Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Phytochemistry 65 (2004) 1369–1381
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Hydrogen and carbon isotopic fractionations of lipid biosynthesis among terrestrial (C3, C4 and CAM) and aquatic plants

Yoshito Chikaraishi [a,*], Hiroshi Naraoka [a], Simon R. Poulson [b]

[a] Department of Chemistry, Tokyo Metropolitan University, 1-1, Minami-Ohsawa, Hachioji, Tokyo 192-0397, Japan
[b] Department of Geological Science, MS-172, University of Nevada-Reno, Reno, NV 89557-0138, USA

Received 14 November 2003; received in revised form 11 March 2004

Abstract

Compound-specific hydrogen and carbon isotopic compositions in *n*-alkanoic acids, phytol and sterols were determined for various plant classes (terrestrial C3-angiosperm; C3-gymnosperm; C4; crassulacean acid metabolism (CAM); and aquatic C3 plants) in order to investigate isotopic fractionations among various plant classes. In all plants, lipid biomolecules are depleted in both D (up to 324‰) and ^{13}C (up to 14.7‰) relative to ambient water and bulk tissue, respectively. In addition, the magnitude of D- and ^{13}C-depletion of lipid biomolecules is distinctive depending on plant classes. For example, C3 angiosperm *n*-alkanoic acids are less depleted in D (95 ± 23‰) and ^{13}C (4.3 ± 2.5‰) relative to ambient water and bulk tissue, respectively, while C4 plant *n*-alkanoic acids are more depleted in D (119 ± 15‰) and ^{13}C (10.2 ± 2.0‰). On the other hand, C3 angiosperm phytol and sterols are much more depleted in D (306 ± 12‰ for phytol, 211 ± 15‰ for sterol) with less depletion in ^{13}C (4.1 ± 1.1‰ for phytol, 1.3 ± 0.9‰ for sterol) relative to ambient water and bulk tissue, respectively, while C4 plant phytol and sterols are less depleted in D (254 ± 7‰ for phytol, 186 ± 13‰ for sterols) with much more depletion in ^{13}C (9.0 ± 1.2‰ for phytol, 5.0 ± 1.1‰ for sterols). Among various plant classes, there is a positive correlation between the D- and ^{13}C-depletion for *n*-alkanoic acids, while a negative correlation was found for phytol and sterols from the same plants.

Keywords: Higher plant; δD; δ^{13}C; Isotopic fractionation; Lipid biosynthesis; Methylerythritol-phosphate; Mevalonic-acid; *n*-Alkanoic acid; Phytol; Sterol

1. Introduction

Knowledge of hydrogen and carbon isotopic compositions of individual lipid biomolecules among various organisms, especially those of primary producers such as terrestrial higher plants and algae, is biologically and geochemically significant (e.g., Hayes, 2001) for the following two reasons: (1) biological individual lipid molecules have various isotopic compositions, in which isotopic fractionations are applied to understand isotope effects during their biochemical processes in the lipid biosynthesis and (2) distinct isotopic compositions (or fractionations) among various organisms are required in order to estimate sources of these lipid molecules in natural geochemical samples such as soils and sediments. In addition to the two reasons, an understanding of hydrogen isotopic fractionations between individual lipid molecules and ambient water would facilitate reconstruction of hydrological cycles in paleoenvironmental studies. There have been many studies on hydrogen isotopic compositions of non-exchangeable (carbon-bound) hydrogen in cellulose (**1**) in order to investigate various paleoenvironmental factors, especially for hydrological cycles (e.g., Epstein et al., 1976; Northfelt et al., 1981; Pendall et al., 1999). However, even carbon-bound hydrogen in cellulose (**1**) may undergo D-enrichment due to isotopic fractionations during biochemical reactions in cellulose (**1**) biosynthesis (Yakir and DeNiro, 1990; Luo and Sternberg, 1991),

* Corresponding author. Present address: Program for Paleoenvironment, Institute for Frontier Research on Earth Evolution (IFREE), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Natsushima-cho 2-15, Yokosuka, Kanagawa 237-0061, Japan, Tel.: +81-46-867-9778; fax: +81-46-867-9775.
E-mail address: ychikaraishi@jamstec.go.jp (Y. Chikaraishi).

0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2004.03.036

which are dependent on plant classes (Sternberg et al., 1984a,b, 1986a,b). Sternberg (1988) insisted that hydrogen isotopic composition of lipid-fraction was well correlated with that of ambient water. In addition, lipid molecules are often easier to find in the sedimentary record than cellulose (**1**). Hence, hydrogen isotopic compositions of individual lipid molecules can supply more precise information on hydrological studies (Xie et al., 2000; Huang et al., 2002).

In higher plants, hydrogen and carbon isotopic fractionations occur during photosynthesis and various lipid biosyntheses. Yakir and DeNiro (1990) reported that the isotopic fractionation between water and the first-formed triose product of photosynthesis was estimated to be −171‰ because of the large isotopic fractionations during nicotinamide adenine dinucleotide phosphate (NADPH) formation. Luo et al. (1991) also reported that hydrogen available for hydrogen transfer to yield NADPH was strongly depleted in D. However, hydrogen isotopic fractionation during photosynthesis has not been studied extensively. In contrast, carbon isotopic fractionation during photosynthesis has been the subject of many studies (e.g., Lerman and Queiroz, 1974; Keeley and Sandquist, 1992; O'Leary, 1993; Lajtha and Marshall, 1994), and different mechanisms of fractionation have been identified among various plant classes such as C3, C4 and crassulacean acid metabolism (CAM) plants (e.g., Deines, 1980; Hayes, 1993). In lipid biosynthetic pathways, hydrogen and carbon isotopic fractionations also occur during various biochemical reactions such as hydrogenation with NADPH (Smith and Esptein, 1970; Luo et al., 1991) and decarboxylation of pyruvate (**2**) to form acetyl-coenzyme A (acetyl-CoA) (**3**) (DeNiro and Epstein, 1977; Monson and Hayes, 1982). In the previous study (Chikaraishi et al., 2004), hydrogen and carbon isotopic fractionations of typical lipid biomolecules are clarified with respect to acetogenic, methylerythritol phosphate (MEP) (**4**) and mevalonic-acid (MVA) (**5**) pathways within a single higher plant (*Cryptomeria japonica*). Several studies have reported that various organisms have distinct isotopic fractionations for the same biosynthesis (e.g., for hydrogen: Sessions et al., 1999; for carbon: Collister et al., 1994; Ballentine et al., 1998). Sessions et al. (1999) reported that hydrogen isotopic fractionations of lipid molecules were variable among various organisms. Collister et al. (1994) and Ballentine et al. (1998) reported that carbon isotopic fractionation during the acetogenic pathway in C4 plant was larger than that in C3 plant. Chikaraishi and Naraoka (2003) also reported that hydrogen and carbon isotopic fractionations during *n*-alkane (**6**) biosynthesis are distinctive among various plant classes. However, detailed isotopic fractionations of various lipid syntheses among different organisms have not yet been studied.

