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Compound-specific δD–$\delta^{13}C$ analyses of *n*-alkanes extracted from terrestrial and aquatic plants

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Abstract

Stable hydrogen and carbon isotopic compositions of individual *n*-alkanes were determined for various terrestrial plants (33 samples including 27 species) and aquatic plants (six species) in natural environments from Japan and Thailand. In C3 plants, *n*-alkanes extracted from angiosperms have a δD value of -152 ± 26‰ (relative to Standard Mean Ocean Water [SMOW]) and $\delta^{13}C$ value of -36.1 ± 2.7‰ (relative to Peedde Belemnite [PDB]), and those from gymnosperms have a δD value of -149 ± 16‰ and $\delta^{13}C$ value of -31.6 ± 1.7‰. Angiosperms have *n*-alkanes depleted in ^{13}C relative to gymnosperms. *n*-Alkanes from C4 plants have a δD value of -171 ± 12‰ and $\delta^{13}C$ value of -20.5 ± 2.1‰, being a little depleted in D and much enriched in ^{13}C compared to C3 plants. *n*-Alkanes of CAM plants are a little depleted in D and vary widely in $\delta^{13}C$ relative to those of C3 and C4 plants. In aquatic plants, *n*-alkanes from freshwater plants have a δD value of -187 ± 16‰ and $\delta^{13}C$ value of -25.3 ± 1.9‰, and those from seaweeds have a δD value of -155 ± 34‰ and $\delta^{13}C$ value of -22.8 ± 1.0‰. All *n*-alkanes from various plant classes are more depleted in D and ^{13}C relative to environmental water and bulk tissue, respectively. In addition, the hydrogen and carbon isotopic fractionations during *n*-alkane synthesis are distinctive for these various plant classes. While C3 plants have smaller isotopic fractionations in both D and ^{13}C, seaweed has larger isotopic fractionations.

Keywords: Terrestrial plant; Aquatic plant; *n*-Alkane; δD; $\delta^{13}C$

1. Introduction

n-Alkanes are one of the most abundant lipid molecules biosynthesized by terrestrial plants, aquatic plants, and certain algae. *n*-Alkanes of terrestrial plants are characterized by strong odd predominance in C_{25}–C_{35} carbon-numbered range (Castillo et al., 1967; Rieley et al., 1991; Collister et al., 1994), whereas aquatic plants are characterized by enrichment of C_{23} and C_{25} *n*-alkanes (Baas et al., 2000; Ficken et al., 2000). Relatively short-chain *n*-alkanes (C_{15}, C_{17} and C_{19}) are often attributed to algae and cyanobacteria (Han et al., 1968; Gelpi et al., 1970). Therefore, sources of *n*-alkanes in natural samples such as soils, sediments, petroleum and coals have been often inferred from their molecular distributions (e.g. Robinson et al., 1984; Cranwell et al., 1987; Rieley et al., 1991). Over the past decade, many studies have employed stable carbon isotopic compositions of individual *n*-alkanes to infer biosynthetic processes, source inputs, and paleoenvironmental conditions (Hayes et al., 1989; Hayes, 1993). Carbon isotopic compositions of biological lipid molecules including *n*-alkanes from several species of terrestrial plants will provide essential background information on isotopic signatures of natural sedimentary lipids (Collister et al., 1994; Lockheart et al., 1997, 1998; Ballentine et al., 1998).

Hydrogen is also an essential element of organic matter, and the isotope effects are commonly large. Besides isotopic fractionation during biosynthesis, hydrogen isotopic composition of organic matter is related to hydrologic variables such as seawater mass and humidity, while the carbon isotopic composition is dependent on carbon cycles as well as ecosystems. There have been many studies on δD of non-exchangeable hydrogen in cellulose as a proxy for various environmental and climatic factors (e.g. Pendall et al., 1999). Furthermore, Sternberg (1988) found that D/H ratios of plant lipid

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fractions were correlated with D/H ratios of environmental water. The lipid fractions contain a variety of organic compounds. Hence the D/H ratios of individual lipid compounds can supply more precise information for environmental and biological studies. Compound-specific hydrogen isotope measurements have been developed during the last few years (Burgøyne and Hayes, 1998; Hilkert et al., 1999). Recently, Sessions et al. (1999) reported the δD values of several lipid compounds, including *n*-alkanes, from various organisms. Xie et al. (2000) used a vertical δD profile of C_{23} *n*-alkane derived from *Sphagnum* species in a peat core sample for a paleoclimatic study. Hydrogen isotopic composition may also be a new means to clarify the sources of sedimentary *n*-alkanes. However, hydrogen isotopic compositions of *n*-alkanes in natural samples are not well known. C3, C4, CAM and aquatic plants have different water-use efficiencies as well as different biochemical reactions during photosynthesis. The purpose of this study is to investigate δD and δ^{13}C distributions of *n*-alkanes from terrestrial (C3, C4, CAM) and aquatic (fresh water and marine) plant leaves in natural environments. In addition to a few previous studies (Collister et al., 1994; Lockheart et al., 1997, 1998; Ballentine et al., 1998; Sessions et al., 1999; Xie et al., 2000), we will provide more detailed isotopic data and clarify hydrogen isotopic fractionation between *n*-alkanes and environmental water.

2. Results and discussion

2.1. Samples

Thirty-three terrestrial plant leaves including 22 C3 plants (18 angiosperms and 4 gymnosperms), seven C4 plants, three CAM plants and one fern were used in this study (Table 1). Six aquatic samples including three freshwater plants and three seaweeds were also analyzed. These samples were collected from suburbs of Tokyo (nine species), Gunma Prefecture (around Lake Haruna: 18 species), Okinawa (one species) and Ogasawara Island (one species) in Japan and from farms in Thailand (four species). Three freshwater plants were collected from about 1 m depth in Lake Haruna in Gunma Prefecture, Japan (36°28′N; 138°52′E). Three seaweeds were collected from about 2 m depth along the seacoast near Tokyo. The environmental water such as precipitation in Tokyo area, the lake water and the seawater were also collected (Table 2).

2.2. Hydrogen isotopic compositions of environmental water

The δD values of the environmental water are summarized in Table 2. Hydrogen isotopic compositions of precipitation in Tokyo area range from −74 to −10‰ with an annual mean of −42‰, and those in Thailand are similar ranging from −76 to −9‰ (IAEA/WMO, 1999). The lake and seawater have δD values of −60 and 0‰, respectively.

2.3. Bulk $\delta^{13}C$

Bulk carbon isotopic compositions of the plant leaves are shown in Table 1. Generally C3 plants use a Calvin–Benson cycle characterized by the use of ribulose biphosphate carboxylase oxidase (RUBISCO), and C4 plants use a Hatch-Slack cycle characterized by phosphoenol-pyruvate carboxylase (PEPC). RUBISCO has the largest kinetic isotope effect (∼26‰) between bulk plant tissue and CO_2. On the other hand, the isotope effect of C4 plants (∼5‰) is much smaller (O'Leary, 1993; Lajtha and Marshall, 1994). CAM plants have both C3- and C4-like characteristics depending on growth environment (Lerman and Queiroz, 1974), and usually have an intermediate isotope effect between C3 and C4 plants (Deines, 1980).

