Carbon isotopic compositions of individual long-chain *n*-fatty acids and *n*-alkanes in sediments from river to open ocean: Multiple origins for their occurrence

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Stable carbon isotopic compositions of individual *n*-fatty acids and *n*-alkanes were determined in six sediments from the Ohtsuchi River to the Pacific Ocean at north Honshu Island, Japan. Long-chain *n*-fatty acids (LCFAs) ranging from C₂₀ to C₃₀ and long-chain *n*-alkanes (LCALs) ranging from C₂₃ to C₃₃ have large isotopic variations from -35 to -25‰ and -35 to -29‰, respectively, although the molecular distributions of LCFAs and LCALs are almost identical in all analyzed samples.

LCFAs are more depleted in ¹³C than total organic carbon (TOC) by about 5 to 12‰. The δ¹³C values are relatively similar between *n*-C₂₀ and *n*-C₂₆, and gradually decrease with increasing molecular weight to *n*-C₃₀ by 3 to 5‰. Each *n*-fatty acid component shows a systematic enrichment in ¹³C from river to open ocean by up to 6‰ (from -32.5 to -26‰ for *n*-C₂₆), and a similar isotopic composition in the open ocean (~-26‰ for *n*-C₂₆). LCALs are also more depleted in ¹³C than TOC by about 6 to 12‰. The δ¹³C values gradually decrease from *n*-C₁₉ to *n*-C₃₁ by up to 6‰, then increase for >*n*-C₃₁. Abundant LCALs, such as *n*-C₂₉ and *n*-C₃₁, show a systematic enrichment in ¹³C from river to open ocean by up to 3‰ (from -34.6 to -31.9‰ for *n*-C₃₁), and a similar isotopic composition in the open ocean (~-31.5‰ for *n*-C₃₁). On the other hand, C₃₅ *n*-alkane has a relatively uniform isotopic composition (~-29‰) for all sediments. Such isotopic variations exhibit good correlations with δ¹³C_{TOC} (-26.4 to -20.4‰) and C/N ratio (12 to 7 by atom) variations as well as amount of sedimentary cutin- and lignin-derived organic compounds of terrestrial higher plant origin.

The isotopic distributions can be explained by a two-component mixture model involving isotopically different terrestrial and marine LCFAs and LCALs as endmember components. Although LCFAs (>*n*-C₂₀) and LCALs (>*n*-C₂₃) in marine sediments have been previously presumed to be derived from terrestrial higher plants, the results presented here may indicate that some of the LCFAs and LCALs in marine sediments are actually originated in the marine environment. Compound-specific isotope signature is important for evaluating the sources, as well as transport and mixing processes for the LCFAs and LCALs in a terrestrial-marine system.

INTRODUCTION

The carbon isotopic composition of sedimentary organic matter has been used to characterize various biogeochemical processes, its terrestrial or marine sources, and global environmental properties such as temporal changes in the partial pressure of atmospheric CO₂ (e.g., Popp *et al.*, 1989; Jasper and Hayes, 1990). For example, the isotopic composition of total organic carbon (δ¹³C_{TOC}) in terrestrial sediments averages -26‰ (relative to

PDB), with a gradual enrichment in ¹³C being observed from bay to coastal sediments, and relatively uniform values (-20‰ on average) typically found in open marine sediments (Sackett and Tompson, 1963; Hunt, 1970). Accordingly, the isotopic signature has been used to assess the relative proportions of terrestrial and marine organic sources in marine sediments (Hedges and Parker, 1976; Jasper and Gagosian, 1990) for a better understanding of carbon cycles on the earth's surface (Hedges *et al.*, 1997).

The widespread biological occurrence of fatty acids complicates the determination of their sources in sediments. Among various fatty acids, n -C₁₆ and n -C₁₈ acids including saturated and unsaturated structures are abundant and can originate from both terrestrial and marine biological sources. However, long-chain n -fatty acids (>C₂₀, LCFAs) have often been used to infer a terrestrial higher plant input to sediments through riverine or eolian transport (e.g., Simoneit, 1977a, 1977b; Prah et al., 1989; Brassell, 1993), since they are usually predominant components in higher plant wax, suberin and cutin (e.g., Kolattukudy, 1980). For example, Simoneit (1977a) used LCFAs as terrestrial indicators in Black Sea sediments, where the major input mechanism was *via* riverine transport. Moreover, Prah et al. (1989) estimated the proportion of terrestrial to marine organic matter in the sediments of northeastern tropical Pacific Ocean, based on the abundance of LCFAs which were presumed to be land-derived by long-range eolian transport. In addition to LCFAs, long-chain n -alkanes (>C₂₃, LCALs) (especially abundant C₂₇, C₂₉ and C₃₁ with a strong odd over even predominance) are usually employed as terrestrial biomarkers in marine sediments (e.g., Simoneit, 1978; Peters and Moldowan, 1993). However, the sources, as well as transpor-

tation and mixing processes, of these compounds in marine sediments have not been well understood because of their common occurrence. For example, Volkman et al. (1980) pointed out a resemblance in the molecular distribution of LCFAs between a intertidal sediment and marine organism cultures, thereby indicating that the use of the molecular distributions of LCFAs in marine environments as terrestrial biomarkers must be carried out with caution.

Using gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) (Hayes et al., 1990), the feasibility of carbon isotopic analysis of small amounts of individual organic compounds in sediments permits this application in the assessment of the sources of such compounds. Recently, we found that the carbon isotopic compositions of LCFAs in a pelagic sediment (SR72 in Fig. 1) could be clearly distinguished from those in a riverine sediment (OR3 in Fig. 1) (Naraoka et al., 1995). The carbon isotope difference of the LCFAs is up to 6‰, which compares well with the isotopic difference of the bulk organic carbon. In this study, we have determined carbon isotopic compositions of individual LCFAs and LCALs in a suite of sediments from river through bay to open ocean from Honshu Island to assess their potential sources and mixing processes.

Fig. 1. Locations of sediment samples from riverine to pelagic used in this study. Sample characteristics are shown in Table 1.

METHODS AND MATERIALS

Sediment samples

Six sediment samples from riverine to pelagic depositional environments were used in this study (Fig. 1). The riverine, bay and coastal sediments (OR3, OB2 and SOB2) were surface sediments. OR3 and OB2 are gray muds, and SOB2 is sandy-mud. Three open ocean sediments, SR65, SR68 and SR72, are from core samples. While SR65 and SR68 are gray muds, SR72 is a siliceous ooze (diatom and radiolaria). The top 30 cm of the cores was used. The locations, water depths and other characteristics of the sediment samples are summarized in Table 1. All sediments were lyophilized and powdered prior to molecular and isotopic analyses.

TOC, nitrogen and $^{13}\text{C}_{\text{TOC}}$ concentrations

After carbonate was removed with 6M HCl treatment, TOC and total nitrogen contents of dry sediments were determined using a CHN analyzer (MT-3, Yanaco). CO_2 for isotopic analysis of TOC was prepared by combustion of HCl-treated samples in evacuated and sealed quartz tubes at ca. 800°C for 2 h, using CuO as oxidant in the presence of Cu and Ag (Boutton *et al.*, 1983). Isotopic measurements were made using a Finnigan MAT delta S dual inlet mass spectrometer. $\delta^{13}\text{C}$ values are given in per mil (‰) relative to PDB (Peedee Belemnite).