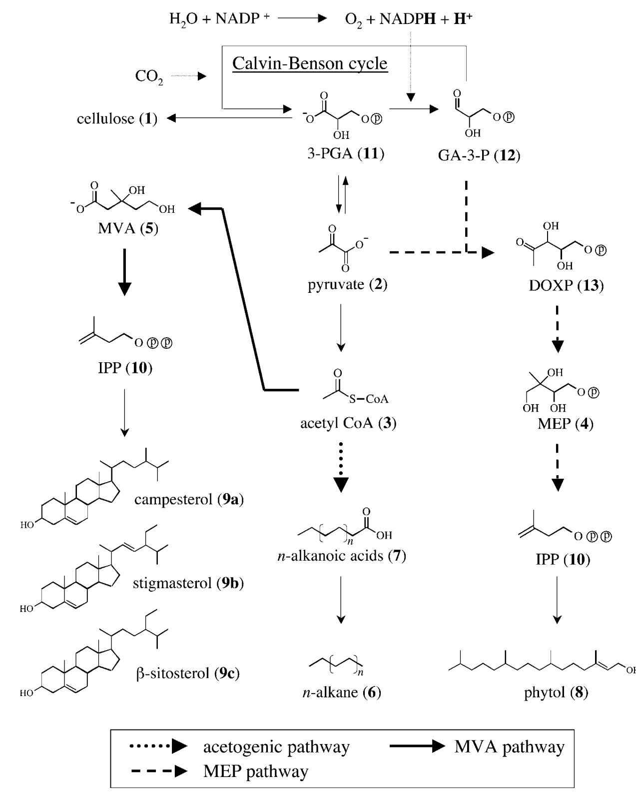

Fig. 1. The biosynthetic relationship among *n*-alkanoic acids, phytol and sterols (after Lichtenthaler, 1999; Cvejic and Rohmer, 2000; Eisenreich et al., 2001). *Abbreviations*: [NADP$^+$, nicotinamide adenine dinucleotide phosphate; acetyl-CoA (**3**), acetyl coenzyme-A; MEP (**4**), 2-C-methyl-D-erythritol-4-phosphate; MVA (**5**), mevalonic acid; IPP (**10**), isopentenyl pyrophosphate; ribulose-1,5-biphosphate; 3-PGA (**11**), 3-phosphoglyceric acid; GA-3-P (**12**), D-glyceraldehyde-3-phosphate; DOXP (**13**), 1-deoxy-D-xylulose-5-phosphate].

The purpose of this study is to clarify hydrogen and carbon isotopic fractionations relative to ambient water and bulk tissue, respectively, with respected to *n*-alkanoic acid (**7**), phytol (**8**) and sterol (**9**) biosyntheses among terrestrial (C3-angiosperm, C3-gymnosperm, C4 and CAM plants) and aquatic C3 plants. *n*-Alkanoic acids (**7**), phytol (**8**) and sterols (**9**) are representative lipid biomolecules associated with acetogenic, MEP (**4**) and MVA (**5**) pathways, respectively (e.g., Lichtenhaler et al., 1997; Lichtenthaler, 1999; Fig. 1). Therefore, hydrogen and carbon isotopic fractionations during these lipid biosyntheses among various plant classes will provide for the better understanding of isotopic distributions in biological and geochemical samples.

2. Results and discussion

2.1. Samples

Twenty-one terrestrial plant leaves including thirteen C3 angiosperm, two C3 gymnosperm, four C4 and two CAM plants and three aquatic C3 plant leaves are used

Table 1
Samples used in this study

Sample	Abbreviation	Type	Date	Location	δD_{cellu}[a]	$\delta^{13}C_{bulk}$[b]
Terrestrial plant species						
C3 plants (angiosperm)						
Acer argutum	AA1	C3	5/99	Japan	−70	−34.2
Acer argutum	AA2	C3	10/99	Japan	−105	−34.8
Acer carpinifolium	AcC1	C3	5/99	Japan	−83	−31.8
Acer carpinifolium	AcC2	C3	10/99	Japan	−100	−33.8
Acer palmatum	AcP	C3	10/00	Japan	−97	−37.5
Artemisia princeps	ArP	C3	10/00	Japan	−116	−32.2
Benthamidia japonica	BJ1	C3	5/99	Japan	−64	−34.3
Benthamidia japonica	BJ2	C3	10/99	Japan	−66	−35.6
Plantago asiatica	PA	C3	5/00	Japan	−129	−36.2
Prunus jamasakura	PJ	C3	10/99	Japan	−101	−32.0
Quercus dentata	QD	C3	10/00	Japan	−113	−30.9
Quercus mongolica	QM	C3	10/00	Japan	−92	−29.7
Taraxacum officinale	TO	C3	5/00	Japan	−104	−32.7
C3 plants (gymnosperm)[c]						
Cryptomeria japonica	CJ1	C3	5/99	Japan	−101	−26.4
Cryptomeria japonica	CJ2	C3	10/99	Japan	−106	−27.9
C4 plants						
Sorghum bicolor	SB	C4	10–12/97	Thailand	−87	−12.1
Saccharum officinarum	SO	C4	10–12/97	Thailand	−52	−12.0
Miscanthus sinensis	MS	C4	10/00	Japan	−63	−11.2
Zea mays	ZM	C4	7/98	Japan	−80	−12.7
CAM plants						
Ananas comosus	AnC	CAM	10–12/97	Thailand	+42	−13.6
Lycoris radiata	LR	CAM	12/98	Japan	−33	−21.9
Aquatic plant species						
aquatic C3 plants						
Hydrilla verticillata	HV	C3	10/00	Japan	−111	−16.5
Potamogeton perfoliatus	PP	C3	10/00	Japan	−104	−15.8
Vallisneria asiatica	VA	C3	10/00	Japan	−124	−14.6

[a] Hydrogen isotopic composition of cellulose (**1**) nitrate (‰ relative to SMOW).
[b] Carbon isotopic composition of bulk tissue (‰ relative to PDB), reported in Chikaraishi and Naraoka (2003).
[c] Reported in Chikaraishi et al. (2004).

in this study (Table 1). Carbon isotopic compositions of bulk tissues from these plants, and hydrogen and carbon isotopic compositions of *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) from a C3-gymnosperm (*C. japonica*) were reported previously (Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2004). In addition to these previous studies, hydrogen and carbon isotopic compositions of *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) from the same 22 plant leaves were determined in this study. These terrestrial plants were collected from suburbs of Tokyo (eighteen species) in Japan and from farms in Thailand (three species). Three aquatic plants were collected from about 1 m depth in Lake Haruna at Gunma Prefecture in Japan. The surfaces of leaves were washed with distilled water to remove contaminants. The collected samples were stored at −20 °C until analysis.

2.2. Molecular distributions

In *n*-alkanoic acids (**7**), all plants show an even-carbon numbered predominance with bimodal abundance maxima at *n*-C_{16} and between *n*-C_{22} and *n*-C_{34}. Generally, *n*-alkanoic acids (**7**) are more abundant in the bound-fraction compared to the free-fraction. Phytol (**8**) and sterols (**9**) occur in both free- and bound-fractions of all plants. The sterols (**9**) are composed mainly of three sterols: 24-methylcholest-5-en-3β-ol (campesterol) (**9a**), 24-ethylcholesta-5,22-dien-3β-ol (stigmasterol) (**9b**) and 24-ethylcholest-5-en-3β-ol (β-sitosterol) (**9c**), with stigmasterol (**9b**) or β-sitosterol (**9c**) the most abundant. These lipid molecules are typically found in higher plants (e.g., Eglinton and Hamilton, 1967; Killops and Killops, 1993). Discrimination of plant classes based solely on their molecular distributions cannot be achieved, consistent with previous studies (e.g., Collister et al., 1994).