In this study, C3 and C4 plants have the bulk δ^{13}C values of −31.5±3.3‰ and −12.0±1.1‰, respectively. In C3 plants, angiosperms (−32.8±2.5‰) are more depleted in ^{13}C than gymnosperms (−26.9±1.1‰). CAM plants have a wide variation in bulk δ^{13}C (−27.1 to −13.6‰), being an intermediate isotopic composition between C3 and C4 plants. Freshwater plants of this study have δ^{13}C values of −15.6±1.0‰, which is similar to C4 plants. It is known that freshwater plants sometimes use ^{13}C-enriched HCO_3^- for carbon fixation, because of low levels of dissolved CO_2 in lake water (Keeley and Sandquist, 1992; Hayes, 1993). In this study, carbon isotopic compositions of freshwater plants can have a C4-like signature. Seaweeds also have δ^{13}C values of −15.6±1.0‰. The δ^{13}C range of this study is consistent with many previous studies (e.g. Deines, 1980).

2.4. Molecular distributions and isotopic profiles of the n-alkanes

The concentrations of *n*-alkanes are summarized in Table 1, along with parameters such as carbon preference index (CPI) and average chain length (ACL). All species show a strong odd carbon-numbered predominance in the range from C_{13} to C_{39}. Most terrestrial plants have an abundance maximum at *n*-C_{29}, *n*-C_{31} or *n*-C_{33}. In freshwater plants, *n*-alkanes range from C_{17} to C_{35} with an abundance maximum at *n*-C_{23} or *n*-C_{25}. These molecular distributions are consistent with many previous studies (e.g. Castillo et al., 1967; Collister et al., 1994; Baas et al., 2000). Three seaweeds have shorter chain *n*-alkanes, ranging from C_{13} to C_{17}, than other samples.

Table 1
Samples used in this study

Sample	Code	Type	Date	L[a]	C_{range}	C_{max}	CPI_{total}[b]	$CPI_{25–33}$[c]	ACL[d]	Bulk tissue $\delta^{13}C$[e]
Terrestrial higher plant species										
Zea mays	ZM	C_4 (a)[f]	7/98	J_T	25–35	33	12.2	11.5	31.0	−12.7
Quercus acutissima	QA	C_3 (a)[g]	7/98	J_T	23–33	29	15.5	15.5	29.2	−32.0
Zoysia japonica	ZJ	C_4 (a)	9/98	J_T	27–37	33	11.1	8.9	32.8	−13.5
Camellia sasanqua	CA	C_3 (a)	10/98	J_T	22–22	29	3.1	3.3	28.7	−28.6
Chamaecyparis obtusa	CO	C_3 (g)[h]	10/98	J_T	23–37	33	11.5	13.4	27.6	−28.3
Pinus thunbergii	PT	C_3 (g)	11/98	J_T	23–33	29	3.8	3.9	33.0	−26.0
Colocasia esculenta	CE	CAM (a)	11/98	J_T	23–33	29	19.8	20.0	29.2	−27.1
Lycoris radiata	LR	CAM (a)	12/98	J_T	23–33	31	11.8	11.8	30.8	−21.9
Miscanthus sinensis	MS1	C_4 (a)	9/99	J_T	22–39	31	4.3	4.0	30.6	−10.1
Saccharum officinarum	SO1	C_4 (a)	3/00	J_{Ok}	22–36	31	3.1	3.2	30.1	−12.6
Albizia julibrissin	AJ	C_3 (a)	9/00	J_{Og}	23–33	29	2.6	2.6	29.5	−33.3
Benthamidia japonica	BJ1	C_3 (a)	5/99	J_G	23–33	29	27.9	29.1	28.1	−34.3
Cryptomeria japonica	CJ1	C_3 (g)	5/99	J_G	25–35	33	9.7	10.9	32.7	−26.4
Acer carpinifolium	AC1	C_3 (a)	5/99	J_G	23–35	31	30.3	30.4	30.2	−31.8
Acer argutum	AA1	C_3 (a)	5/99	J_G	23–35	31	16.7	17.0	28.7	−34.2
Phrogmites communis	PC	C_3 (a)	10/99	J_G	22–35	29	1.4	1.4	28.4	−32.3
Benthamidia japonica	BJ2	C_3 (a)	10/99	J_G	23–33	29	10.3	10.7	28.6	−35.6
Prunus jamasakura	PJ	C_3 (a)	10/99	J_G	21–33	29	19.1	19.9	29.7	−32.0
Cryptomeria japonica	CJ2	C_3 (g)	10/99	J_G	25–35	33	12.0	14.3	32.7	−27.9
Acer carpinifolium	AC2	C_3 (a)	10/99	J_G	23–35	31	14.0	14.0	30.5	−33.8
Acer argutum	AA2	C_3 (a)	10/99	J_G	23–35	31	8.1	8.1	29.6	−34.8
Taraxacum officinale	TO	C_3 (a)	5/00	J_G	23–35	29	13.0	13.1	27.6	−32.7
Plantago asiatica	PA	C_3 (a)	5/00	J_G	25–35	31	11.3	11.3	30.1	−36.2
Artemisia princeps	ArP	C_3 (a)	10/00	J_G	25–35	31	32.4	32.5	30.4	−32.2
Miscanthus sinensis	MS2	C_4 (a)	10/00	J_G	21–39	29	3.6	3.4	29.8	−11.2
Acer palmatum	AcP	C_3 (a)	10/00	J_G	23–35	31	6.3	6.4	30.0	−37.5
Quercus mongolica	QM	C_3 (a)	10/00	J_G	23–33	29	5.5	5.4	27.8	−29.7
Quercus dentata	QP	C_3 (a)	10/00	J_G	23–33	29	4.6	4.8	28.0	−30.9
Manihot utilissima	MU	C_3 (a)	10-12/97	T	26–35	31	5.1	5.1	31.1	−28.5
Saccharum officinarum	SO2	C_4 (a)	10-12/97	T	25–37	33	3.8	3.4	32.0	−12.0
Sorghum bicolor	SB	C_4 (a)	10-12/97	T	25–39	31	8.1	6.3	31.7	−12.1
Ananas comsus	AnC	CAM (a)	10-12/97	T	23–37	31	2.7	2.6	30.2	−13.6
Pterophyta		fern	10/00	J_G	23–35	31	5.7	5.8	30.2	−32.0
Freshwater plant species										
Vallisneria asiatica	VA	C_3	10/00	J_G	17–35	23	4.9	4.0	26.2	−14.6
Potamogeton perfoliatus	PP	C_3	10/00	J_G	17–35	25	1.8	1.4	24.6	−15.8
Hydrilla verticillata	HV	C_3	10/00	J_G	17–33	23	9.6	6.7	23.0	−16.5
Seaweed species										
Gelidium japonicum	GJ	–[i]	2/01	J_T	15–17	17	–	–	16.9	−15.1
Binghamia californica	BC	–	2/01	J_T	13–17	15	246.7	–	15.0	−11.8
Undaria pinnatifida	UP	–	2/01	J_T	15–17	15	–	–	10.8	−13.1