Extraction procedure and molecular analyses

Powdered dry sediments (2–18 g) were saponified with 0.5 M KOH/methanol (30–180 ml) solution by reflux (3 h). The supernatant was removed after centrifugation, and the residue further extracted by sonication (15 min) with benzene/methanol (3:1 v/v; 2 × 6 ml). The extracts were combined and were reduced in volume to a few ml by rotary evaporation, then H_2O (8 ml) was added. Neutral organic compounds were recovered with *n*-hexane/diethyl ether (9:1 v/v; 3 × 30 ml) and separated by silica gel (5% H_2O w/w deactivated) column chromatography to obtain alkanes (hexane fraction). Following acidification of the aqueous fraction with distilled HCl/ H_2O azeo-

Table 1. Sediment samples used in this study

Sample	Environment	Location (see Fig. 1)	Approximate distance from Honshu Island	Water depth	org.C (wt%/dry sed.)	N (wt%/dry sed.)	C/N (by atomic)	$\delta^{13}\text{C}_{\text{TOC}}$ (‰)	Lignin-derived compounds (mg/g org.C)*
OR3	riverine	Ohtsuchi River		surface	3.19	0.30	12.4	-26.4	28.6
OB2	bay	Ohtsuchi Bay		27 m	3.10	0.27	13.4	-25.5	31.9
SOB2	continental shelf	Pacific Ocean (out of Ohtsuchi Bay)	3.5 km	101 m	0.88	0.12	8.6	-22.2	22.3
SR65	shelf edge	Pacific Ocean (39°06.6' N; 142°23.8' E)	35 km	988 m	2.62	0.33	9.3	-20.8	3.2
SR68	hemipelagic	Pacific Ocean (38°29.9' N; 143°28.4' E)	170 km	2775 m	1.85	0.24	9.0	-20.6	1.9
SR72	pelagic	Pacific Ocean (39°11.4' N; 147°06.2' E)	420 km	5360 m	0.67	0.09	8.7	-20.4	0.3

*Total amounts of vanillin, acetovanillone, vanillic acid, syringaldehyde, acetosyringone and syringic acid (S + V).

Table 2. Concentrations and isotopic compositions of individual a) *n*-fatty acids and b) *n*-alkanes in the sediments from Ohtsuchi River to Pacific Ocean

a) *n*-fatty acids

Sample Carbon number	OR3			OB2			SOB2		
	($\mu\text{g/g sed.}$)	($\mu\text{g/org.C}$)	(‰)* <i>s</i>	($\mu\text{g/g sed.}$)	($\mu\text{g/org.C}$)	(‰)* <i>s</i>	($\mu\text{g/g sed.}$)	($\mu\text{g/org.C}$)	(‰)* <i>s</i>
14	15.9	498	-28.4 1.1	12.9	416	-29.6 0.3	7.1	807	-24.8 0.2
15	3.4	107	-25.0 0.9	2.6	84	-25.2 0.7	1.4	159	-22.8 0.8
16	46.6	1461	-24.9 1.3	31.6	1019	-30.6 0.7	12.9	1466	-25.3 0.1
17	3.0	94	-27.5 2.9	0.6	19	-29.2 0.0	0.9	102	-24.9 0.5
18	10.2	320	-27.1 1.4	9.1	294	-27.0 0.4	2.7	307	-26.0 0.2
19	0.7	22	— —	0.8	26	— —	0.3	34	— —
20	5.3	166	-32.4 2.1	4.6	148	-31.1 0.0	1.2	136	-27.0 1.1
21	0.9	28	-32.5 1.7	0.7	23	-31.6 0.4	0.9	102	-25.9 0.6
22	9.7	304	-31.9 0.7	9.3	300	-32.2 0.1	1.8	205	-29.8 0.2
23	3.4	107	-34.5 0.8	3.8	123	-33.4 0.4	0.9	102	-29.7 1.1
24	8.7	273	-32.0 0.2	11.2	361	-30.3 0.2	1.9	216	-29.1 0.2
25	2.6	82	-35.4 2.0	4.0	129	-32.4 0.2	0.8	91	-31.3 0.7
26	4.5	141	-32.5 0.1	6.6	213	-30.7 0.2	1.3	148	-28.7 0.1
27	0.8	25	-35.0 1.6	1.0	32	-32.4 0.0	0.3	34	-31.3 1.1
28	3.4	107	-33.5 0.1	5.9	190	-32.2 0.2	0.9	102	-31.0 0.0
29	0.9	28	-36.5 0.9	1.7	55	-33.8 0.1	0.2	23	-32.2 1.1
30	2.7	85	-35.2 0.6	5.1	165	-35.0 2.2	0.6	68	-32.9 0.1
FA (total)	122.7	3846		111.5	3597		36.1	4102	
FA (C ₂₀₋₃₀)	42.9	1345		53.9	1739		10.8	1227	
CPI _{FA} **	3.7			3.5			2.3		

Table 2. (continued)

Sample Carbon number	SR65			SR68			SR72		
	($\mu\text{g/g sed.}$)	($\mu\text{g/org.C}$)	(‰)* <i>s</i>	($\mu\text{g/g sed.}$)	($\mu\text{g/org.C}$)	(‰)* <i>s</i>	($\mu\text{g/g sed.}$)	($\mu\text{g/org.C}$)	(‰)* <i>s</i>
14	17.8	679	-22.2	10.2	551	-25.7	1.9	284	-23.6
15	1.7	65	-19.1	1.8	97	-22.7	0.4	60	-21.3
16	23.1	882	-18.1	14.3	773	-24.3	3.8	567	-21.6
17	1.4	53	-25.4	1.0	54	-25.9	0.2	30	-24.2
18	5.4	206	-23.0	3.9	211	-25.9	1.2	179	-24.3
19	0.4	15	—	0.5	27	—	0.1	15	—
20	2.0	76	-26.1	1.5	81	-26.9	0.4	60	-26.9
21	0.6	23	-28.7	0.4	22	-28.2	0.1	15	-27.0
22	2.7	103	-28.6	2.0	108	-27.4	0.5	75	-26.6
23	0.7	27	-30.5	0.6	32	-29.6	0.2	30	-29.5
24	3.8	145	-24.5	3.4	184	-26.7	0.9	134	-25.9
25	0.5	19	-29.3	0.5	27	-30.3	0.2	30	-28.4
26	2.6	99	-26.3	2.7	146	-26.6	1.0	149	-25.7
27	0.4	15	-32.6	0.5	27	-34.4	0.3	45	-29.7
28	1.4	53	-29.0	1.5	81	-28.9	0.8	119	-28.5
29	0.3	11	-30.5	0.3	16	-34.5	0.2	30	-29.0
30	0.8	31	-32.5	0.7	38	-32.3	0.4	60	-30.5
FA (total)	65.6	2504		45.8	2476		12.6	1881	
FA (C ₂₀₋₃₀)	15.8	603		14.1	762		5.0	746	
CPI _{FA} **	4.9			4.6			3.8		

s; standard deviation based on duplicate or triplicate injections (see text).

—; not determined.

*Relatively large uncertainty could be associated with the carbon isotopic compositions of the minor odd-carbon-numbered *n*-fatty acids due to the low concentration during GC/C/IRMS analyses.

**CPI (Carbon Preference Index) value of *n*-fatty acids (CPI_{FA}) is defined as $\text{CPI}_{\text{FA}} = 2\sum(\text{even } C_{20} \text{ to } C_{28}) / (\sum(\text{odd } C_{19} \text{ to } C_{27}) + \sum(\text{odd } C_{21} \text{ to } C_{29}))$.

Table 2. (continued)

b) *n*-alkanes

Sample Carbon number	OR3			OB2			SOB2		
	(ng/g sed.)	($\mu\text{g/org.C}$)	(‰)	(ng/g sed.)	($\mu\text{g/org.C}$)	(‰)	(ng/g sed.)	($\mu\text{g/org.C}$)	(‰)
15									
16	22	0.7	—	37	1.2	-28.3	—	—	—
17	116	3.6	-29.4	72	2.3	-26.8	0.2	—	—
18	54	1.7	-26.4	132	4.3	-27.7	0.3	14	1.5
19	61	1.9	-28.6	72	2.3	-27.9	0.2	9	1.1
20	62	2.0	-28.4	73	2.3	-28.5	0.6	19	2.1
21	86	2.7	-29.7	46	1.5	-28.1	0.8	15	1.7
22	64	2.0	-30.2	72	2.3	-30.1	0.1	31	3.5
23	144	4.5	-30.6	53	1.7	-30.4	0.2	20	2.3
24	85	2.7	-31.8	121	3.9	-31.6	0.6	46	5.2
25	231	7.2	-32.5	62	2.0	-32.7	0.1	29	3.3
26	66	2.1	-31.4	193	6.2	-32.0	0.1	83	9.5
27	402	12.6	-32.7	75	2.4	-31.4	0.4	39	4.4
28	80	2.5	-33.3	472	15.2	-31.9	0.1	201	22.9
29	644	20.2	-33.8	96	3.1	-33.4	0.2	54	6.1
30	77	2.4	-33.9	740	23.9	-32.8	0.0	336	38.2
31	816	25.6	-34.6	81	2.6	-33.9	0.6	48	5.4
32	60	1.9	-35.1	760	24.5	-33.6	0.0	304	34.5
33	268	8.4	-33.0	59	1.9	-33.7	0.1	35	4.0
34	23	0.7	—	279	9.0	-32.9	0.6	123	13.9
35	100	3.1	-29.5	21	0.7	—	—	20	2.3
36	15	0.5	—	102	3.3	-29.3	0.7	55	6.2
37	23	0.7	—	12	0.4	—	—	15	1.6
38	14	0.4	—	20	0.6	—	—	17	1.9
39	14	0.4	—	10	0.3	—	—	14	1.6
40	13	0.4	—	11	0.3	—	—	13	1.5
				9	0.3	—	—	13	1.5
HC (total)	3541	111		3644	118			1552	176
HC (C ₂₃₋₃₃)	2873	90		2939	95			1298	147
CPI _{HC} *	6.1			6.3				4.9	