2.3. Isotopic fractionations

Hydrogen isotopic fractionation of the cellulose (**1**) nitrate or lipid biomolecule (**7**–**9**) relative to ambient water (ε_{water}) is defined in Eq. (1). Similarly, carbon isotopic fractionation of lipid biomolecule (**7–9**) relative to bulk tissue (ε_{bulk}) is defined in Eq. (2).

$$\varepsilon_{water} = 1000[(\delta D_{(cellu\ or\ lipid)} + 1000)/(\delta D_{water} + 1000) - 1], \tag{1}$$

$$\varepsilon_{bulk} = 1000[(\delta^{13}C_{lipid} + 1000)/(\delta^{13}C_{bulk} + 1000) - 1], \tag{2}$$

where δD values of ambient water (δD_{water}) are available in the previous report (Chikaraishi and Naraoka, 2003), being δD_{water} values of −42‰ for Japan and −40‰ for Thailand as annual mean values of precipitations, and −60‰ as an annual mean value of lake water. δD values of cellulose (**1**) nitrate (δD_{cellu}) and $\delta^{13}C$ values of bulk tissues ($\delta^{13}C_{bulk}$) are summarized in Table 1. Compound-specific δD_{lipid} and $\delta^{13}C_{lipid}$ data from C3-angiosperm, C4, CAM and aquatic C3 plants are summarized in Tables 2 and 3, respectively. The ε_{water}–ε_{bulk} cross plots of *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) for various plant classes are shown in Fig. 2. In all plants, *n*-alkanoic acids (**7**) are less depleted in D with a wider range of ε_{bulk} values relative to phytol (**8**) and sterols (**9**). Phytol (**8**) is more depleted in ^{13}C relative to sterols (**9**). For example, *n*-alkanoic acids (**7**) from C3-angiosperms (Fig. 2(a)) have ε_{water} values of −95 ± 23‰, while phytol (**8**) and sterols (**9**) have the ε_{water} values of −306 ± 12‰ and −212 ± 15‰, respectively. The ε_{bulk} variation of *n*-alkanoic acids (**7**) is very large (∼15‰), while those of phytol (**8**) and sterols (**9**) have a relatively narrow range (approximately 5‰ and 4‰, respectively). This isotopic trend among *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) is common to all plants. Though the ε_{water} and ε_{bulk} values of the same biomolecules vary among various plant classes (see below), this ε_{water}–ε_{bulk} signature clearly indicates the distinct discrimination in D and ^{13}C among acetogenic, MEP and MVA pathways for all plants. The relationships between ε_{water} and ε_{bulk} values of various lipid biomolecules with respect to their biosynthetic pathways within a single higher plant have been described in more detail in the previous report (Chikaraishi et al., 2004).

2.3.1. Cellulose (1) nitrate (ε_{water})

The ε_{water} values of cellulose (**1**) nitrate are shown in Fig. 3. The ε_{water} values of C3 (−23‰ to −91‰ for angiosperms and −62‰ to −67‰ for gymnosperms), C4 (−12‰ to −49‰) and aquatic C3 plants (−47‰ to −68‰) demonstrate that cellulose (**1**) is depleted in D relative to ambient water for these plants, while the ε_{water} values of the CAM cellulose (**1**) nitrates (+9‰ and +86‰) show extreme D-enrichment relative to ambient water. These ε_{water} values of cellulose (**1**) nitrates from C3, C4 and CAM plants are consistent with those of previous reports (e.g., Sternberg et al., 1984a–c). In the previous studies, isotope signatures of cellulose (**1**) nitrate may not indicate D-enriched leaf water due to evapotranspiration, but rather indicate different isotopic fractionations during biochemical reactions in cellulose (**1**) biosynthesis depending on plant class (Sternberg et al., 1984a,b, 1986b), because δD values of the leaf water were not substantially different among C3, C4 and CAM plants (e.g., Ziegler et al., 1976; Sternberg et al., 1986a,b). If the evapotranspiration is a significant factor for the D-enrichment of cellulose (**1**), cellulose (**1**) of all terrestrial plants would be more enriched in D relative to that of aquatic plants, because aquatic plants should have no D-enrichment by evapotranspiration. However, the ε_{water} values of aquatic C3 plants cannot be distinguished from those of terrestrial C3 and C4 plants in this study, supporting the hypothesis that evapotranspiration is a minor factor controlling the ε_{water} values of cellulose (**1**).

2.3.2. n-Alkanoic acids (7) (ε_{water}–ε_{bulk})

The ε_{water} and ε_{bulk} values of *n*-alkanoic acids (**7**) are distinctive depending on plant classes, with no significant difference between the free- and bound-fractions within a single plant species (Fig. 4). For example, the ε_{water} value of the free-fraction (−96 ± 23‰) from C3-angiosperms is close to that of the bound-fraction (−94 ± 23‰), showing less depletion in D relative to C4 plants (−122 ± 14‰ for the free-fraction and −116 ± 15‰ for the bound-fraction). The ε_{bulk} value of the free-fraction (−3.9 ± 2.4‰) from C3-angiosperms is also close to that of the bound-fraction (−4.8 ± 2.6‰), showing less depletion in ^{13}C relative to C4 plants (−9.9 ± 2.0‰ for the free-fraction and −10.4 ± 1.9‰ for the bound-fraction). For *n*-alkyl lipids including *n*-alkanes (**6**) and *n*-alkanoic acids (**7**), the larger ε_{bulk} values of C4 plants relative to those of C3 plants have been observed previously (Collister et al., 1994; Ballentine et al., 1998; Chikaraishi and Naraoka, 2003). The ε_{water} values of *n*-alkanoic acids (**7**) have a positive correlation ($R^2 = 0.63$) with the ε_{bulk} values among various plant classes. This positive ε_{water}–ε_{bulk} correlation of *n*-alkanoic acids is consistent with that of *n*-alkanes (**6**) in the previous study (Chikaraishi and Naraoka, 2003), as expected since *n*-alkanes (**6**) are biosynthesized from *n*-alkanoic acids (**7**) (e.g., Cheesbrough and Kolattukudy, 1984; Post-Beittenmiler, 1996; Fig. 1).

2.3.3. Phytol (8) (ε_{water}–ε_{bulk})

The ε_{water} and ε_{bulk} values of phytol (**8**) are also distinctive depending on plant classes, with no significant difference between the free- and bound-fractions within a single plant species (Fig. 4). The ε_{water} values of phytol (**8**) have a negative correlation ($R^2 = 0.68$) with the ε_{bulk} values among various plant classes, which indicate different isotopic fractionations for those of *n*-alkanes (**6**) and *n*-alkanoic acids (**7**). This suggests that the mechanism of isotopic fractionation during *n*-alkanoic acid (**7**)

Table 2
Compound-specific hydrogen isotopic compositions of *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) from terrestrial and aquatic plants