[a] L = Location/collection area; J_T = Tokyo-Japan, J_{Ok} = Okinawa-Japan, J_{Og} = Ogasawara-Japan, J_G = Gunma-Japan, T = Thailand.
[b] CPI_{total}, Carbon Preference Index, $= \Sigma_{odd}\, Cn/\Sigma_{even}\, Cn$.
[c] $CPI_{25–33}$, CPI of *n*-alkane in the range C_{25}–C_{33}, $= 2\Sigma_{odd}\, C_{25}–C_{33}/(\Sigma_{even}\, C_{24}–C_{32} + \Sigma_{even}\, C_{26}–C_{34})$.
[d] ACL, average chain length, $= (\Sigma Cn \cdot n)/\Sigma Cn$. Cn is the concentration of *n*-alkane containing *n* carbon atoms.
[e] $\delta^{13}C$, ‰ relative to PDB.
[f] C_4 (a), C_4 plant-angiosperm.
[g] C_3 (a), C_3 plant-angiosperm.
[h] C_3 (g), C_3 plant-gymnosperm.
[i] Not determined.

Individual hydrogen and carbon isotopic compositions of *n*-alkanes are shown in Tables 3 and 4. All *n*-alkanes are much depleted in both D and ^{13}C relative to corresponding environmental water and bulk plant tissues, respectively. Isotopic compositions of *n*-alkanes are widely variable in each plant. In some cases (e.g. *Phrogmites communis*), these isotopic differences are up to 93‰ for δD and 16.0‰ for $\delta^{13}C$. Several plants have a zigzag pattern in δD and $\delta^{13}C$ value dependent on carbon number. In these plants, odd carbon-numbered *n*-alkanes are enriched in ^{13}C relative to even carbon-numbered *n*-alkanes. These zigzag patterns for $\delta^{13}C$ of

Table 2
Hydrogen isotopic compositions of environmental water

Sample	Collect		δD (‰)	S.D.
	d/m/y	area		
Rain	21/10/98	Tokyo	−47.4	0.8
Rain	30/11/98	Tokyo	−68.1	0.4
Rain	07/12/98	Tokyo	−67.9	0.7
Rain	25/01/99	Tokyo	−65.0	0.6
Rain	11/02/99	Tokyo	−72.5	1.0
Rain	09/03/99	Tokyo	−50.8	–
Rain	19/03/99	Tokyo	−46.1	0.1
Rain	06/04/99	Tokyo	−34.5	–
Rain	04/05/99	Tokyo	−23.6	0.1
Rain	18/06/99	Tokyo	−74.0	0.1
Rain	30/06/99	Tokyo	−17.9	0.7
Rain	07/09/99	Tokyo	−17.5	0.1
Rain	06/08/99	Tokyo	−9.7	0.8
Rain	13/08/99	Tokyo	−15.1	0.4
Rain	07/09/99	Tokyo	−33.7	2.3
Rain	13/09/99	Tokyo	−28.9	0.6
		Average	−42.0	22.4
Sea water	18/02/99	Tokyo	−0.4	0.4
Lake water	27/05/99	Gunma	−59.0	–
	21/10/99	Gunma	−61.0	1.4
		Average	−60.0	1.4
Rain[a]	1995–97	Thailand	−9.2 to−76.4	–
		Average	−40.1	−21.9

[a] Source: the Global Network of Isotopes in Precipitation (GNIP) Database. Release 3, October 1999, from International Atomic Energy Agency/World Meteorological Organization (IAEA/WMO).

n-alkanes from terrestrial plants have been reported previously (Collister et al., 1994). In the case of δD, however, the enrichment or depletion in D is independent of carbon-number of *n*-alkanes. In terrestrial plants, the substantial difference is not found in both δD and $\delta^{13}C$ values at any sampling area.

2.5. Isotopic fractionations during n-alkane biosynthesis

In general, δD values of biomolecules in plants are expected to be dependent on δD values of environmental water (e.g. Sternberg, 1988; Sauer et al., 2001). For example, Sauer et al. (2001) reported a positive correlation of hydrogen isotopic compositions between sedimentary sterols and environmental water. Therefore, when comparing various plants, it is important to report hydrogen isotopic fractionation between *n*-alkane and environmental water (ε_{water}), which is calculated using Eq. (1) as shown below.

$$\varepsilon_{water} = 1000[(\delta D_{n\text{-alkane}} + 1000)/(\delta D_{water} + 1000) - 1] \quad (1)$$

where δD values of environmental water are used from Table 2. During lipid biosynthesis carbon isotopic fractionation (ε_{bulk}) also occurs, which is calculated between $\delta^{13}C$ of *n*-alkanes and bulk tissue using Eq. (2).

$$\varepsilon_{bulk} = 1000\left[\left(\delta^{13}C_{n\text{-alkane}} + 1000\right)/\left(\delta^{13}C_{bulk} + 1000\right) - 1\right] \quad (2)$$

The average ε_{water} and ε_{bulk} values for various plant classes are shown in Fig. 1. The ε_{water} and ε_{bulk} values of C3-angiosperm are −117±27‰ and −3.1±2.0‰, respectively. While the ε_{water} value of C3 gymnosperms (−116±13‰) shows no difference compared to that of C3 angiosperms, the ε_{bulk} value of gymnosperms (−4.7±2.2‰) shows larger fractionation than that of angiosperms. The ε_{water} and ε_{bulk} values of C4 plants are −132±12‰ and −8.9±1.7‰, respectively. C4 plants have larger fractionations in both D and ^{13}C than do C3 plants. A larger carbon isotopic fractionation in C4 plants was previously reported between total wax and bulk plant tissue (Ballentine et al., 1998; Collister et al., 1994).

In previous studies (e.g. Sternberg et al., 1984a, 1984b), using non-exchangeable hydrogen of cellulose, CAM plants had a significant D-enrichment of cellulose relative to C3 and C4 plants. However, δD values of the saponifiable lipid fraction are not substantially different among C3, C4 and CAM plants within a range of −170 to −120‰ (Sternberg et al., 1984c). This could be explained by isotopically different hydrogen pools for cellulose biosynthesis and for lipid biosynthesis (Sternberg et al., 1984c). In the case of *n*-alkanes in this study, the ε_{water} value of CAM plants (−147±10‰) is likely to be a little smaller than that of C3 plants (−116±25‰) and C4 plants (−133±12‰) as shown in Fig. 2. The ε_{bulk} value of CAM plants is −7.7±1.4‰, an intermediate isotopic fractionation between C3 and C4 plants. The ε_{water} and ε_{bulk} values of ferns are −131±6‰ and −4.6±1.1‰, respectively, which are close to C4 plants for hydrogen and close to gymnosperms for carbon.