Table 2. (continued)

Sample Carbon number	SR65			SR68			SR72		
	(ng/g sed.)	($\mu\text{g}/\text{org.C}$)	(‰)	(ng/g sed.)	($\mu\text{g}/\text{org.C}$)	(‰)	(ng/g sed.)	($\mu\text{g}/\text{org.C}$)	(‰)
15	45	1.7	—	—	—	—	—	—	—
16	61	2.3	-26.1	43	2.3	—	3	0.4	—
17	61	2.3	-26.0	33	1.8	—	2	0.2	—
18	36	1.4	-27.1	24	1.3	—	2	0.3	—
19	41	1.5	-29.2	29	1.6	—	2	0.2	—
20	30	1.1	-29.2	20	1.1	—	2	0.2	—
21	52	2.0	-28.0	35	1.9	-29.0	3	0.4	-28.6
22	40	1.5	-29.1	28	1.5	-29.1	3	0.4	-29.1
23	93	3.6	-29.1	66	3.6	-29.1	5	0.7	-29.2
24	55	2.1	-31.5	42	2.2	-30.2	4	0.6	-29.2
25	156	6.0	-30.4	106	5.7	-30.6	8	1.1	-29.6
26	70	2.7	-30.7	48	2.6	-30.6	4	0.6	-30.2
27	349	13.3	-30.9	215	11.6	-30.9	14	2.1	-30.8
28	83	3.2	-31.2	54	2.9	-30.7	4	0.7	-30.7
29	492	18.8	-31.6	300	16.2	-31.2	24	3.5	-31.3
30	108	4.1	-31.5	41	2.2	-31.8	3	0.5	-31.2
31	466	17.8	-31.9	282	15.3	-31.5	26	3.8	-31.5
32	39	1.5	-32.0	21	1.2	-30.0	2	0.3	—
33	159	6.0	-31.4	93	5.0	-29.8	7	1.1	-30.6
34	17	0.6	—	9	0.5	—	1	0.1	—
35	45	1.7	-28.1	26	1.4	-29.2	2	0.2	—
36	0	0.0	—	4	0.2	—	0	0.0	—
37	13	0.5	—	6	0.3	—	—	—	—
38	—	—	—	—	—	—	—	—	—
39	—	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—	—
HC (total)	2464	94	—	1525	82	—	118	18	—
HC (C ₂₃₋₃₃)	2070	79	—	1268	69	—	101	15	—
CPI _{HC} *	4.5	—	—	4.7	—	—	4.3	—	—

s; standard deviation based on duplicate or triplicate injections (see text).

—; not determined.

*CPI (Carbon Preference Index) value of *n*-alkanes (CPI_{HC}) is defined as $\text{CPI}_{\text{HC}} = 2\sum(\text{odd } C_{23} \text{ to } C_{33}) / (\sum(\text{even } C_{22} \text{ to } C_{32}) + \sum(\text{odd } C_{24} \text{ to } C_{34}))$.

trope to $\text{pH} < 1$, the resultant free fatty acids were extracted with *n*-hexane (50 ml and 30 ml) and then *n*-hexane/diethyl ether (9:1 v/v; 2 $\times$ 30 ml). A portion of the combined extracts, which contained monocarboxylic, dicarboxylic and hydroxycarboxylic acids, was treated with *N,O*-bis(trimethylsilyl)acetamide (BSA; 75°C, 1 h) to form the trimethylsilyl (TMS) ether derivatives for GC and GC/MS analyses (as described below). On the basis of GC/MS analyses, *n*-fatty acid-methyl esters in the neutral compound fraction were observed to be less than 1/30 in concentration compared to those in the acid compound fraction. So *n*-fatty acid-methyl esters in the neutral compound fraction are not considered in this study.

Lignin-derived phenolic compounds were also analyzed by cupric oxide oxidation according to Hedges and Parker (1976). Briefly, powdered dry sediment (~100 mg) was mixed with Mohr's salt (45 mg), powdered cupric oxide (500 mg) and aqueous 8% NaOH (6 ml) in a glass tube. The solution was degassed by N_2 -bubbling and sealed under N_2 atmosphere. The reaction was carried out at 170°C in an oven for 3 h. The reaction mixture was acidified with 6M HCl (3 ml) and extracted with ether (3 $\times$ 15 ml). The ether extract was dried with anhydrous Na_2SO_4 . After the solvent was removed, the residue containing phenolic compounds was derivatized with BSA for GC and GC/MS analyses.

Alkane, fatty acid-TMS and lignin-derived phenol-TMS fractions were analyzed using an HP5890 gas chromatograph equipped with an FID and a DB-5 fused silica capillary column (J&W Scientific, 30 m $\times$ 0.32 mm i.d., 0.25 μm liquid phase thickness). Mass spectral characterization was carried out using a Varian 3400 gas chromatograph equipped with a DB-5 capillary column and interfaced with a Finnigan MAT INCOS 50 mass spectrometer. Compound identifications were performed by mass spectral comparison of with those of authentic standards and mass spectral interpretation as well as standard library spectra search using INCOS 50 software.

A separate aliquot of the fatty acid and alkane fractions was prepared for isotope analysis by gas

chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS), according to the procedures reported elsewhere (Naraoka *et al.*, 1994; Yamada *et al.*, 1994). Briefly, the fatty acid fraction was esterified with 14% BF_3/MeOH to convert free fatty acids to their methyl ester derivatives, then subjected to silica gel column chromatography to obtain a monocarboxylic methyl ester fraction. Subsequently, a saturated *n*-fatty acid fraction was isolated using $\text{AgNO}_3/\text{silica gel}$ (10% w/w) column chromatography. The alkane fraction was subjected to $\text{AgNO}_3/\text{silica gel}$ (10% w/w) column chromatography to remove alkenes, and treated with molecular sieve (5A) to isolate *n*-alkanes. Using this technique, *n*-alkanes were separated from isoprenoid alkanes such as lycopane. GC/C/IRMS analysis was conducted using a Finnigan MAT delta S mass spectrometer equipped with a Varian 3400 GC and CuO/Pt microvolume combustion furnace interface (Hayes *et al.*, 1990; Merritt *et al.*, 1995). Ion currents for masses 44, 45 and 46 were recorded every 0.125 sec. The DB-5 GC column was programmed from 50 to 160°C at 30°C/min and from 160 to 310°C at 2°C/min for fatty acids, and from 50 to 120°C at 30°C/min and from 120 to 310°C at 5°C/min for alkanes. Helium was used as carrier gas. The $\delta^{13}\text{C}$ values were calibrated by coinjected three *n*-alkanes ($\text{C}_{18}\text{H}_{38}$, Aldrich®; -27.14‰, $\text{C}_{24}\text{D}_{50}$, MSD ISOTOPES®; -26.09‰ and $\text{C}_{34}\text{H}_{70}$, Aldrich®; -28.54‰) for fatty acids, and *n*- $\text{C}_{24}\text{D}_{50}$ (MSD ISOTOPES®; -26.09‰) for alkanes using ISODAT software. Isotopic compositions of the standard were determined by a conventional method as described above (Boutton *et al.*, 1983). In the case of *n*-fatty acids, $\delta^{13}\text{C}$ values obtained were corrected for the effects of the additional carbon atom from the CH_3OH ($\delta^{13}\text{C} = -37.4\text{‰}$) used in their derivatization, because no isotopic fractionation was observed during the derivatization (Naraoka *et al.*, 1994). Reported carbon isotopic compositions represent averaged values based on duplicate or triplicate injections (Table 2). Standard deviations were generally less than 0.7‰, although minor compounds such as odd-carbon number *n*-fatty acids and even-carbon

number *n*-alkanes have higher standard deviations (Table 2).