Compound	C3 plant (angiosperm)												
	AA1	AA2	AcC1	AcC2	AcP	ArP	BJ1	BJ2	PA	PJ	QD	QM	TO
n-Alkanoic acid (**7**) (free-fraction)													
12		−170								−163			
14	−213	−103	−124	−108	−140	−182		−139	−169	−157	−140	−154	−125
16	−119	−123	−94	−103	−140	−146	−125	−131	−153	−139	−123	−164	−159
18	−110	−131	−123	−88	−133	−141	−138	−128	−152	−123	−132	−114	−140
20	−104	−121				−152	−104	−134	−148	−139	−148	−134	
22	−110		−92	−94	−118	−145	−118	−127	−136	−139	−143	−146	−128
24	−105		−91	−104	−127	−141	−120	−134	−146	−144	−158	−148	−131
26			−111	−104	−145	−164	−125	−130		−146	−150	−149	−139
28	−112		−124	−103	−142	−157	−130	−134	−157	−131	−168	−175	−128
30	−119	−119	−116	−116	−134	−153	−130	−144	−148	−154	−160	−175	−142
32		−110	−91	−101			−140	−156		−153	−160	−162	
34				−109									
36													
n-Alkanoic acid (**7**) (bound-fraction)													
12				−91						−176	−190	−144	
14	−179	−135	107	−139		−146	−109	−120	−181	−162	−150	−136	−120
16	−107	−125	−114	−127	−138	−130	−128	−135	−159	−125	−143	−143	−126
18	−102	−103	−90	−122	−140	−144	−113	−123	−139	−110	−132	−114	−133
20						−146	−124	−124	−145	−138	−143	−138	
22	−118	−131	−101	−124	−99	−145	−121	−134	−109	−138	−141	−145	−130
24	−101	−127	−96	−121	−133	−137	−112	−122	−142	−142	−155	−155	−127
26			−99		−124	−151	−115	−132	−125	−163	−150	−154	−132
28		−75	−118	−101	−150	−159	−128	−136	−151	−145	−174	−172	−126
30		−114	−117	−125	−134	−153	−124	−138		−159	−169	−175	−138
32		−108	−81	−97	−152		−136	−155			−161	−112	−142
34				−122									
Isoprenoid (free-fraction)													
Phytol (**8**)	−323	−343	−333	−337	−341	−333	−342	−342	−330	−352	−339	−344	−330
Campesterol (**9a**)						−230							
Stigmasterol (**9b**)	−218	−246	−232	−244	−262	−242					−237	−261	−236
β-sitosterol (**9c**)	−227	−250	−227	−259	−256	−240	−267	−283	−225	−249	−234	−256	−237
Isoprenoid (bound-fraction)													
Phytol (**8**)	−325	−347	−338	−341	−339	−342	−332	−350	−320	−295	−333	−340	−335
Campesterol (**9a**)						−231							
Stigmasterol (**9b**)	−220	−235	−237	−241	−242	−240					−250	−262	
β-sitosterol (**9c**)	−236	−255	−229	−236	−250	−235	−272	−271	−229	−257	−245	−261	−240

	C4 plant				CAM plant		Aquatic c3 plant		
	SB	SO	MS	ZM	AnC	LR	HV	PP	VA
n-Alkanoic acid (**7**) (free-fraction)									
12	−186	−182	−167	−173	−208			−184	
14	−161	−185	−150	−171	−200	−190	−322	−238	−201
16	−163	−171	−152	−139	−182	−195	−232	−239	−189
18	−160	−167	−174	−147	−192	−199	−213	−147	−153
20	−164	−153	−159	−175	−193	−191	−217	−172	−148
22	−167	−154	−145	−155	−204	−189	−215	−183	−152
24	−166	−157	−137	−140	−203	−167	−209	−154	−150
26	−163	−143	−135	−142	−194	−166	−189	−179	−150
28	−167	−160	−154	−155	−176	−187	−191	−204	
30	−166	−158	−149	−148	−182	−174		−198	−146
32	−156	−168	−130	−147					−156
34	−157	−177	−160	−158					
36			−146						
n-Alkanoic acid (**7**) (bound-fraction)									
12	−170	−181	−161	−140				−170	−140
14	−161	−178	−137	−148	−199	−194	−318	−222	−194
16	−163	−160	−139	−151	−180	−187	−224	−198	−188

(*continued on next page*)

Table 2 (*continued*)

	C4 plant				CAM plant		Aquatic c3 plant		
	SB	SO	MS	ZM	AnC	LR	HV	PP	VA
18	−163	−156	−135	−142	−188	−184	−221	−161	−155
20	−156	−155	−120	−151	−183	−175	−216	−160	−164
22	−157	−155	−131	−141	−198	−183	−220	−151	−166
24	−157	−151	−142	−135	−205	−178	−204	−161	−165
26		−156	−159	−143	−184		−202	−171	
28	−165	−179	−164	−159	−180	−189	−206		
30	−147	−175	−147	−147	−183	−176			−170
32	−161	−158	−132	−143		−173			−128
34		−172	−129						−168
Isoprenoid (free-fraction)									
Phytol (**8**)	−278	−278	−290	−285	−328	−317	−343	−304	−320
Campesterol (**9a**)	−218	−212	−234	−244	−220		−256	−274	
Stigmasterol (**9b**)	−218	−215	−209	−210	−235		−263	−264	
β-sitosterol (**9c**)	−215	−209	−242	−230	−241	−222	−261	−252	−221
Isoprenoid (bound-fraction)									
Phytol (**8**)	−280	−277	−295	−295	−301	−333	−343	−352	−319
Campesterol (**9a**)	−196	−215		−227		−219		−246	
Stigmasterol (**9b**)	−209	−197		−225				−267	−242
β-sitosterol (**9c**)	−219	−215	−237	−231	−238	−220	−255	−257	−235

Plant abbreviation taken from Table 1.
Standard deviations of replicate (2–4 times) analyses range from 3‰ to 11‰ (4‰ in average).

biosynthesis is different from that of phytol (**8**) biosynthesis. The ε_{water} values of C3-angiosperms (-309 ± 8‰ for the free-fraction and -304 ± 15‰ for the bound-fraction) are much smaller relative to those of C4 plants (-252 ± 6‰ for the free-fraction and -256 ± 9‰ for the bound-fraction), while ε_{bulk} values of C3-angiosperms (-3.9 ± 1.0‰ for the free-fraction and -4.4 ± 1.1‰ for the bound-fraction) are less smaller relative to those of C4 plants (-9.1 ± 0.9‰ for the free-fraction and -8.8 ± 1.6‰ for the bound-fraction).

2.3.4. Sterols (9) (ε_{water}–ε_{bulk})

The ε_{water} and ε_{bulk} values of sterols (**9**) are also distinctive depending on plant classes, with no significant difference between the free- and bound-fractions within a single plant species (Fig. 4). The ε_{water} values of sterols (**9**) have a negative correlation ($R^2 = 0.75$) with the ε_{bulk} values among various plant classes, which is similar to phytol (**8**) but different from *n*-alkyl lipids (**6** and **7**). The ε_{water} values of C3-angiosperms (-211 ± 16‰ for the free-fraction and -211 ± 14‰ for the bound-fraction) are much smaller relative to those of C4 plants (-188 ± 13‰ for the free-fraction and -184 ± 14‰ for the bound-fraction), while ε_{bulk} values of C3-angiosperms (-1.2 ± 0.9‰ for the free-fraction and -1.4 ± 0.9‰ for the bound-fraction) are less smaller relative to those of C4 plants (-4.1 ± 1.1‰ for the free-fraction and -5.6 ± 0.7‰ for the bound fraction).