The ε_{water} values of freshwater plants and seaweed are −135±17‰ and −155±34‰, respectively. *n*-Alkanes from seaweed are the most D-depleted relative to environmental water. A similar hydrogen isotopic fractionation of *n*-alkanes has been reported in aquatic plants (Sessions et al., 1999). The ε_{bulk} values of freshwater plant and seaweed *n*-alkanes are −9.8±2.0‰ and −9.6±2.7‰, respectively, the largest ε_{bulk} values in this study. These observations indicate that hydrogen and carbon isotopic fractionations during the *n*-alkane biosynthesis are distinctive for each plant class as shown in Fig. 2, where C3 plants are the least depleted in both D and ^{13}C and seaweed is the most depleted. Other plants fall between those two plant classes. This isotopic discrepancy has not been observed in measurements of bulk lipid fractions. This is probably due to the fact that total lipid fractions are mixtures of many lipid molecules such as acetogenic (*n*-alkyl) lipids and polyisoprenoid lipids.

Table 3
Individual hydrogen isotopic compositions of *n*-alkanes (‰, relative to SMOW)

Carbon number	ZM 7/98	S.D.	QA 7/98	S.D.	ZJ 9/98	S.D.	CA 10/98	S.D.	CO 10/98	S.D.	PT 11/98	S.D.	CE 11/98	S.D.	LR 12/98	S.D.	MS1 9/99	S.D.	SO1 3/00	S.D.	AJ 9/00	S.D.
23											−155	3										
24											−136	0										
25	−144	13					−167	5			−166	0					−157	4	−182	4	−133	3
26	−142	3					−168	4			−145	3					−171	2	−187	3	−126	3
27	−160	1	−81	7			−166	4	−180	3	−167	3	−170	4			−181	4	−186	3	−123	1
28	−156	0	−119	8	−176	4	−162	1			−140	2	−166	5			−173	3	−188	3	−117	5
29	−153	8	−145	2	−177	2	−167	3	−150	5	−164	2	−196	3	−188	7	−173	6	−188	4	−124	4
30	−157	1	−142	4	−178	5	−157	2					−180	4	−189	4	−164	5	−188	2	−117	1
31	−156	1	−142	6	−180	2	−159	5	−134	1	−151	4	−179	2	−186	6	−168	8	−185	2	−128	5
32	−149	5			−169	2			−121	5							−150	4	−182	2	−116	3
33	−158	5	−133	14	−180	5	−146	16	−123	2	−146	1	−165	2			−154	6	−169	4	−126	3
34					−160	3			−118	3												
35	−158	1			−177	4			−125	1							−156	3				
36																						
37	−158	1			−147	1																
WA[a]	−156		−136		−178		−165		−128		−157		−187		−186		−165		−183		−123	

Carbon number	BJ1 5/99	S.D.	CJ1 5/99	S.D.	AC1 5/99	S.D.	AA1 5/99	S.D.	PC 10/99	S.D.	BJ2 10/99	S.D.	PJ 10/99	S.D.	CJ2 10/99	S.D.	AC2 10/99	S.D.	AA2 10/99	S.D.	TO 5/00	S.D.
23																					−160	5
24																						
25	−140	0	−169	5			−185	1							−175	4					−162	3
26			−159	5			−118	7	−113	7					−154	2						
27	−157	6	−165	0	−120	4	−119	7	−142	4	−149	7	−159	2	−178	3	−105	2	−93	5	−151	4
28	−164	10	−146	6		4	−119	10	−191	5	−141	0	−153	3	−167	1			−128	7		
29	−158	2	−156	6	−134	8	−119	7	−206	3	−170	5	−183	1	−168	1	−161	5	−123	5	−155	4
30	−176	12			−126	7	−122	9	−191	0	−156	2	−169	6			−126	2	−143	9		
31	−168	11	−141	5	−111	5	−111	6	−196	4	−176	0	−183	5	−153	1	−127	5	−116	2	−155	4
32			−130	3	−121	3	−115	3					−177	15	−150	4	−133	4	−147	1		
33			−140	5	−107	7	−113	10	−178	4			−183	3	−146	1	−128	6	−133	3		
34			−134	4											−143	5						
35			−141	2											−144	10						
WA	−156		−142		−115		−128		−187		−165		−182		−149		−134		−117		−157	

Carbon number	PA 5/00	S.D.	ArP 10/00	S.D.	MS2 10/00	S.D.	AcP 10/00	S.D.	QM 10/00	S.D.	QD 10/00	S.D.	MU 10–12/97	S.D.	SO2 10–12/97	S.D.	SB 10–12/97	S.D.	AnC 10–12/97	S.D.	Fern 10/00	S.D.
23					−165	3			−171	1												
24					−177	3			−170	8	−163	7										
25	−177	1			−171	3	−158	9	−173	4	−168	1										
26					−189	7			−180	7	−182	8										
27	−177	2	−172	4	−191	1	−174	4	−186	1	−194	2	−129	4			−162	2	−184	5	−166	2
28	−169	0			−183	4	−165	5	−180	3	−179	8	−116	10			−157	6	−173	2	−158	1
29	−178	2	−157	4	−186	1	−171	2	−188	6	−193	4	−134	1	−173	1	−171	10	−190	1	−170	5
30	−168	2	−156	4	−172	5	−171	7	−169	1	−180	4	−123	6	−177	6	−172	4	−187	3	−169	3
31	−176	0	−163	2	−179	1	−179	1	−172	4	−184	6	−133	3	−176	0	−176	4	−194	0	−175	2
32	−164	0			−166	0	−171	5					−121	6	−176	5	−173	2	−178	3	−164	7
33	−169	1	−161	4	−173	1	−177	5					−138	3	−179	1	−172	5	−186	4	−174	3
34															−175	5	−171	2				
35															−177	4	−171	4	−180	1		
WA	−175		−161		−182		−175		−182		−188		−133		−177		−172		−193		−171	

Carbon number	VA 10/00	S.D.	PP 10/00	S.D.	HV 10/00	S.D.	GJ 2/01	S.D.	BC 2/01	S.D.	UP 2/01	S.D.
15									−160	7	−186	2
17	−157	7	−196	0	−198	9	−118	5				
19					−195	3						
21	−198	4	−191	18	−199	7						
22	−171	3	−193	7	−196	5						
23	−184	4	−207	3	−211	5						
24	−173	1	−203	6	−192	6						
25	−175	5	−214	6	−197	2						