RESULTS AND DISCUSSION

TOC contents, C/N ratios and $\delta^{13}\text{C}_{\text{TOC}}$ of the sediments

TOC contents vary from 3.2 wt% (OR3) to 0.7 wt% (SR72) (Table 1), gradually decreasing from riverine to pelagic samples except for SOB2. A relatively low TOC content in SOB2 may be due to a locally high contribution of inorganic components, as suggested by the lithology (sandy-mud). C/N ratios are relatively high in riverine (OR3; 12.4 by atomic) and bay sediments (OB2; 13.4), and gradually decrease to the pelagic sediment (SR72; 8.7) (Table 1). C/N ratios have been often used to estimate the relative proportions of deposited terrestrial and marine organic matter (e.g., Jasper and Gagosian, 1990). The rationale for the use of C/N ratios is that marine organisms usually have C/N ratios in the range of 4 to 8, while terrestrial higher plants have C/N ratios >10 (Muller, 1977).

Bulk isotopic compositions ($\delta^{13}\text{C}_{\text{TOC}}$) exhibit a gradual enrichment in ^{13}C from riverine (OR3; -26.4‰) to pelagic samples (SR72; -20.4‰) (Table 1). Open ocean samples (SR65, SR68 and SR72) are characterized by a relatively uniform isotopic composition ($\sim -20\text{‰}$). The observed isotopic variation is quite consistent with a previous study at the Ohtsuchi watershed (Wada *et al.*, 1987). Bulk terrestrial organic matter ($\sim -26\text{‰}$) is generally more depleted in ^{13}C compared to bulk marine one ($\sim -20\text{‰}$) (Deines, 1980). This is due to a different isotopic fractionation during carbon fixation (Farquhar *et al.*, 1982; Hayes, 1993). An intermediate isotopic composition has been usually interpreted as a mixing of terrestrial and marine organic matter (Sackett and Tompson, 1963; Hunt, 1970; Hedges and Mann, 1979).

The Ohtsuchi River is a small river with a rather low-mass flow ($\sim 8 \times 10^7 \text{ m}^3/\text{yr}$; Wada *et al.*, 1987), and its contribution of terrestrial organic matter to the Pacific Ocean is estimated to be quite small. However, according to many previous

studies (e.g., Sackett, 1989; Jasper and Gagosian, 1990), the C/N (12.4 to 8.7) and $\delta^{13}\text{C}_{\text{TOC}}$ (-26.4 to -20.4‰) variations indicate that the samples used in this study have typical bulk chemical characteristics for a transect of terrestrial to marine sediments. Therefore, the bulk chemical composition is considered to represent a two-endmember mixing between terrestrial and marine organic matter.

Molecular distribution with relevance to the sources and mixing processes

(i) *n*-fatty acid and *n*-alkane Total concentrations of *n*-fatty acids ranging from C_{14} to C_{30} vary between 123 ($\mu\text{g/g}$ dry sediment) (OR3) and 12.6 (SR72) (Table 2). When the concentrations are normalized to organic carbon contents, riverine to coastal samples (OR3 to SOB2) contain relatively high amounts (~ 3.6 to 4.1 mg/g org.C). Whereas open ocean samples (SR65 to SR72) have low amounts (~ 1.9 to 2.5 mg/g org.C). The same feature is applicable for LCFAs ($\geq \text{C}_{20}$); the former group exhibits high (~ 1.2 to 1.7 mg/g org.C) and the latter low (~ 0.60 to 0.76 mg/g org.C) concentrations (Table 2).

The relative abundance of *n*-fatty acids does not show significant differences among the sediments (Fig. 2a, normalized to $n\text{-C}_{16}$), which exhibits two maxima, one at $n\text{-C}_{16}$, and another at $n\text{-C}_{22}$, $n\text{-C}_{24}$ or $n\text{-C}_{26}$. Such a bimodal distribution with an even-carbon number predominance has been widely recognized in various sediments (e.g., Simoneit, 1978). The carbon preference index of LCFAs (CPI_{FA} , Table 2) ranges between 2.3 and 4.9, but shows no systematic lateral change. Although both OR3 and OB2 sediments have slightly lower CPI_{FA} values (3.7 and 3.5) than those of SR65 and SR68 (4.9 and 4.6), they match that of pelagic sample SR72 (3.8).

The amounts of *n*-alkanes range from 3.68 ($\mu\text{g/g}$ dry sediment) (OB2) to 0.12 (SR72) for the $\text{C}_{15}\text{--}\text{C}_{40}$ homologs (Table 2), being about 1–10% in amount of *n*-fatty acid. The relative abundances differ little among the six sediments (Fig. 2b); with $n\text{-C}_{29}$ and $n\text{-C}_{31}$ being the most predominant homologs. The molecular distributions show a strong

Fig. 2. Relative abundance of (a) fatty acids and (b) $n\text{-alkanes}$ in the sediments, where amount of $n\text{-C}_{16}$ fatty acid and $n\text{-C}_{31}$ alkane is normalized to 100, respectively. C_n shows the carbon number of molecules, and $C_{n:1}$ represents monounsaturated fatty acid. The terms, $n\text{-}$, anteiso-, iso-, $\omega\text{OH-}$ and diacid-represent saturated normal-, anteiso-, iso-, ω -hydroxycarboxylic and α,ω -dicarboxylic fatty acid structures, respectively.

odd-carbon number predominance with the carbon preference index for LCALs (CPI_{HC}) varying between 6.3 and 4.3. OR3 and OB2 sediments exhibit higher CPI_{HC} values (6.1 and 6.3) than the rest of the sediments (4.3 to 4.9). Such higher CPI_{HC} values in OR3 and OB2 samples compared to those in open marine sediments may suggest different sources for the $n\text{-alkanes}$.

(ii) *Cutin- and suberin-derived acid, and lignin-derived phenol* Some ω -hydroxycarboxylic- and α,ω -dicarboxylic acids have been reported to derive from cutin and suberin of terrestrial higher plants (e.g., Kolattukudy, 1980). In particular, 18-hydroxyoctadecenoic acid ($\omega\text{OH-C}_{18:1}$) and 18-carboxyloctadecenoic acid (diacid- $\text{C}_{18:1}$) are typical components of cutin and suberin, and have

been previously employed as terrestrial biomarkers (Goni and Hedges, 1990). In this study, ω -hydroxycarboxylic- and α,ω -dicarboxylic acids ranging from C_{14} to C_{26} were also identified in the sediments (Fig. 2a). The concentrations show a marked variation, being most abundant in the OR3 and OB2 sediments, gradually becoming less abundant from SOB2 to SR68 sediment and quite small in the SR72 sediment (Fig. 2a).

Lignin-derived compounds, such as vanillyl (V) and syringyl (S) phenols with aldehyde, ketone and carboxylic acid functional groups, have been also used as terrestrial biomarkers (e.g., Hedges and Parker, 1976; Goni and Hedges, 1990; Opsahl and Benner, 1997), because they occur exclusively in vascular land plants. The total concentrations of these compounds are relatively high in the riverine (28.6 mg/g org.C, OR3) and bay sediments (31.9 mg/g org.C, OB2), and gradually decrease seawards (Table 1). In the SR72 pelagic sediment, the concentration of lignin-derived compounds is extremely low (0.3 mg/g org.C). The molecular abundance is similar between cutin- and lignin-derived compounds, but different from that of LCFAs and LCALs.

Figure 3a shows relationships of the molecular abundance between lignin-derived phenols (S + V) and LCFAs (n - C_{26} and n - C_{30}) including ω OH- $C_{18:1}$ acid. The cutin-derived ω OH- $C_{18:1}$ acid is quantitatively correlated with lignin-derived phenols (correlation coefficient $r^2 = 0.99$) where the y-intercept is nearly 0. On the other hand, concentrations of n - C_{26} and n - C_{30} components do not exhibit good correlations with those of lignin-derived phenols. In addition, the y-intercepts of n - C_{26} and n - C_{30} in the fatty acid-lignin phenol plots are positive and dependent on molecular species, with the y-intercept of n - C_{30} being closer to 0. In the case of LCALs, while the content of n - C_{35} gradually decreases seaward, and correlates in concentration with lignin-derived phenols (V + S) ($r^2 = 0.50$) with the y-intercept ~ 0 , the most abundant LCALs (n - C_{29} and n - C_{31}) show an abrupt decrease in concentration at SR72 sediment (Fig. 3b).