2.4. Isotopic fractionations among various plant classes

For CAM plants, significant D-enrichment found in cellulose (**1**) nitrate (ε_{water} values of +9‰ to +86‰) compared to other plant classes (Fig. 3) is not observed in lipid molecules (**7**–**9**) (Fig. 4). These hydrogen isotopic signatures for C3, C4 and CAM plants are consistent with the δD of saponifiable lipid-fractions in several previous studies (e.g., Sternberg et al., 1984c, 1986a). This result supports the assumption that the D-enriched ε_{water} values of cellulose (**1**) nitrate may not indicate D-enriched leaf water due to evapotranspiration but rather indicate different isotopic fractionations during cellulose (**1**) biosynthesis depending on plant class (Sternberg et al., 1984a,b, 1986b). The ε_{water} values of the same lipid molecules (**7**–**9**) are very similar ($< \pm 15$‰) between two CAM plants (*Ananas comosus* and *Lycoris radiata*), while the ε_{water} value of cellulose (**1**) nitrate from *A. comosus* (+86‰) is much larger than that from *L. radiata* (+9‰). This result suggests different isotopic fractionations during cellulose (**1**) biosynthesis even within a single plant class, probably depending on growth conditions.

For *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**), the magnitude of D- and ^{13}C-depletion is distinctive depending on plant classes (Fig. 4). Although the specific mechanisms responsible for those differences have not been known, this could be due to the following five factors depending on plant classes: (1) molecular flux among *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) including intermediate molecules, with respect to pool size and division of flow; (2) leaf growth rate; (3) water-efficiency; (4) kinetic isotope effects of key biochemical reactions (branching points) and (5) D-enrichment of leaf water during evapotranspiration, which can cause distinct δD values of hydrogen sources (e.g., NADPH and cell water). Among these factors, the difference in

Table 3
Compound-specific carbon isotopic compositions of *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) from terrestrial and aquatic plants

Compound	C3 plant (angiosperm)												
	AA1	AA2	AcC1	AcC2	AcP	ArP	BJ1	BJ2	PA	PJ	QD	QM	TO
n-Alkanoic acid (**7**) (free-fraction)													
12	−37.8	−40.9								−40.2	−39.6		
14	−37.2	−32.8	−30.8	−42.0	−42.3	−39.5		−45.6	−35.4	−37.2	−39.7	−38.6	−35.3
16	−35.9	−38.2	−34.5	−39.2	−40.8	−39.2	−34.4	−37.4	−33.3	−36.8	−37.5	−35.8	−34.9
18	−35.1		−35.2	−37.0	−40.7	−39.6	−34.2	−38.0	−35.6	−36.2	−37.2	−33.5	−37.7
20	−34.8	−41.6	−36.5	−40.6	−38.4	−40.2	−35.8	−38.2	−37.3	−33.6	−34.8	−36.3	−37.5
22	−36.3	−40.8	−37.1	−41.3	−38.4	−38.6	−34.8	−37.6	−37.1	−33.6	−34.0	−35.8	−35.1
24	−36.1		−35.9	−37.9	−39.7	−38.8	−36.1	−35.9	−37.5	−35.8	−35.4	−36.9	−35.7
26	−36.2	−37.6	−36.0	−35.1	−38.3	−37.4	−38.4	−39.0	−38.9	−35.4	−34.2	−34.0	−36.0
28	−36.8	−35.6	−35.6	−37.5	−38.7	−36.3	−38.7	−39.6	−39.9	−36.0	−33.4	−34.4	−36.9
30	−36.0	−37.0	−35.7	−38.1	−41.8	−37.3	−37.5	−40.4	−40.2	−35.5	−34.9	−36.9	−37.9
32		−37.2	−36.1	−37.7	−42.3	−36.6	−38.0	−38.3	−40.2	−33.8	−37.5	−36.8	
34				−38.4									
36													
n-Alkanoic acid (**7**) (bound-fraction)													
12		−43.1		−43.4						−39.0	−39.8	−41.0	
14	−43.3	−38.1	−35.3	−42.6		−36.6	−41.5	−47.7	−36.4	−36.8	−39.8	−39.6	−38.7
16	−36.1	−37.5	−35.0	−39.9	−40.0	−39.3	−36.0	−37.8	−33.7	−38.2	−37.7	−36.4	−35.4
18	−36.4		−36.4	−39.5	−41.1	−40.3	−36.8	−38.4	−34.8	−36.9	−38.0	−34.4	−35.7
20	−37.3	−40.0		−42.7		−37.2	−38.3	−40.0	−37.9	−33.6	−38.0	−38.0	−37.7
22	−36.5	−40.5	−36.7	−42.5	−39.4	−37.9	−38.4	−38.7	−36.9	−33.6	−38.3	−38.6	−35.0
24	−36.5	−40.1	−36.5	−41.3	−40.1	−38.7	−38.7	−38.4	−37.9	−36.0	−36.0	−37.8	−35.8
26		−38.4	−37.3	−37.7	−38.8	−37.6	−39.1	−39.3	−39.2	−35.2	−35.8	−38.3	−36.5
28		−36.3	−35.6	−35.9	−39.2	−37.5	−38.1	−39.8	−39.4	−35.1	−34.5	−37.7	−36.4
30		−38.0	−35.8	−38.3	−42.8	−37.7	−38.5	−39.5	−39.8	−35.1	−35.6	−37.4	−37.6
32		−38.1	−36.4	−38.1	−44.5		−38.4	−38.4		−34.3	−35.8	−35.8	−37.3
34				−38.6									
Isoprenoid (free-fraction)													
Phytol (**8**)	−37.9	−37.5	−36.9	−38.8	−41.1	−35.7	−36.5	−38.0	−41.3	−36.0	−35.6	−33.6	−35.8
Campesterol (**9a**)	−35.2	−37.3	−33.4	−35.3		−33.0	−34.9	−39.3			−31.6		−33.6
Sigmasterol (**9b**)	−34.3	−34.8	−33.2	−34.1	−38.2	−34.0		−36.3			−33.0	−31.9	−34.0
β-sitosterol (**9c**)	−35.6	−35.5	−33.6	−36.0	−38.1	−33.7	−34.5	−37.2	−37.1	−32.2	−31.4	−31.9	−33.6
Isoprenoid (bound-fraction)													
Phytol (**8**)	−40.6	−38.1	−37.8	−37.9	−41.3	−35.7	−38.0	−38.5	−41.5	−35.7	−35.6	−33.5	−36.7
Campesterol (**9a**)	−34.6	−36.7	−33.6	−35.7	−38.0	−33.5					−31.4		
Stigmasterol (**9b**)	−34.1	−35.0	−34.3	−34.1	−37.7	−33.8	−35.4	−39.2			−33.4	−32.7	
β-sitosterol (**9c**)	−34.8	−36.3	−34.4	−35.6	−38.6	−33.4	−35.5	−36.5	−37.3	−32.5	−32.2	−31.6	−33.9

