

helps to identify important gaps in our knowledge of hydrogen exchange and fractionation processes.

The reactions shown in Figure 8, and their sequence, are representative rather than definitive. The starting compound is assumed to have δD = −250‰, typical of sterol compounds measured by Sessions et al. (1999) for organisms growing in seawater with δD ≈ 0‰. Because no detailed information is available, we further assume that no intramolecular isotopic ordering exists. Equilibrium fractionation factors from Table 6 were used to calculate the δD of exchanged hydrogens.

Before any structural changes, exchange of hydrogen at C-2, C-3 and C-4 is possible on short time-scales because the acidity of these positions is greatly increased by proximity to the electron-withdrawing hydroxyl group. No information about the rate of exchange at such positions is currently available, so we assume that all five hydrogens have exchanged in Figure 8.

One of the first diagenetic reactions affecting cholesterol will be dehydration to form the $\Delta^{3,5}$ diene, in which no hydrogen is gained but a previously-exchanged hydrogen at C-4 is lost (de Leeuw et al., 1989). Laboratory studies of the hydrogen isotope effect on catalytic dehydration of alcohols consistently measure values of $k_H/k_D \approx 2$, regardless of temperature (Dabbagh et al., 1991) and catalytic substrate (Shi et al., 2002). The measured isotope effect refers to hydrogen on carbon atoms adjacent to the hydroxyl group and indicates that elimination of H—rather than loss of OH—is the rate-limiting step. Assuming a similar isotope effect for the thermal dehydration of sterols, the hydrogen remaining at C-4 would be enriched in D by a factor of 2, equivalent to a 750‰ increase if the molecule started with δD = −250‰. This is a very large effect, even when averaged across the 45 hydrogens in a cholesterol molecule, and should be directly observable in comparisons of sterols and steradienes.

Next, selective hydrogenation of the Δ^3 position produces the Δ^5-sterene with the addition of two hydrogens. A recent report by Andersen et al. (unpublished data) compared δD values for carotane, derived from β-carotene by the saturation of 11 double bonds, with pristane to estimate the isotopic composition of added hydrogen at −590‰. This value is quite sensitive to the assumption that pristane and β-carotene had the same initial isotopic compositions, which is in turn rather uncertain (e.g., Sessions et al., 1999).

Cholestene will slowly isomerize between the Δ^4 and Δ^5 forms, potentially exchanging one hydrogen with each flip of the double bond. If this process is repeated, all hydrogens on C-4 and C-6 could eventually exchange. Isomeric equilibrium between Δ^4 and Δ^5 cholestene does not, however, imply isotopic equilibrium as indicated by our observation that <50% of cholest-4-ene obtained a deuterium label in incubation experiments. There may also be steric effects which govern whether axial or equatorial positions are both equally subject to exchange. Figure 8 assumes that all hydrogen at C-4 and C-6 has exchanged, an assumption which leads to replacement of the strongly D-enriched hydrogen at C-4 resulting from dehydration.

An alternative pathway to Δ^5, endorsed by Peakman and Maxwell (1988) and others, is hydrogenation of cholesterol to cholestanol, dehydration to Δ^2 cholestene, then isomerization via Δ^3 and Δ^4 to Δ^5. An important uncertainty in this pathway is whether the hydrogen at C-2, which is strongly enriched in D as a result of fractionation during dehydration, is subject to exchange during double-bond migration. Figure 8 assumes that it is not, with the result that the δD value of cholest-4-ene is slightly heavier when following this pathway.

Rearrangement of the sterene to diasterene results, on average, in the exchange of five additional hydrogens. The number of positions affected may vary substantially with the reaction conditions, but our data suggest that the extent of this constitutional exchange is relatively constant for a given mineral substrate. Inversion at C-20 can result in stereochemical exchange of one additional hydrogen, and hydrogenation of the double bond will add two additional, strongly depleted hydrogens. Following these diagenetic alterations, all of the hydrogen on cholestane and diacholestane is still subject to pure exchange, albeit on uncertain timescales. Based on data in Table 6, we estimate δD = −160‰ for a sterane (and diasterane) in isotopic equilibrium at 30°C with water having δD = 0‰.

Several conclusions can be drawn from Figure 8. First, the change in δD values across the entire sequence of diagenetic products is relatively small. Out of 48 hydrogen atoms in diacholestane, three were added by hydrogenation, approximately twelve exchanged with water, and 33 remain from the parent cholesterol. Thus any of the steroid products may serve as crude indicators of primary D/H ratios. When intact sterols (as opposed to sterenes or stanols) are found, the number of hydrogen atoms subject to exchange drops to five, with none due to addition and none fractionated by dehydration.

Second, sterene molecules represent potentially problematic targets for developing paleoclimatic records. The extent of hydrogen exchange resulting from isomerization cannot be determined from ratios of Δ^4/Δ^5 molecules. Identical molecules produced by a different series, or even a different order, of diagenetic reactions might have different δD values. Similar problems exist for isomerization of $\Delta^{8(14)}$ and Δ^{14} sterenes, and possibly for isomerization of Δ^7 and $\Delta^{8(14)}$ (de Leeuw et al., 1989).

Third, because diagenetic changes in δD are not large, it is

conceivable that different sterane skeletons (or steranes and hopanes) deriving from different biologic sources might preserve large differences in δD. Because the reactions depicted in Figure 8 are essentially unidirectional, any downcore convergence in δD values between those molecules would then provide evidence for ongoing hydrogen exchange.

5. CONCLUSIONS

Coincident values of δD in *n*-alkyl and isoprenoid carbon skeletons extracted from bitumen and petroleum samples suggest that exchange of carbon-bound hydrogen is extensive in many ancient (>340 Ma) rocks. Similar studies on young (<20 Ma), immature sediments suggest little hydrogen exchange (Andersen et al., 2001; Yang and Huang, 2003). The significance of hydrogen exchange in samples of intermediate age remains unexplored. Experimental and empirical data regarding exchange rates are scarce, and uncertainties cover several orders of magnitude. Available evidence indicates that both temperature and lithology are likely to be important variables in determining exchange rates.

Several tests have been proposed for identifying the effects of exchange in geologic samples. Comparison of δD values for isoprenoid and *n*-alkyl lipids is a potentially useful, but probably insensitive, assay. Comparison of δD values for lipids and water can only be used when the distribution of D in organic matter and water started far from equilibrium. Identification of large differences in δD between homologous compounds or consecutive samples is currently the most promising approach, although it can never exclude the possibility that some exchange has occurred. A possibility that deserves further investigation is that structural changes, such as stereochemical inversion or rearrangement of sterenes to diasterenes, could serve as a proxy for hydrogen exchange.

The likelihood that hydrogen exchange is significant over geologic timescales increases both the richness and complexity of information recorded in isotopic records of ancient organic matter. It also provides many new opportunities. For example, differential rates of exchange might be used either as a chronometer, or to determine—with temporal resolution—the isotopic compositions of fluids. Where complete exchange is achieved, the temperature dependence of equilibrium isotope effects could be exploited. In this context, we should not be concerned that, unlike carbon, hydrogen isotopic compositions of primary products are not well preserved over long timescales. Rather, we should view organic hydrogen as a continuously evolving system that can provide information about geologic conditions and processes during its burial in sedimentary rocks.

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