Fig. 3. Relationships of molecular abundance between (a) lignin-derived phenolic compounds (S + V) and LCFAs (n - C_{26} and n - C_{30}) including cutin-derived acid (ω OH- $C_{18:1}$), and (b) S + V and LCALs (n - C_{29} , n - C_{31} and n - C_{35}).

The positive y-intercept might suggest that LCFAs are more resistant to degradation or that a different transport process could have resulted in the fatty acids being preferentially transported seawards compared to cutin- and lignin-derived compounds. However, cutin- and lignin-derived compounds seem unlikely to be preferentially degraded compared to LCFAs and LCALs, because these compounds are bound to higher-molecular weight and inert structures (de Leeuw and Largeau, 1993). In addition, different molecular signatures for the same molecular homologs (i.e., n - C_{26} and n - C_{30} for LCFAs, n - C_{29} and n - C_{35} for LCALs, and

the different positive y-intercepts) may indicate different sources for some LCFAs and LCALs. The use of the molecular distributions of *n*-fatty acids and *n*-alkanes alone, in this study, is deemed insufficient to clarify the sources and mixing processes.

Carbon isotopic compositions of individual LCFAs and LCALs

(i) *LCFAs* The carbon isotopic compositions of the even numbered LCFAs exhibited large variation from -35 to -25‰ (Table 2a and Fig. 4a). All LCFAs are more depleted in ^{13}C than TOC by

ca. 5 to 12‰, a result consistent with previous studies of lipid fractions (Degens, 1969) and fatty acids (Parker, 1962, 1964). This is attributed to isotope fractionation during their biosynthesis (DeNiro and Epstein, 1977). In the OR3 and SR65 sediments, shorter-chain *n*-fatty acids such as *n*-C₁₆ are more enriched in ^{13}C than TOC by a few per mil (Table 2). This may be due to the isotopic enrichment during dietary processes by heterotrophs (DeNiro and Epstein, 1978).

Each LCFA becomes gradually enriched in ^{13}C from riverine to open ocean by *ca.* 3 to 6‰, but remains relatively uniform in the open ocean (Fig.

Fig. 4. Carbon isotope distributions of individual long-chain (a) *n*-fatty acids (even carbon-numbered ranging from C₂₀ to C₃₀) and (b) *n*-alkanes (odd carbon-numbered ranging from C₁₉ to C₃₅) in the sediments. Standard deviations are shown in Table 2.

5). For example, *n*-C₂₆ gradually becomes enriched in ¹³C from riverine (−32.5‰; OR3) to open ocean (−26.3‰; SR65) by *ca.* 6‰, but varies little between open ocean sediments (−26.6‰ in SR68; −25.7‰ in SR72). A similar trend is observed for all LCFAs. However, the extent of enrichment in ¹³C for *n*-C₃₀ from riverine to pelagic is only about 3‰ (Fig. 5).

In each sediment, isotopic compositions are relatively similar between *n*-C₂₀ and *n*-C₂₆, then show a gradual depletion in ¹³C with increasing molecular weight to *n*-C₃₀ by *ca.* 3 to 5‰ (Fig. 4a). For example, the $\delta^{13}\text{C}$ values in OR3 are relatively uniform (−31.9 to −32.5‰) between *n*-C₂₀ and *n*-C₂₆, but decrease to −33.5‰ for *n*-C₂₈ and −35.2‰ for *n*-C₃₀. In spite of various location, the relatively similar pattern may be due a fundamental isotopic regulation for LCFA biosynthesis (i.e., elongation) (Kolattukudy, 1976; Walker and Harwood, 1986).

Carbon isotopic differences between each LCFA and TOC ($\Delta_{\text{LCFA-TOC}}$) vary from 5 to 8‰ (*n*-C₂₀ to *n*-C₂₆), 7 to 9‰ (*n*-C₂₈), and 9 to 12‰ (*n*-C₃₀) for a series of sediments (Fig. 6a). The relatively uniform $\Delta_{\text{LCFA-TOC}}$ value for each LCFA in the sediment sequence would indicate that

$\delta^{13}\text{C}_{\text{LCFA}}$ values may be related to the $\delta^{13}\text{C}_{\text{TOC}}$ values. A minor but distinctive feature is that the isotopic difference between *n*-C₂₆ and *n*-C₃₀ is somewhat smaller in riverine (2.7‰) and bay (4.3‰) sediment than in open ocean sediments (ave. 5.5‰). Such isotopic difference suggests a different proportion of contribution among the LCFAs. The different distribution between *n*-C₂₆ and *n*-C₃₀ is consistent with the molecular abundance (Fig. 3a). The relative contribution of the more ¹³C-depleted *n*-C₃₀ is probably higher in open ocean sediment than it is in riverine sediments.

(ii) *LCALs* The carbon isotopic compositions of the odd numbered *n*-alkanes from C₁₉ to C₃₁ also vary widely from −35‰ to −27‰ (Table 2b and Fig. 4b), being more depleted in ¹³C than TOC by *ca.* 6 to 11‰, a similar extent of depletion as observed in LCFAs. Their pattern of isotopic distribution, however, is different from that of LCFAs. The isotopic compositions are continuously depleted in ¹³C from *n*-C₁₉ to *n*-C₃₁ by 3 to 6‰, and then gradually more enriched in ¹³C from *n*-C₃₃ to *n*-C₃₅. For example, in the river sediment (OR3) the $\delta^{13}\text{C}$ values decrease from −28.6‰ (*n*-C₁₉) to −34.6‰ (*n*-C₃₁) by 6‰, and then to −33.0‰ (*n*-C₃₃) and −29.5‰ (*n*-C₃₅). In open marine sediments, however, the carbon isotopic difference between *n*-C₂₁ and *n*-C₃₁ is only *ca.* 3‰ (Table 2b and Fig. 4b). Such a similar decrease of ~3.5‰ between *n*-C₂₁ and *n*-C₃₁ was previously reported from a marine sediment (Bakel *et al.*, 1994).

The most abundant *n*-alkanes (C₂₉ and C₃₁) show a systematic isotopic change from riverine to pelagic samples that is similar to that of LCFAs. For example, the $\delta^{13}\text{C}$ value of *n*-C₃₁ alkane increases from −34.6‰ (OR3) to −31.9‰ (SR65), and becomes relatively uniform (~−31.5‰) in open marine sediments (Fig. 5). However, the isotopic difference of *n*-C₂₉ and *n*-C₃₁ alkanes between riverine and pelagic sediment (~3‰) is smaller than that of C₂₆ *n*-fatty acid (~6‰), being rather similar to that of C₃₀ *n*-fatty acid (~4‰).

Using $\Delta_{\text{LCAL-TOC}}$ values, the most predominant

Fig. 5. Carbon isotope distributions of *n*-C₂₆ and *n*-C₃₀ fatty acids, and *n*-C₂₉ and *n*-C₃₁ alkanes in the sediments from riverine to pelagic. Approximate distance of sediment location from the Honshu Island is shown in Table 1.

Fig. 6. Carbon isotope difference between the $\delta^{13}\text{C}$ values of TOC and (a) *n*-fatty acids ($\Delta_{\text{LCFA-TOC}}$), and (b) *n*-alkanes ($\Delta_{\text{LCAL-TOC}}$) in the sediments.

n-C₂₉ and *n*-C₃₁ alkanes may be classified into two groups corresponding to sediment location. In riverine and bay sediments the values are higher by 3 to 4‰ than those in the open marine sediments (Fig. 6b). This observed discrepancy is consistent with CPI_{HC} values (~6.2 for riverine and bay; ~4.5 for open ocean) (see above). On the other hand, isotopic compositions of *n*-C₃₅ are similar ($-29.0 \pm 1.0\text{‰}$) for all sediments (Table 2). The isotopic difference between *n*-C₃₅ and abundant alkanes (such as *n*-C₂₉ and *n*-C₃₁) implies distinct sources. The isotopically uniform C₃₅ *n*-alkane may be derived from a common source of land plant, because the content gradually de-

creased seaward.