	C4 plant				CAM plant		Aquatic C3 plant		
	SB	SO	MS	ZM	AnC	LR	HV	PP	VA
n-Alkanoic acid (**7**) (free-fraction)									
12	−23.3	−23.0	−22.4	−26.5				−25.1	−21.8
14	−24.3	−24.1	−22.9	−26.5	−25.4	−35.9	−19.0	−21.7	−19.4
16	−22.5	−24.4	−22.8	−23.7	−26.5	−32.7	−21.1	−20.4	−20.6
18	−22.2	−22.6	−18.7	−24.1	−25.4	−28.7	−23.6	−24.7	−22.6
20	−22.0	−21.4	−16.3	−17.5	−22.9	−29.5	−26.1	−27.2	−25.3
22	−20.6	−22.6	−16.9	−20.2	−22.3	−29.1	−24.9	−26.4	−25.3
24	−21.6	−22.2	−20.8	−23.2	−23.2	−31.6	−24.7	−25.4	−24.9
26	−22.0	−21.6	−18.7	−24.4	−24.1	−27.1	−27.5	−25.4	−25.6
28	−23.7	−23.9	−18.0	−22.3	−25.7	−28.8	−22.0	−25.6	
30	−19.7	−20.8	−19.3	−21.6	−23.7	−28.1	−23.6	−26.4	−24.0
32	−20.9	−21.0	−20.2	−22.8	−22.4				−25.3
34	−21.0	−22.7	−20.3						
36			−20.7						
n-Alkanoic acid (**7**) (bound-fraction)									
12	−25.2	−23.7	−22.3	−26.6				−23.7	−21.4
14	−25.5	−24.3	−22.4	−27.2	−21.5	−35.2	−22.9	−22.5	−21.0
16	−22.1	−22.6	−21.2	−24.3	−26.4	−31.8	−22.8	−24.7	−20.5

(*continued on next page*)

Table 3 (*continued*)

	C4 plant				CAM plant		Aquatic C3 plant		
	SB	SO	MS	ZM	AnC	LR	HV	PP	VA
18	−22.5	−22.1	−21.0	−25.4	−24.1	−29.4	−24.1	−26.0	−22.7
20	−23.4	−22.0	−17.6	−21.9	−20.5	−30.0	−25.6	−28.7	−25.2
22	−22.3	−23.0	−18.8	−23.2	−19.1	−30.1	−25.1	−29.0	−25.2
24	−22.8	−22.6	−19.1	−25.7	−23.4	−31.3	−24.9	−25.8	−24.3
26	−21.9	−20.4	−18.5	−23.8	−23.3	−31.6	−27.0	−27.5	
28	−22.2	−23.2	−19.2	−21.7	−23.4	−29.5	−24.7		
30	−20.2	−21.6	−18.8	−21.6	−22.3	−29.0			−26.5
32	−21.2	−20.5	−20.0	−25.1	−21.9	−29.8			−25.4
34	−21.2	−24.6	−20.0						
Isoprenoid (free-fraction)									
Phytol (**8**)	−20.8	−20.0	−20.4	−22.9	−20.5	−29.3	−20.5	−19.4	−20.2
Campesterol (**9a**)	−16.2	−16.0	−16.7	−17.8	−17.9		−17.5	−16.9	−15.8
Stigmasterol (**9b**)	−14.8	−17.3	−14.5	−17.0	−16.6		−17.2	−16.1	−15.1
β-sitosterol (**9c**)	−17.3	−16.1	−14.1	−18.6	−18.7	−24.5	−16.9	−16.5	−16.7
Isoprenoid (bound-fraction)									
Phytol (**8**)	−21.8	−19.9	−18.0	−23.0	−20.4	−28.2	−19.8	−19.6	−20.1
Campesterol (**9a**)	−16.7	−17.6	−16.5	−19.0	−17.2	−25.0		−16.9	−15.3
Stigmasterol (**9b**)	−16.2	−18.5	−17.3	−18.3	−17.2		−17.5	−16.2	−15.7
β-sitosterol (9c)	−17.7	−17.5	−16.9	−18.1	−17.6	−25.8	−18.3	−17.1	−15.4

Plant abbreviation taken from Table 1.

Standard deviations of replicate (2–4 times) analyses range from 0.0‰ to 1.8‰ (0.4‰ in average).

molecular flux among *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) may be an unlikely explanation for the ε_{water}–ε_{bulk} difference, since the molecular distributions of these lipid molecules cannot be distinguished among various plant classes. Differences in leaf growth rate, water-efficiency and D-enrichment of leaf water during evapotranspiration are also unlikely explanations. Since, *n*-alkanoic acids (**7**) of C3-angiosperms have larger ε_{water} values than those of C4 plants, while phytol (**8**) and sterols (**9**) of C3-angiosperms have smaller ε_{water} values than those of C4 plants (Fig. 4). If the isotopic fractionations are controlled mainly by leaf growth rate or water-efficiency as well as D-enrichment of leaf water during evapotranspiration, the magnitude of D-depletion depending on plant classes must have the similar fractionation trends among *n*-alkanoic acid (**7**), phytol (**8**) and sterol (**9**) biosyntheses.

As a result, the distinctive D- and ^{13}C-depletion among various plant classes is probably attributable to the different kinetic isotope effects of key biochemical reactions of each plant class. For example, it has been known that isotope effect of ribulose biphosphate carboxylase oxidase (RUBISCO) associated with carbon fixation is distinctive depending on plant classes (e.g., Estep et al., 1978; Goericke et al., 1994; Hayes, 2001). Similarly, it could be common that different plant classes utilize the same enzymes with different isotope effects during the biochemical reactions in the lipid biosyntheses. In addition, it has been demonstrated that ^{13}C-depletion of lipid molecules relative to bulk tissue is due to isotopic fractionations at acetyl-CoA (**3**) formation from pyruvate (**2**) (DeNiro and Epstein, 1977; Monson and Hayes, 1982) and at the following branching points in lipid biosynthesis (Monson and Hayes, 1980; Collister et al., 1994). Biosynthetic pathways of *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) are independent, in which *n*-alkanoic acids (**7**) are formed in the acetogenic pathway as an acetyl-CoA (**3**) precursor while phytol (**8**) and sterols (**9**) are formed using isopentenyl pyrophoshate (IPP) (**10**) as an intermediate produced independently by MEP (**4**) and MVA (**5**) pathways, respectively (Fig. 1). When one or more enzymatic reaction points exist independently in each lipid biosynthesis involved with different kinetic isotope effects of the same enzymes, the distinctive D- and ^{13}C-depletion could occur among various plant classes.

Among various plant classes, there was a positive correlation between ε_{water} and ε_{bulk} values for *n*-alkanoic acids, while a negative correlation was found for phytol and sterols from the same plants. This suggests that the isotopically fractionated mechanisms of *n*-alkanoic acid (**7**) synthesis is clearly different from those of phytol (**8**) and sterol (**9**) syntheses. If the isotopic fractionation steps might include contrast reactions (e.g., hydrogenation vs. dehydrogenation) between *n*-alkanoic acids (**7**) and isoprenoids [phytol (**8**) and sterol (**9**)], it could be a likely explanation for the observed correlations. If the kinetic isotope effect causes preferential hydrogenation using D-depleted hydrogen from hydrogen pools such as cell water and NADPH, the slope of ε_{water}–ε_{bulk} correlation could be positive. On the other hand, if dehydrogenation condenses D-enriched hydrogen on lipid molecules as a result of preferential elimination of D-depleted hydrogen, the slope of ε_{water}–ε_{bulk} correlation