(*continued on next page*)

Table 3 (*continued*)

Carbon number	VA 10/00	S.D.	PP 10/00	S.D.	HV 10/00	S.D.	GJ 2/01	S.D.	BC 2/01	S.D.	UP 2/01	S.D.
26	−167	9	−211	2	−192	2						
27	−175	10	−209	2	−192	4						
28	−177	6	−203	4	−185	3						
29	−167	10	−202	4	−190	6						
30	−173	4	−190	9								
31	−156	4	−183	7	−171	8						
32	−158	5										
33	−168	11	−180	2								
WA	−173		−210		−201		−118		−160		−186	

ZM = *Zea mays*; QA = *Quercus acutissima*; ZJ = *Zoysia japonica*; CA = *Camellia sasanqua*; CO = *Chamaecyparis obtuse*; PT = *Pinus thunbergii*; CE = *Colocasia esculenta*; LR = *Lycoris radiata*; MS1 = *Miscanthus sinensis*; SO1 = *Saccharum officinarum*; AJ = *Albizia julibrissin*; BJ1 = *Benthamidia japonica*; CJ1 = *Cryptomeria japonica*; AC1 = *Acer carpinifolium*; AA1 = *Acer argutum*; PC = *Phrogmites communis*; BJ2 = *Benthamidia japonica*; PJ = *Prunus jamasakura*; CJ2 = *Cryptomeria japonica*; AC2 = *Acer carpinifolium*; AA2 = *Acer argutum*; TO = *Taraxacum officinale*; PA = *Plantago asiatica*; ArP = *Artemisia princeps*; MS2 = *Miscanthus sinensis*; AcP = *Acer palmatum*; QM = *Quercus mongolica*; QD = *Quercus dentate*; MU = *Manihot utilissima*; SO2 = *Saccharum officinarum*; SB = *Sorghum bicolour*; AnC = *Ananas comsus*; Fern = *Pterophyta*; VA = *Vallisneria asiatica*; PP = *Potamogeton perfoliatus*; HV = *Hydrilla verticillata*; GJ = *Gelidium japonicum*; BC = *Binghamia californica*; UP = *Undaria pinnatifida*. C*n* and *δn* are the concentration and isotopic composition of *n*-alkane containing *n* carbon atoms, respectively.

[a] WA, weighted mean average, $= \Sigma Cn \cdot \delta n / \Sigma Cn$.

Table 4
Individual carbon isotopic compositions of *n*-alkanes (‰, relative to PDB)

Carbon number	ZM 7/98	S.D.	QA 7/98	S.D.	ZJ 9/98	S.D.	CA 10/98	S.D.	CO 10/98	S.D.	PT 11/98	S.D.	CE 11/98	S.D.	LR 12/98	S.D.	MS1 9/99	S.D.	SO1 3/00	S.D.	AJ 9/00	S.D.
23											−30.9	0.2					−20.5	0.6	−21.3	0.3	−34.0	0.3
24											−30.8	0.0					−18.2	0.0	−20.6	0.1	−36.8	0.9
25	−23.8	0.1	−34.6	0.7			−32.7	0.4			−32.0	0.2					−17.6	0.1	−20.4	0.2	−34.6	0.1
26	−22.4						−31.7	0.5			−31.7	0.6					−17.4	0.5	−20.4	0.5	−36.3	0.0
27	−21.0	0.0	−35.5	0.5			−32.5	0.1	−31.1	0.3	−32.7	0.2	−34.1	0.3			−16.7	0.1	−20.2	0.1	−35.8	0.1
28	−21.8	0.3	−37.5	0.3	−24.5		−32.4	0.2					−33.1	0.1			−18.2	0.8	−20.5	0.1	−35.8	0.0
29	−20.9	0.1	−34.7	0.0	−24.1	0.6	−31.3	0.2	−30.6	0.8	−33.5	0.1	−33.2	0.0	−28.0	0.1	−17.2	0.1	−20.6	0.1	−35.9	0.0
30	−23.7	0.3	−37.4	0.1	−23.9	0.4	−33.2	0.3					−36.7	0.2	−28.4	0.2	−18.7	0.6	−20.7	0.1	−37.6	0.1
31	−21.7	0.1	−34.8	0.8	−24.2	0.2	−33.1	0.2	−30.0	0.3	−34.1	0.1	−34.0	0.2	−27.8	0.2	−17.3	0.1	−20.7	0.2	−37.8	0.1
32	−21.3	0.4		0.3	−24.0	0.4					−31.8	0.4					−20.4	1.1	−21.9	0.4	−38.1	0.1
33	−21.1	0.2	−34.9	0.2	−22.3	0.1			−29.2	0.6	−34.6	0.3	−37.2	0.3			−18.0	0.2	−22.3	0.0	−36.1	0.1
34					−22.8	0.3			−29.5	0.3							−20.7	1.2				
35	−19.8	0.4			−22.6	0.2			−27.6	0.2							−19.0	0.2	−23.1	0.2		
36																						
37					−23.0	0.5											−18.6	0.2				
WA[a]	−20.1		−35.0		−23.0		−32.0		−29.0		−32.7		−33.7		−27.9		−17.8		−21.0		−36.4	

	BJ1 5/99	S.D.	CJ1 5/99	S.D.	AC1 5/99	S.D.	AA1 5/99	S.D.	PC 10/99	S.D.	BJ2 10/99	S.D.	PJ 10/99	S.D.	CJ2 10/99	S.D.	AC2 10/99	S.D.	AA2 10/99	S.D.	TO 5/00	S.D.
23	−33.0	0.0							−33.8	0.9	−35.1	0.0	−34.2	0.0							−35.6	0.3
24									−37.4	1.0	−41.5	0.0									−35.7	0.3
25	−38.1	0.1			−35.5	0.2	−36.2	0.1	−35.3	0.4	−38.1	0.5	−35.2	0.0	−34.4	0.2			−34.6	0.2	−35.8	0.0
26	−39.4	0.0					−35.9	0.7	−34.8	0.3	−37.4	0.7	−35.4	0.0					−35.1	0.2	−34.7	0.3
27	−37.2	0.2	−32.2	0.2	−35.5	0.1	−35.7	0.0	−34.9	0.1	−38.8	0.1	−33.9	0.5	−32.7	0.3	−35.5	0.3	−34.9	0.1	−36.0	0.1
28	−38.0	0.6	−34.0	0.0			−35.8	0.7	−34.0	0.2	−38.8	0.3	−35.4	0.7					−36.5	0.2	−36.3	0.3
29	−36.5	0.2	−32.3	0.2	−35.5	0.1	−35.9	0.2	−34.6	0.1	−38.8	0.0	−34.2	0.1	−32.9	0.3	−37.3	0.1	−35.6	0.0	−37.0	0.0
30	−40.5	0.0	−31.8	0.0	−38.3	0.6	−37.9	1.7	−35.6	0.3	−38.8	0.7	−35.4	0.4			−38.6	0.4	−37.5	0.3	−36.5	0.1
31	−36.5	0.1	−30.4	0.3	−35.4	0.5	−36.4	0.2	−38.1	0.3	−37.3	0.2	−33.5	0.1	−32.5	0.5	−37.1	0.0	−36.0	0.0	−36.4	0.1
32			−31.7	0.2	−38.6	0.0	−39.7	0.9	−36.5	0.4			−35.3		−31.6		−40.1	0.3	−38.6	1.2	−33.4	0.0
33			−29.5	0.4	−35.2	0.3	−34.7	0.5	−39.0	0.5			−33.2	0.3	−31.5	0.1	−37.0	0.0	−34.0	0.0	−36.0	0.1
34			−31.4	0.4			−30.8	0.9							−32.2	0.3			−29.3			
35			−29.8	0.4											−30.3	0.4						
WA	−36.7		−30.0		−35.5		−36.1		−34.9		−38.2		−34.0		−31.5		−37.2		−35.6		−36.2	