(iii) $\delta^{13}\text{C}$ relationship between LCFAs and LCALs
Abundant LCFAs such as *n*-C₂₆ and *n*-C₂₈ are well correlated isotopically with abundant LCALs such as *n*-C₂₉ and *n*-C₃₁ ($r^2 > 0.96$) in this study. Normal alkanes (*C_{n-1}*) are proposed to originate from the corresponding *n*-fatty acids (*C_n*) by biogenic decarboxylation (e.g., Wettstein-Knowles, 1979), and so they are expected to correlate isotopically. Carbon isotopic compositions of C₂₆, C₂₈ and C₃₀ *n*-fatty acid correlate well with those of C₂₅ ($r^2 = 0.86$), C₂₇ ($r^2 = 0.89$) and C₂₉ ($r^2 = 0.80$) *n*-alkane, respectively. The regression lines have a slope less than 1, and cross the 1:1 ($y = x$) line at

~-32‰ (Fig. 7). Obviously, *n*-fatty acids with $\delta^{13}\text{C}$ values lower than -32‰ are more depleted in ^{13}C than the corresponding *n*-alkanes, whereas *n*-fatty acids with $\delta^{13}\text{C}$ values higher than -32‰ are enriched in ^{13}C than the corresponding *n*-alkanes. This implies an inconsistent isotopic behavior between the LCFAs and LCALs in spite of the same molecular homologs.

Isotopic differences between *n*-fatty acid and the corresponding *n*-alkane ($\Delta_{n\text{FA}-(n-1)\text{AL}}$) in the riverine sediment (OR3) vary from -3.8 to 0‰. In the pelagic sediment (SR72), the $\Delta_{n\text{FA}-(n-1)\text{AL}}$ values are positive (+0.8 to +3.9‰). This difference might be due to a different extent of isotopic fractionation during decarboxylation between terrestrial and marine fatty acids. Given that the carboxyl carbon of the higher plant-derived saturated *n*-fatty acids (C_{14} and C_{16}) is usually ^{13}C -depleted by a few per mil compared with other carbons (Vogler and Hayes, 1980), the resultant *n*-alkane is expected to be isotopically heavier than the corresponding *n*-fatty acid. On the other hand, the carboxyl carbon in algal fatty acids is more ^{13}C -enriched than the molecular average (Vogler and Hayes, 1980). However, biologically formed

carboxyl groups of *n*- C_{14} and *n*- C_{16} fatty acids are isotopically different to the molecular average only within 10‰ (Vogler and Hayes, 1980; Monson and Hayes, 1982). So, decarboxylation of fatty acids could contribute to a change in ^{13}C less than a few per mil for the whole molecule. The isotopic relationship between LCFAs and LCALs could not be explained only by the decarboxylation. Some additional ^{13}C -depleted LCAL input compared to LCFA is therefore plausible from a different origin (i.e., probably terrigenous) for the open marine sediments.

Geochemical implications of the occurrence of LCFAs and LCALs in marine sediments

(i) A mixing model of two endmember components

The isotopic distributions of LCFAs and LCALs can be explained by a mixing model between terrestrial and marine endmember. The mixing model is supported by various relationships between the compound-specific isotope ratio and terrestrial biomarkers as well as bulk chemical characteristics. For example, the $\delta^{13}\text{C}$ values of *n*- C_{26} show a strong correlation with the concentrations of $\omega\text{OH-C}_{18:1}$ (Fig. 8a) and lignin-derived compounds (Fig. 8b). In addition, the good correlation for $\delta^{13}\text{C}$ values between TOC and *n*- C_{26} ($r^2 = 0.96$, Fig. 9a) suggests that the LCFAs are derived from isotopically different carbon sources, and/or mixing of terrestrial and marine in origin as endmember components. The $\delta^{13}\text{C}$ value of *n*- C_{26} correlates with the C/N ratios ($r^2 = 0.70$, Fig. 9b).

If the $\delta^{13}\text{C}$ values of riverine (OR3) and pelagic (SR72) sediments are presumed to represent simple terrestrial and marine endmembers respectively, a binary mixing model can express the $\delta^{13}\text{C}$ value in locations ($\delta_{\text{meas.}}$) with varying contributions from these two sources using the following equation:

$$\delta_{\text{meas.}} = f \cdot \delta_{\text{t}} + (1 - f) \cdot \delta_{\text{m}}$$

where, δ_{t} and δ_{m} represent the $\delta^{13}\text{C}$ values of terrestrial and marine endmembers, respectively. The term f reflects the ratio of terrestrial to marine contribution for the total LCFA or LCAL. When

Fig. 7. Relationships of carbon isotope ratios between *n*- C_{26} , *n*- C_{28} and *n*- C_{30} fatty acid and *n*- C_{25} , *n*- C_{27} and *n*- C_{29} alkane, respectively, in all analyzed sediments. Regression lines have a slope less than 1, and cross the 1:1 ($y = x$) line at ~-32‰ (see text).

Fig. 8. Relationship between the $\delta^{13}\text{C}$ values of $n\text{-C}_{26}$ fatty acid and (a) content of 18-hydroxyoctadecenoic acid ($\omega\text{OH-C}_{18:1}$) which may be derived from cutin, and (b) total contents of lignin-derived compounds ($S + V$).

this equation is applied to C_{26} n -fatty acid and C_{31} n -alkane, f values vary from 0.74 and 0.68 at OB2, 0.44 and 0.48 at SOB2, 0.09 and 0.13 at SR65, to 0.13 and 0.00 at SR68, respectively. Similar f values are obtained at each location using other abundant LCFA and LCAL. These f values are also in accordance with those calculated by means of isotopic compositions of TOC (0.85 at OB2, 0.30 at SOB2, 0.07 at SR65, and 0.03 at SR68). Using the f values, the terrestrial-derived concentrations in sediments can be calculated for each LCFA and LCAL. The corrected concentrations have better correlations ($r^2 > 0.76$) with those of lignin-derived compounds (Fig. 10) than the

uncorrected concentrations have (Fig. 3).

(ii) *Isotopic effects by biological reworking and early diagenesis* In order to characterize sources and mixing process, sedimentary LCFAs and LCALs must preserve their original isotopic signature during transportation and deposition. The isotopic modification by biological reworking and early diagenesis is poorly known. LCFAs, unlike other common fatty acids such as $n\text{-C}_{14}$, $n\text{-C}_{16}$ and $n\text{-C}_{18}$, are not digested well by organisms (Nishimura and Baker, 1987). Similarly, LCALs such as $n\text{-C}_{29}$ and $n\text{-C}_{31}$ do not seem to be readily metabolized. In petroleum samples, microbial degradation of alkanes has been often reported,

Fig. 9. Relationships between the $\delta^{13}\text{C}$ values of $n\text{-C}_{26}$ fatty acid and (a) those of TOC, and (b) C/N ratios in the sediments.

which is characterized by unresolved complex mixture (UCM) on a gas chromatogram of hydrocarbon fraction (Hunt, 1995). The samples used in this study, however, are recent sediments, and have no significant UCM on the chromatogram. Such microbial degradation must not have to be considered in this study.

Even when some organisms could utilize these compounds, it is unlikely that they would discriminate against isotopically light or heavy LCFAs and LCALs during their dietary processes. Furthermore, diagenesis involving decarboxylation of fatty acids may not result in significant changes in the carbon isotopic composition of the molecule as mentioned above, even if the reaction is followed by oxidation to produce other shorter chain

Fig. 10. Relationship in concentration between the calculated terrestrial LCFAs and LCALs, and lignin-derived compounds (S + V) (see text).

fatty acids. These considerations may indicate that LCFAs and LCALs in sediments are likely to retain their original isotopic composition, despite biological reworking and early diagenesis.

(iii) *LCFAs and LCALs as terrestrial biomarkers*
To date, the occurrence of predominantly even-numbered *n*-fatty acids $>\text{C}_{20}$ and odd-numbered *n*-alkanes $>\text{C}_{23}$ has been suggested to reflect contributions from allochthonous higher plant waxes (e.g., Simoneit, 1978; Peters and Moldowan, 1993; Madureira *et al.*, 1997). In this study, LCFAs and LCALs are more abundant in river and bay sediments than in open marine sediments (Table 2). In spite of the similar high CPI values, however, the isotopic compositions exhibit a lot of varia-

tion (up to 6‰) from river to open ocean, thereby dismissing a similar source in the series of sediments.