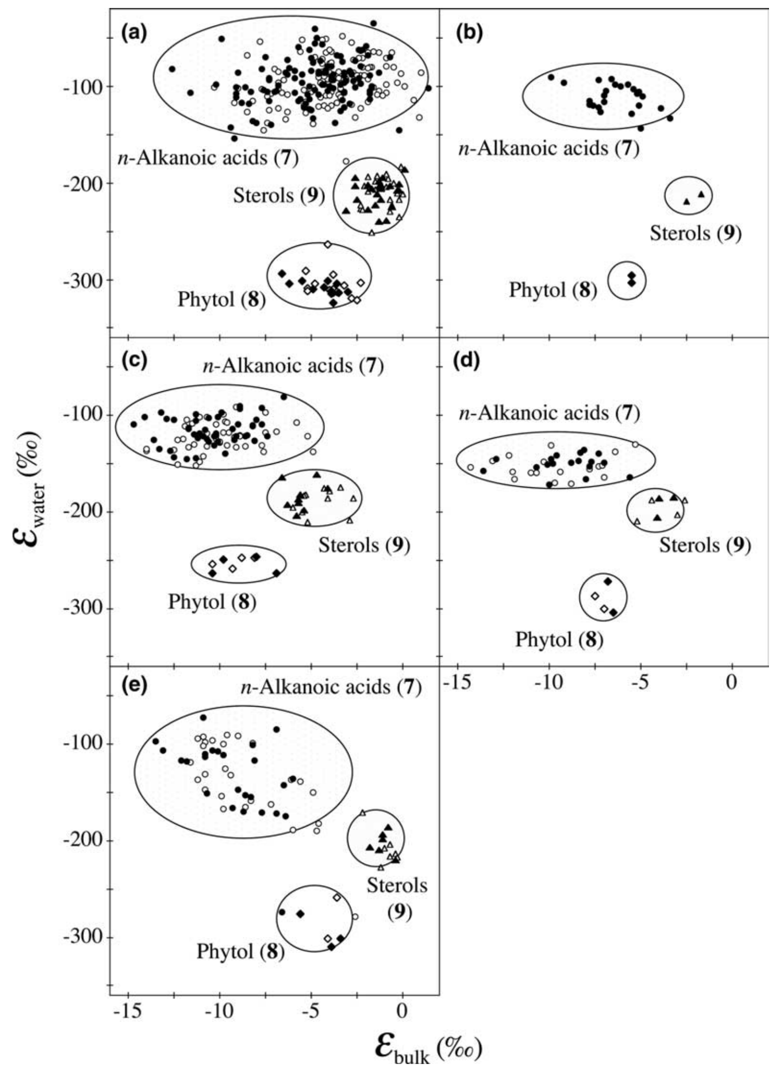

Fig. 2. The ε_{water}–ε_{bulk} plot of *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**) extracted from (a) C3-angiosperm, (b) C3-gymnosperm, (c) C4, (d) CAM and (e) aquatic C3 plants. Circles, diamond and triangle indicate *n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**), respectively. Open and filled symbols indicate the free- and bound-fractions, respectively.

could be negative. Apparent biosynthesis of phytol (**8**) is independent of that of sterols (**9**), being consistent with the different carbon isotopic signatures between phytol (**8**) and sterols (**9**) (Fig. 2). However, the relative ε_{water}–ε_{bulk} distribution for various plant classes is similar between phytol (**8**) and sterols (**9**) (Fig. 4), which probably suggests that a part of the biochemical reactions with different isotopic fractionations, are common in both biosyntheses.

For *n*-alkanoic acids, phytol and sterols, the positive or negative correlation among various plant classes should suggest a close relation among their mechanisms of isotopic fractionations, though the specific mechanisms responsible for the different fractionation have yet been unclear. Comparative studies of possible factors such as the isotope effects, pool sizes and division of flow in reactants among various plant classes is needed to clarify the observed ε_{water}–ε_{bulk} correlation. In particular,

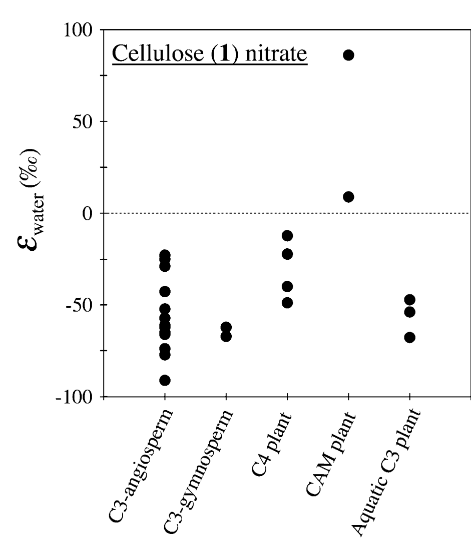

Fig. 3. The ε_{water} values of cellulose (**1**) nitrate from C3, C4, CAM and aquatic C3 plants.

such studies should investigate the isotope effects on the same enzyme from different plant classes.

3. Concluding remarks

Compound-specific dual (δD–$\delta^{13}C$) isotopic data of representative lipid molecules [*n*-alkanoic acids (**7**), phytol (**8**) and sterols (**9**)] associated with three biosynthetic pathways [acetogenic, MEP (**4**) and MVA (**5**) pathways, respectively] from terrestrial and aquatic plants provided more detailed information on distinct isotopic fractionations among various plant classes than previous studies (e.g, Collister et al., 1994; Lockheart et al., 1997; Ballentine et al., 1998; Sessions et al., 1999; Chikaraishi and Naraoka, 2003). In all plants, lipid molecules were depleted in both D (up to 324‰) and ^{13}C (up to 14.7‰) relative to ambient water and bulk tissue, respectively. Different D- and ^{13}C-depletions among *n*-alkanoic acids, phytol and sterols were observed in all plants, in which the fractionation trends were consistent with different isotopic fractionations among acetogenic, MEP and MVA pathways within a single plant (*C. japonica*), as reported previously (Chikaraishi et al., 2004). In addition, for *n*-alkanoic acid (**7**), phytol (**8**) and sterol (**9**) syntheses, the magnitude of D- and ^{13}C-depletions was distinctive depending on plant classes (Fig. 4). This is probably because the magnitude of kinetic isotope effects in key enzymatic reactions is different among various plant classes. In all plant classes examined, *n*-alkanoic

Cheesbrough, T.M., Kolattukudy, P.E., 1984. Alkane biosynthesis by decarbonylation of aldehydes catalyzed by a particulate preparation from *Pisum sativum*. Proceedings of the National Academy of Sciences of the United States of America 81, 6613–6617.

Chikaraishi, Y., Naraoka, H., 2003. Compound-specific δD-δ^{13}C analysis of *n*-alkanes extracted from terrestrial and aquatic plants. Phytochemistry 63, 361–371.

Chikaraishi, Y., Naraoka, H., Poulson, S.R., 2004. Carbon and hydrogen isotopic fractionation during lipid biosynthesis in a higher plant (*Cryptomeria japonica*). Phytochemistry 65, 323–330.

Collister, J.W., Rieley, G., Stern, B., Eglinton, G., Fry, B., 1994. Compound-specific δ^{13}C analyses of leaf lipids from plants with differing carbon dioxide metabolisms. Organic Geochemistry 21, 619–627.

Cvejic, J.H., Rohmer, M., 2000. CO_2 as main carbon source for isoprenoid biosynthesis via the mevalonate-independent methylerythritol 4-phosphate route in the marine diatoms *Phaeodactylum tricornutum* and *Nitzschia ovalis*. Phytochemistry 53, 21–28.

Deines, P., 1980. The isotope composition of reduced organic carbon. In: Fritz, P., Fontes, J.Ch. (Eds.), Handbook of Environmental Isotope Geochemistry, The Terrestrial Environment, A. Elsevier, Amsterdam, Oxford, New York, pp. 329–406.

DeNiro, M.J., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197, 177–185.