(*continued on next page*)

Table 4 (*continued*)

Carbon number	PA 5/00	S.D.	ArP 10/00	S.D.	MS2 10/00	S.D.	AcP 10/00	S.D.	QM 10/00	S.D.	QD 10/00	S.D.	MU 10–12/97	S.D.	SO2 10–12/97	S.D.	SB 10–12/97	S.D.	AnC 10–12/97	S.D.	Fern 10/00	S.D.
23					−20.8	0.6			−33.6	0.7	−30.5	0.5										
24					−18.3	0.8			−36.2	0.3	−36.2	0.4										
25	−38.6	0.1	−36.7	0.5	−17.6	0.4	−36.9	0.2	−33.6	0.7	−34.9	0.2					−27.4					
26	−38.8	0.1			−17.3	0.3	−39.2	1.3	−35.3	0.9	−35.3	0.4	−25.9	2.2	−24.0		−25.0	0.8	−22.5	0.2		
27	−39.0	0.1	−35.9	0.2	−17.1	0.1	−38.5	0.2	−33.2	0.8	−34.1	0.1	−25.0	0.7	−23.0	0.3	−22.7	0.4	−22.9	0.1	−34.9	0.3
28	−39.3	0.2	−36.6	0.1	−18.5	0.5	−41.0	0.3	−34.0	0.4	−35.6	0.2	−28.9	0.4	−21.6	0.9	−22.6	0.0	−23.0	0.3	−35.2	0.8
29	−39.6	0.2	−36.5	0.1	−17.5	0.3	−40.5	0.1	−33.4	0.6	−33.5	0.1	−30.8	0.1	−20.0	0.6	−18.9	0.0	−21.7	0.1	−36.0	0.1
30	−40.0	0.1	−36.8	0.3	−19.9	0.2	−42.1	0.9	−37.7	0.2	−34.7	0.2	−31.8	0.0	−19.1	0.4	−20.6	0.2	−20.6	0.2	−37.1	0.3
31	−39.8	0.3	−35.2	0.0	−18.6	0.1	−41.8	0.2	−32.2	0.5	−34.4	0.4	−32.0	0.0	−19.3	0.7	−19.6	0.3	−20.5	0.1	−36.9	0.2
32	−40.1	0.2	−37.6	0.1	−21.5	0.5	−42.2	0.6	−33.4	0.5	−36.5	0.7	−33.1	0.2	−20.9	0.4	−22.7	0.5	−21.5	0.4	−37.7	0.5
33	−40.3	0.4	−35.4	0.0	−18.9	0.4	−41.7	0.5	−32.9	0.1			−30.7	0.1	−20.1	0.4	−19.8	0.1	−20.5	0.1	−37.4	0.3
34					−22.0	1.0									−21.6	0.3						
35	−38.8	0.1			−18.1	0.2									−21.1	0.3	−19.8	0.3	−22.0	0.4		
36																						
37					−18.7	0.4											−20.7	0.1				
WA*[1]	−39.7		−35.7		−18.2		−41.1		−33.5		−34.2		−31.0		−20.2		−20.0		−21.3		−36.5	

Carbon number	VA 10/00	S.D.	PP 10/00	S.D.	HV 10/00	S.D.	GJ 2/01	S.D.	BC 2/01	S.D.	UP 2/01	S.D.
15									−23.8	0.1	−22.6	0.2
17	−24.2	0.8	−23.3	0.6	−22.7	1.2	−21.8	0.1				
19					−22.7	1.1						
20					−20.3	0.9						
21	−23.6	0.2	−24.4	1.4	−24.0	0.8						
22	−24.5	0.4	−24.8	0.4	−23.2	1.1						
23	−23.4	0.1	−26.9	0.3	−24.4	0.2						
24	−24.2	0.3	−26.7	0.6	−26.3	0.4						
25	−23.2	0.1	−27.5	0.0	−25.5	0.1						
26	−24.2	0.3	−29.0	0.6	−26.6	0.6						
27	−23.5	0.1	−28.8	0.3	−25.4	0.6						
28	−25.0	0.3	−28.8	0.4	−25.8	0.8						
29	−23.9	0.1	−26.6	0.3	−26.2	0.9						
30	−24.9	0.4	−24.6	0.3	−28.6	1.1						
31	−23.4	0.1	−27.4	1.0	−30.0	0.7						
32	−26.2	0.3										
33	−24.6	0.6	−24.9	1.4	−28.2	0.6						
WA	−23.7		−26.9		−24.8		−21.8		−23.8		−22.8	

ZM = *Zea mays*; QA = *Quercus acutissima*; ZJ = *Zoysia japonica*; CA = *Camellia sasanqua*; CO = *Chamaecyparis obtuse*; PT = *Pinus thunbergii*; CE = *Colocasia esculenta*; LR = *Lycoris radiata*; MS1 = *Miscanthus sinensis*; SO1 = *Saccharum officinarum*; AJ = *Albizia julibrissin*; BJ1 = *Benthamidia japonica*; CJ1 = *Cryptomeria japonica*; AC1 = *Acer carpinifolium*; AA1 = *Acer argutum*; PC = *Phrogmites communis*; BJ2 = *Benthamidia japonica*; PJ = *Prunus jamasakura*; CJ2 = *Cryptomeria japonica*; AC2 = *Acer carpinifolium*; AA2 = *Acer argutum*; TO = *Taraxacum officinale*; PA = *Plantago asiatica*; ArP = *Artemisia princeps*; MS2 = *Miscanthus sinensis*; AcP = *Acer palmatum*; QM = *Quercus mongolica*; QD = *Quercus dentate*; MU = *Manihot utilissima*; SO2 = *Saccharum officinarum*; SB = *Sorghum bicolour*; AnC = *Ananas comsus*; Fern = *Pterophyta*; VA = *Vallisneria asiatica*; PP = *Potamogeton perfoliatus*; HV = *Hydrilla verticillata*; GJ = *Gelidium japonicum*; BC = *Binghamia californica*; UP = *Undaria pinnatifida*. C*n* and *δn* are the concentration and isotopic composition of *n*-alkane containing *n* carbon atoms, respectively.