The major contribution of the carbon source may be representatively reflected by the $\delta^{13}\text{C}_{\text{TOC}}$ values (Hedges *et al.*, 1997). Since the $\delta^{13}\text{C}_{\text{TOC}}$ distributions in this study are consistent with normal values reported thus far for terrestrial to marine sediments (Sackett and Tompson, 1963; Hunt, 1970), and because the LCFAs and LCALs are related isotopically to TOC, the LCFAs and LCALs are not likely to be contributed from peculiar organisms, for example, chemotrophic bacteria (CoBabe and Pratt, 1995).

It is known that the lipid fraction and individual lipid components are more ^{13}C -depleted by several per mil relative to the bulk organic matter (e.g., Degens, 1969; DeNiro and Epstein, 1977). However, the $\delta^{13}\text{C}$ values of C_{20} – C_{26} *n*-fatty acids in open marine sediments of this study range from –27 to –26‰, being similar to the average bulk isotopic composition of terrestrial C_3 plants (Deines, 1980). Such a lack of isotopic fractionation between C_{20} – C_{26} *n*-fatty acids in the marine sediments and the bulk composition of putative terrestrial C_3 higher plants may indicate that the LCFAs of this study could have been derived from sources other than terrestrial C_3 higher plants. On the other hand, minor odd-numbered *n*-fatty acids are more depleted in ^{13}C than the adjacent even-numbered *n*-fatty acids by up to several per mil in open marine sediments (Table 2). The $\delta^{13}\text{C}$ values of odd-numbered *n*-fatty acids (–34.5 to –29‰) may be consistent with the putative terrestrial C_3 higher plants.

Some recent studies reveal that the $\delta^{13}\text{C}$ values of C_{27} – C_{33} *n*-alkanes from C_4 plant leaves range from –25 to –18‰, more enriched by 12‰ than C_3 plant-derived *n*-alkanes (Collister *et al.*, 1994; Naraoka *et al.*, 1997). Therefore, although C_4 plant contribution or hydrodynamic segregation from C_4 -derived to C_3 -derived compounds during transport (Goni *et al.*, 1997) could be considered in this study, extensive vegetation of terrestrial C_4 plants is not observed along the Japanese Islands as well as in the Ohtsuchi watershed. This obser-

vation is also supported by the isotope ratios of various Ohtsuchi River sediments (~–26‰; this study and Wada *et al.*, 1987). Wada *et al.* (1987) reported an occurrence of C_4 -based marine seagrass and considered its contribution to sediments in the Ohtsuchi Bay. However, the contribution is small and local; the sediment signature can be explained by a terrestrial-marine mixing process based on a bulk C and N isotope study.

In the open marine environment, especially in pelagic sediments, eolian transport of terrestrial organic matter may be important (Simoneit, 1977b, 1997; Gagosian *et al.*, 1981; Brassell, 1993). The carbon isotope distribution of bulk organic matter of aerosol was studied at a site of the western Pacific (Cachier *et al.*, 1986). The isotope ratios (~–26‰) suggested that terrestrially-derived organic matter originated from C_3 plants, which is inconsistent with the isotopic signature of this study. Furthermore, both LCFAs and LCALs become more depleted in ^{13}C in bay and coastal environments, where eolian transport is not important compared to riverine input. The implication is that all the LCFAs and LCALs in the marine sediments cannot be derived from terrestrial higher plants.

(iv) *Marine LCFAs and LCALs in origin* The suggestion of LCFAs as marine in origin may be supported by previous studies. Volkman *et al.* (1980) reported a resemblance between the molecular distribution of LCFAs in an intertidal sediment and a diatom culture. LCFAs were also found in a diatomaceous ooze with the preference of even carbon-numbered *n*-fatty acids (Boon *et al.*, 1975). Because the SR72 sediment is a siliceous ooze composed mainly of diatom and radiolaria, diatoms are probably one of the candidates for the source of the LCFAs in the SR72 sediment. In the bay and coastal environments, LCFAs may have another sources such as seagrass in addition to diatom, because some seagrass species were found to contain even-carbon predominant *n*-fatty acids (Khotimchenko, 1993).

In addition to LCFAs, LCALs such as *n*- C_{29} and *n*- C_{31} also seem to have terrestrial and marine isotopic endmember components, although

some LCALs of terrigenous sources could be recognized in open marine sediments based on the observation that the LCALs are more depleted in ^{13}C than the corresponding LCFAs (Fig. 7). McCaffrey *et al.* (1991) suggested that sedimentary *n*-alkanes (C_{25} to C_{33}) of Peru margin represent mixing of a marine and terrestrial endmember by a detailed CPI study. In addition, Lichtfouse *et al.* (1994) suggested a possible algal origin for odd-carbon predominant LCALs, produced by the reduction of corresponding *n*-alkadiene (C_{29} and C_{31}) in immature marine sediments by a molecular and isotopic study. Freeman *et al.* (1994) also reported C_{29} and C_{31} *n*-alkanes of marine origin in the water column of the Black Sea based on molecular and isotope distributions.

CONCLUSIONS

Stable carbon isotopic compositions of individual LCFAs and LCALs show a systematic enrichment in ^{13}C in a series of sediments from the Ohtsuchi River to Ohtsuchi Bay and then the Pacific Ocean. The $\delta^{13}\text{C}$ values of LCFAs and LCALs gradually increased from riverine to open marine sediment by up to ca. 6‰ and 3‰, respectively. Those of open marine sediments are isotopically similar. The isotope ratios of abundant LCFAs such as *n*- C_{26} and *n*- C_{28} are positively well correlated with those of the abundant LCALs such as *n*- C_{29} and *n*- C_{31} ($r^2 > 0.96$). These isotope ratios also have good correlations with those of $\delta^{13}\text{C}_{\text{TOC}}$ ($r^2 = 0.96$), C/N ratios ($r^2 = 0.70$), and the amount of sedimentary cutin- and lignin-derived compounds ($r^2 > 0.88$) of terrestrial higher plant origin. The isotopic variations infer a two-endmember mixing of LCFAs and LCALs between terrigenous and marine origins.

Because LCFAs and LCALs are more abundant in river and bay sediments than in open marine sediments, they should represent important terrestrial biomarkers. The molecular abundances and isotope signatures revealed in this study, however, indicate that LCFAs and LCALs in the marine sediments could have had multiple origins besides from terrestrial higher plants. Instead, the

extent of contribution of LCFAs and LCALs of marine organisms in origin may be much more significant than previously envisaged. Therefore, a caution may be needed when interpreting the source of LCFAs and LCALs as a terrestrial biomarker in marine environments. On the other hand, C_{35} *n*-alkane has a similar isotopic composition in a series of sediments, and hence may be an appropriate terrigenous indicator in this study. Compound-specific isotope analysis of sedimentary LCFAs and LCALs should provide useful information with respect to source identification and understanding of mixing processes.