DeNiro, M.J., 1981. The effects of different methods of preparing cellulose nitrate on the determination of the D/H ratios of non-exchangeable hydrogen of cellulose. Earth and Planetary Science Letter 54, 177–185.

Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. Science 156, 1322–1335.

Eisenreich, W., Rohdich, F., Bacher, A., 2001. Deoxyxylulose phosphate pathway to terpenoids. Trends in Plant Science 6, 78–84.

Epstein, S., Yapp, C.J., Hall, J.H., 1976. The determination of D/H ratios of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. Earth and Planetary Science Letter 30, 241–251.

Estep, M.F., Tabita, F.R., Parker, P.L., Baalen, C.V., 1978. Carbon isotope fractionation by ribulose-1,5-bisphosphate carboxylase from various organisms. Plant Physiology 61, 680–687.

Goericke, R., Montoya, J.P., Fry, B., 1994. Physiology of isotopic fractionation in algae and cyanobacteria. In: Lajtha, K., Michener, H. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publication, Oxford, pp. 187–221.

Hayes, J.M., Freeman, K.H., Popp, B.N., Hoham, C.H., 1990. Compound-specific isotopic analyses: a novel tool reconstruction of ancient biogeochemical processes. Organic Geochemistry 16, 115–1128.

Hayes, J.M., 1993. Factors controlling ^{13}C contents of sedimentary organic compounds: principles and evidence. Marine Geology 113, 111–125.

Hayes, J.M., 2001. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. Reviews in Mineralogy and Geochemistry 43, 225–277.

Hilkert, A.W., Douthitt, C.B., Schlüter, H.J., Brand, W.A, 1999. Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry. Rapid Communications in Mass Spectrometry 13, 1226–1230.

Huang, Y., Shuman, B., Wang, Y., Webb III, T., 2002. Hydrogen isotope ratios of palmitic acid in lacustrine sediments record late quaternary climate variations. Geology 30, 1103–1106.

Killops, S.D., Killops, V.J., 1993. Chemical composition of biogenic matter. In: Gill, R.C.O. (Ed.), An Introduction to Organic Geochemistry. Wiley, New York, pp. 22–62.

Keeley, J.E., Sandquist, D.R., 1992. Carbon: freshwater plants. Plant, Cell and Environment 15, 1021–1035.

Lajtha, K., Marshall, J.D., 1994. Sources of variation in the stable isotopic composition of plants. In: Lajtha,, K., Michener, R.H. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Science, Publishment, Oxford, pp. 1–21.

Lerman, J.C., Queiroz, O., 1974. Carbon fixation and isotope discrimination by a crassulacean plant: dependence on the photoperiod. Science 22, 1207–1209.

Lichtenthaler, H.K., Rohmer, M., Schwender, J., 1997. Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. Physiologia Plantarum 101, 643–652.

Lichtenthaler, H.K., 1999. The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plant. Annual Reviews of Plant Physiology and Plant Molecular Biology 50, 47–65.

Lockheart, M.J., van Bergen, P.F., Evershed, R.P., 1997. Variations in the stable carbon isotope compositions of individual lipids from the leaves of modern angiosperms: implications for the study of higher land plant-derived sedimentary organic matter. Organic Geochemistry 26, 137–153.

Luo, Y.-H., Sternberg, L.dS.L., 1991. Deuterium heterogeneity in starch and cellulose nitrate of CAM and C3 plants. Phytochemistry 30, 1095–1098.

Luo, Y.-H., Sternberg, L.dS.L., Suda, S., Kumazawa, S., Mitsui, A., 1991. Extremely low D/H ratios of photoproduced hydrogen by cyanobacteria. Plant Cell Physiology 32, 897–900.

Monson, K.D., Hayes, J.M., 1980. Biosynthetic control of the natural abundance of carbon 13 at specific positions within fatty acids in *Escherichia coli*. The Journal of Biological Chemistry 255, 11435–11441.

Monson, K.D., Hayes, J.M., 1982. Carbon isotope fractionation in biosynthesis of bacterial fatty acids. Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. Geochimica et Cosmochimica Acta 46, 139–149.

Northfelt, D.W., DeNiro, M.J., Epstein, S., 1981. Hydrogen and carbon isotopic ratios of the cellulose nitrate and saponifiable lipid fractions prepared from annual growth rings of a California redwood. Geochimica et Cosmochimica Acta 45, 1895–1898.

O'Leary, M.H., 1993. Biochemical basis of carbon isotope fractionation. In: Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (Eds.), Stable Isotopes and Plant Carbon–Water Relations. Academic Press, San Diego, pp. 19–28.

Pendall, E., Betancourt, J.L., Leavitt, S.W., 1999. Paleoclimatic significance of δD and δ^{13}C values in piñon pine needles from packrat middens spanning the last 40,000 years. Palaeogeography Palaeoclimatology Palaeoecology 147, 53–72.

Post-Beittenmiler, D., 1996. Biochemistry and molecular biology of wax production in plants. Annual Reviews of Plant Physiology and Plant Molecular Biology 47, 405–430.

Rieley, G., 1994. Derivatization of organic compounds prior to gas chromatographic-combustion-isotope ratio mass spectrometric analysis: identification of isotope fractionation processes. Analyst 199, 915–919.

Sessions, A.L., Burgoyne, T.W., Schimmelman, A., 1999. Fractionation of hydrogen isotope in lipid biosynthesis. Organic Geochemistry 30, 1193–1200.

Smith, B.N., Esptein, S., 1970. Biogeochemistry of the stable isotopes of hydrogen and carbon in salt marsh biota. Plant Physiology 46, 738–742.

Sternberg, L.dS.L., DeNiro, M.J., Ting, I.P., 1984a. Carbon, hydrogen, and oxygen isotope ratios of cellulose from plants having intermediary photosynthetic modes. Plant Physiology 74, 104–107.

Sternberg, L.dS.L., DeNiro, M.J., Johnson, H.B., 1984b. Isotope ratios of cellulose from plants having different photosynthetic pathways. Plant Physiology 74, 557–561.

Sternberg, L.dS.L., DeNiro, M.J., Ajie, H., 1984c. Stable hydrogen isotope ratios of saponifiable lipids and cellulose nitrate from CAM, C3 and C4 plants. Phytochemistry 23, 2475–2477.

Sternberg, L.dS.L., DeNiro, M.J., Ajie, H.O., 1986a. Isotope relationships between saponifiable lipids and cellulose nitrate prepared from red, brown and green algae. Planta 169, 320–324.

Sternberg, L.dS.L., DeNiro, M.J., Johnson, H.B., 1986b. Oxygen and hydrogen isotope ratios of water from phytosynthetic tissued of CAM and C3 plants. Plant Physiology 82, 428–431.

Sternberg, L.dS.L., 1988. D/H ratios of environmental water recorded by D/H ratios of plant lipids. Nature 333, 59–61.

Xie, S., Nott, C.J., Avsejs, L.A., Volders, F., Maddy, D., Chambers, F.M., Gledhill, A., Carter, J.F., Evershed, R.P., 2000. Palaeclimate records in compound-specific δD values of a lipid biomarker in ombrotrophic peat. Organic Geochemistry 31, 1053–1057.

Yakir, D., DeNiro, M.J., 1990. Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba L*. Plant Physiology 93, 325–332.

Ziegler, H., Osmond, C.B., Stichler, W., Trimborn, P., 1976. Hydrogen isotope distribution in higher plants: correlations with phytosynthetic pathway and environment. Planta 128, 85–92.