[a] WA, weighted mean average, $= \Sigma Cn \cdot \delta n / \Sigma Cn$.

In higher plants, while all acetogenic lipids including *n*-alkanes, *n*-alkanols and *n*-fatty acids are formed via the fatty acid biosynthetic pathway, polyisoprenoid lipids are formed by two distinctive biosynthetic pathways: mevalonic acid pathway for sterols (Killops and Killops, 1993) and non-mevalonic acid pathway (1-deoxy-D-xylulose-5-phosphate pathway) for phytol (Kleining, 1989; Lichtenthaler, 1999). Hence acetogenic lipids are expected to show different isotopic signature compared with polyisoprenoid lipids (Hayes, 1993). Sessions et al. (1999) reported that isoprenoids such as phytol from terrestrial plants are depleted in D by ~200‰ more than acetogenic lipids.

For the biosynthesis of *n*-alkanes, hydrogen and carbon isotopic fractionations occur during enzymatic reactions such as hydrogenation with NADPH (Deines, 1980; Sessions et al., 1999) and decarboxylation of pyruvate to form acetate (DeNiro and Epstein, 1977; Monson and Hayes, 1982). Generally, lipid compounds are biosynthesized from ^{13}C-depleted acetate precursors, and additional fractionations occur at biosynthetic branch points (Hayes, 1993). However, the extent

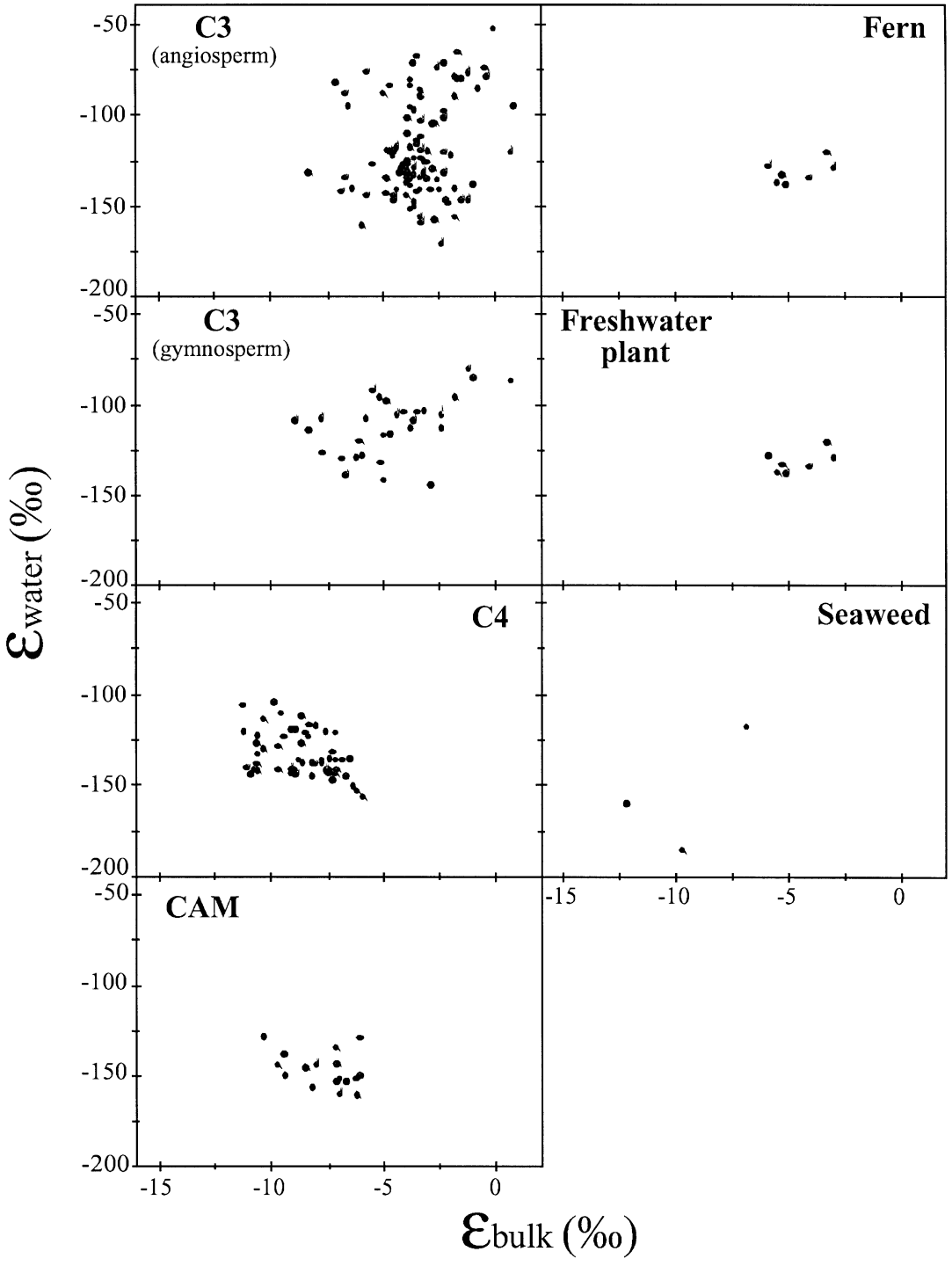

Fig. 1. ε_{water}–ε_{bulk} diagrams for various plant classes. Filled circle symbols indicate hydrogen and carbon isotopic compositions of all measured *n*-alkanes. The diagrams for terrestrial plants are composed of eighteen C3-angiosperms, four C3-gymnosperms, seven C4 plants, three CAM plants and one fern. The diagrams of aquatic plants are also composed of three freshwater plants and three seaweeds.

of hydrogen and carbon isotopic fractionation has not been clarified for each lipid molecule yet. Besides *n*-alkane biosynthesis, δD values of *n*-alkanes will be controlled by the isotopic composition of leaf water at the time they are formed. The δD of leaf water may vary relative to that of environmental water for a variety of reasons such as evaporation, use of groundwater and seasonality of precipitation. The smaller ε_{water} in C3 plants of this study could be related to increased evapotranspiration in those plants, while aquatic plants have larger ε_{water} possibly due to their least evaporation effect in leaf water.

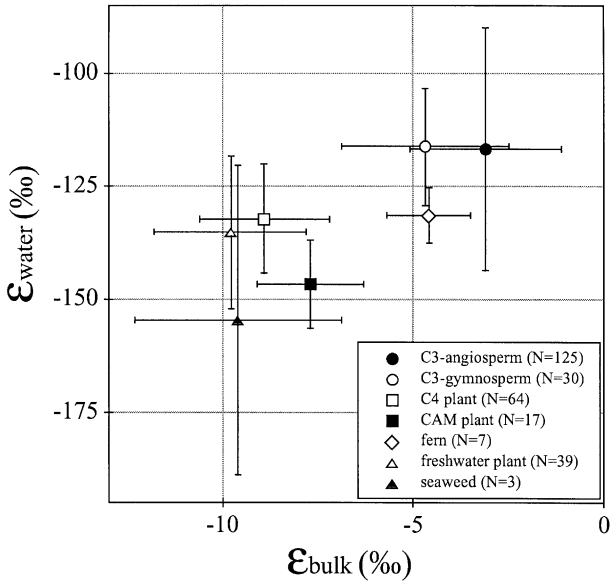

Fig. 2. Hydrogen and carbon isotopic fractionations during *n*-alkane biosynthesis are distinguishable for various plant classes. Each symbol indicates a mean value for each plant with bars of standard deviation (1σ) of the mean values.

Even though ε_{water} and ε_{bulk} values apparently represent unrelated phenomena (i.e. ε_{water} combines the effect of water-use efficiency, evapotranspiration and *n*-alkane biosynthesis, while ε_{bulk} represents only *n*-alkane biosynthesis), ε_{water} and ε_{bulk} distributions of *n*-alkanes in this study suggest that each plant class has distinguishable isotope effects for hydrogen and carbon during *n*-alkane synthesis. For applications, the ε_{bulk}–ε_{water} plot should be useful to distinguish the sources of sedimentary *n*-alkanes especially if postulating δD of environmental water, as well as to reconstruct δD values of environmental water in an aquatic environment.

3. Concluding remarks

Hydrogen and carbon isotopic compositions of individual *n*-alkanes were determined for various terrestrial and aquatic plants. Their characteristics are summarized as follows:

1. C3 plants: *n*-alkanes from angiosperms have a ε_{water} value of -117 ± 27‰ and ε_{bulk} value of -3.1 ± 2.0‰. While the ε_{water} value of C3 gymnosperms (-116 ± 13‰) shows no difference compared to that of C3 angiosperms, the ε_{bulk} value of gymnosperms (-4.7 ± 2.2‰) shows larger fractionation than that of angiosperm (-3.1 ± 2.0‰).
2. C4 plants: *n*-alkanes have a ε_{water} value of -132 ± 12‰ and ε_{bulk} value of -8.9 ± 1.7‰, larger fractionations in both D and ^{13}C than found in C3 plants.
3. CAM plants: *n*-alkanes are a little depleted in D (ε_{water} value of -147 ± 10‰) than C3 and C4 plants and are intermediate in ^{13}C (-7.7 ± 1.4‰) between C3 and C4 plants.
4. Fern: *n*-alkanes have ε_{water} values of -131 ± 6‰ and ε_{bulk} values of -4.6 ± 1.1‰, which are close to C4 plants for hydrogen and close to gymnosperms for carbon.
5. Aquatic plants: *n*-alkanes from freshwater plants have a ε_{water} value of -135 ± 17‰ and ε_{bulk} value of -9.8 ± 2.0‰, and from seaweeds have a ε_{water} value of -155 ± 34‰ and ε_{bulk} value of -9.6 ± 2.7‰.

Hydrogen and carbon isotopic fractionations during *n*-alkane synthesis are distinguishable for each plant class (Fig. 2). For example, in both D and ^{13}C, C3 plant is the least depleted and seaweed is the most depleted relative to environmental water and bulk tissue respectively during *n*-alkane synthesis. These differences may arise from different isotope effects associated with evapotranspiration for hydrogen and associated with *n*-alkane biosynthesis for both hydrogen and carbon. A two-dimensional cross plot using ε_{water} and ε_{bulk} values provides basic information on not only the biosynthetic pathway of *n*-alkanes in more detail compared to only carbon isotopes, but also inferring the sources of sedimentary *n*-alkanes and reconstruction of the isotopic composition of environmental water.

4. Experimental

4.1. Bulk isotopic analysis

The surface of leaves, freshwater plants and seaweeds were cleaned with distilled water to remove contaminants. The collected samples were stored at -20 °C until analysis. All samples were freeze-dried and crushed to a fine powder. Powdered plant tissue (bulk) was combusted in an evacuated and sealed quartz tube at ca. 800 °C for 3 h in the presence of CuO. Evolved CO_2 was separated cryogenically, and analyzed for isotopic compositions using a dual inlet mass spectrometer (Finnigan delta S). δ^{13}C values are given in permil (‰) relative to PDB. Standard deviations of carbon isotope measurements were generally better than 0.2‰. Isotopic precision was tested using NIST Reference Material 8540 (Polyethylene Foil 1, PEF1), and gave accurate values within the analytical standard deviations.

4.2. Molecular analysis

The powdered plant tissue was extracted by sonication with *n*-hexane (15 min×4). The resulting lipid extract was further separated by silica gel column chromatography

using *n*-hexane to obtain a hydrocarbon fraction, which was composed of saturated and unsaturated straight-chain hydrocarbons based on gas chromatography/mass spectrometry analysis. The terpenoids and anteiso-alkanes were also identified in several hydrocarbon fractions of terrestrial plants. The hydrocarbon fraction was separated into saturated and unsaturated hydrocarbon fractions by silver nitrate (10%, w/w) impregnated silica gel column chromatography. The saturated fraction was eluted by *n*-hexane, and the unsaturated fraction was subsequently eluted by *n*-hexane/diethylether (2/1 by volume). *n*-Alkanes were further isolated from the saturated hydrocarbon fraction by molecular sieve treatment (Yamada et al., 1994).

4.3. Compound-specific δD and $\delta^{13}C$ analyses

Compound-specific hydrogen isotopic analyses were carried out by gas chromatography/pyrolysis/isotope ratio mass spectrometry (GC/pyrolysis/IRMS) using a Finnigan (Delta plus XL) mass spectrometer combined with Hewlett Packard 6890GC. Carbon isotopic analyses were carried out by GC/combustion/IRMS using a Finnigan delta S combined with HP5890GC. Pyrolysis was performed in a microvolume ceramic tube with graphite at 1440 °C (Hilkert et al., 1999). Combustion was performed in a microvolume ceramic tube with CuO and Pt wires at 840 °C (Hayes et al., 1989). Isotopic compositions were calibrated by coinjected fatty acid methyl esters for δD measurement and deuterated *n*-alkanes for $\delta^{13}C$ measurement. δD values of the fatty acid methyl esters were determined by reference to *n*-alkane standards on GC/pyroysis/IRMS analysis. Standard deviations of hydrogen and carbon isotope measurements were generally better than 7‰ (~3‰ in average) and 0.5‰ (~0.3‰ in average), respectively.

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