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REFERENCES

- Bakel, A. J., Ostrom, P. H. and Ostrom, N. E. (1994) Carbon isotopic analysis of individual *n*-alkanes: evaluation of accuracy and application to marine particulate organic material. *Org. Geochem.* **21**, 595–602.
- Boon, J. J., de Leeuw, J. W. and Schenck, P. A. (1975) Organic geochemistry of Walvis Bay diatomaceous ooze—I. Occurrence and significance of the fatty acids. *Geochim. Cosmochim. Acta* **39**, 1559–1565.
- Boutton, T. W., Wong, W. W., Hachey, D. L., Lee, L. S., Cabrera, M. P. and Klein, P. D. (1983) Comparison of quartz and Pyrex tubes for combustion of organic samples for stable carbon isotope analysis. *Anal. Chem.* **55**, 1832–1833.
- Brassell, S. C. (1993) Applications of biomarkers for delineating marine paleoclimatic fluctuations during the Pleistocene. *Organic Geochemistry: Principles and Applications* (Engel, M. H. and Macko, S. A., eds.), 705–738, Plenum Press, New York.
- Cachier, H., Buat-Menard, P., Fontugne, M. and Chesselet, R. (1986) Long-range transport of conti-

- nentally-derived particulate carbon in the marine atmosphere: evidence from stable carbon isotope studies. *Tellus* **38B**, 161–177.
- CoBabe, E. A. and Pratt, L. M. (1995) Molecular and isotopic compositions of lipids in bivalve shells: A new prospect for molecular paleontology. *Geochim. Cosmochim. Acta* **59**, 87–95.
- Collister, J. W., Rieley, G., Stern, B., Eglinton, G. and Fry, B. (1994) Compound-specific $\delta^{13}\text{C}$ analyses of leaf lipids from plants with differing carbon dioxide metabolisms. *Org. Geochem.* **21**, 619–627.
- Degens, E. T. (1969) Biogeochemistry of stable carbon isotopes. *Organic Geochemistry; Methods and Results* (Eglinton, G. and Murphy, M. T. J., eds.), 304–329, Springer-Verlag, Berlin, Heidelberg.
- Deines, P. (1980) The isotopic composition of reduced organic carbon. *Handbook of Environmental Isotope Geochemistry* (Fritz, P. and Fontes, J. Ch., eds.), 329–406, Elsevier, Amsterdam.
- de Leeuw, J. W. and Largeau, C. (1993) A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal, and petroleum formation. *Organic Geochemistry; Principles and Applications* (Engel, M. H. and Macko, S. A., eds.), 23–72, Plenum Press, New York.
- DeNiro, M. J. and Epstein, S. (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **197**, 261–263.
- DeNiro, M. J. and Epstein, S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **42**, 495–506.
- Farquhar, G. D., O'Leary, M. H. and Berry, J. A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* **9**, 121–137.
- Freeman, K. H., Wakeham, S. G. and Hayes, J. M. (1994) Predictive isotopic biogeochemistry: Hydrocarbons from anoxic marine basins. *Org. Geochem.* **21**, 629–644.
- Gagosian, R. B., Peltzer, E. T. and Zafiriou, O. C. (1981) Atmospheric transport of continentally derived lipids to the tropical North Pacific. *Nature* **291**, 312–314.
- Goni, M. A. and Hedges, J. I. (1990) The diagenetic behavior of cutin acids in buried conifer needles and sediments from a coastal marine environment. *Geochim. Cosmochim. Acta* **54**, 3073–3081.
- Goni, M. A., Ruttenberg, K. C. and Eglinton, T. I. (1997) Sources and contribution of terrigenous organic carbon to surface sediments in the Gulf of Mexico. *Nature* **389**, 275–278.
- Hayes, J. M. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: principles and evidence. *Mar. Geol.* **113**, 111–125.
- Hayes, J. M., Freeman, K. H., Popp, B. N. and Hoham, C. H. (1990) Compound-specific isotope analyses: A novel tool for reconstruction of ancient biogeochemical processes. *Org. Geochem.* **16**, 1115–1128.
- Hedges, J. I. and Mann, D. C. (1979) The lignin geochemistry of marine sediments from the southern Washington coast. *Geochim. Cosmochim. Acta* **43**, 1809–1818.
- Hedges, J. I. and Parker, P. L. (1976) Land-derived organic matter in surface sediments from the Gulf of Mexico. *Geochim. Cosmochim. Acta* **40**, 1019–1029.
- Hedges, J. I., Keil, R. G. and Benner, R. (1997) What happens to terrestrial organic matter in the ocean? *Org. Geochem.* **27**, 195–212.
- Hunt, J. M. (1970) The significance of carbon isotope variations in marine sediment. *Advances in Organic Geochemistry, 1966* (Hobson, G. O. and Speers, G. C., eds.), 27–35, Pergamon, London.
- Hunt, J. M. (1995) *Petroleum Geochemistry and Geology*. 2nd ed., W. H. Freeman and Company, New York.
- Jasper, J. P. and Gagosian, R. B. (1990) The sources and deposition of organic matter in the Late Quaternary Pigmy Basin, Gulf of Mexico. *Geochim. Cosmochim. Acta* **54**, 1117–1132.
- Jasper, J. P. and Hayes, J. M. (1990) A carbon isotope record of CO_2 levels during the late Quaternary. *Nature* **347**, 462–464.
- Khotimchenko, S. V. (1993) Fatty acids and polar lipids of seagrasses from the sea of Japan. *Phytochemistry* **33**, 369–372.
- Kolattukudy, P. E. (ed.) (1976) *Chemistry and Biochemistry of Natural Waxes*. Elsevier, Amsterdam, 439 pp.
- Kolattukudy, P. E. (1980) Cutin, Suberin, and Waxes. *The Biochemistry of Plants, Vol. 4* (Stumpf, P. E. and Conn, E. E., eds.), 571–645, Academic Press, London.
- Lichtfouse, E., Derenne, S., Mariotti, A. and Largeau, C. (1994) Possible algal origin of long chain odd *n*-alkanes in immature sediments as revealed by distributions and carbon isotope ratios. *Org. Geochem.* **22**, 1023–1027.
- Madureira, L. A. S., van Kreveld, S. A., Eglinton, G., Conte, M. H., Ganssen, G., van Hinte, J. E. and Ottens, J. J. (1997) Late Quaternary high-resolution biomarker and other sedimentary climate proxies in a northeast Atlantic core. *Paleoceanogr.* **12**, 255–269.
- McCaffrey, M. A., Farrington, J. W. and Repeta, D. J. (1991) The organic geochemistry of Peru margin surface sediments: II. Paleoenvironmental implications of hydrocarbon and alcohol profiles. *Geochim. Cosmochim. Acta* **55**, 483–498.

- Merritt, D. A., Freeman, K. H., Ricci, M. P., Studley, S. A. and Hayes, J. M. (1995) Performance and optimization of a combustion interface for isotope ratio monitoring gas chromatography/mass spectrometry. *Anal. Chem.* **67**, 2461–2473.
- Monson, K. D. and Hayes, J. M. (1982) Carbon isotopic fractionation in the biosynthesis of bacterial fatty acids. Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. *Geochim. Cosmochim. Acta* **46**, 139–149.
- Muller, P. J. (1977) C/N ratios in Pacific deep-sea sediments: Effect of inorganic ammonium and organic nitrogen compounds sorbed by clays. *Geochim. Cosmochim. Acta* **41**, 765–776.
- Naraoka, H., Yamada, K. and Ishiwatari, R. (1994) Stable carbon isotope measurement of individual fatty acids using gas chromatography/isotope ratio monitoring mass spectrometry. *J. Mass Spectrom. Soc. Jpn.* **42**, 315–323.
- Naraoka, H., Yamada, K. and Ishiwatari, R. (1995) Carbon isotopic difference of saturated long-chain *n*-fatty acids between a terrestrial and a marine sediment. *Geochem. J.* **29**, 189–195.
- Naraoka, H., Yamada, K., Matsumoto, K. and Ishiwatari, R. (1997) Compound-specific isotope analysis of light elements using gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) and its application to geochemistry. *Chikyukagaku (Geochemistry)* **31**, 193–210 (in Japanese with an English abstract).
- Nishimura, M. and Baker, E. W. (1987) Compositional similarities of non-solvent extractable fatty acids from recent marine sediments deposited in differing environments. *Geochim. Cosmochim. Acta* **51**, 1365–1378.
- Opsahl, S. and Benner, R. (1997) Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature* **386**, 480–482.
- Parker, P. L. (1962) The isotopic composition of the carbon of fatty acids. *Carnegie Inst. Washington Yearbook* **61**, 187–190.
- Parker, P. L. (1964) The biogeochemistry of the stable isotopes of carbon in a marine bay. *Geochim. Cosmochim. Acta* **28**, 1155–1164.
- Peters, K. E. and Moldowan, J. M. (1993) *The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments*. Prentice-Hall, New Jersey.
- Popp, B. N., Takigiku, R., Hayes, J. M., Louda, J. W. and Baker, E. W. (1989) The post-Paleozoic chronology and mechanism of ^{13}C depletion in primary marine organic matter. *Amer. J. Sci.* **289**, 436–454.
- Prahl, F. G., Muehlhausen, L. A. and Lyle, M. (1989) An organic geochemical assessment of oceanographic conditions at MANOP site C over the past 26,000 years. *Paleoceanogr.* **4**, 495–510.
- Sackett, W. M. (1989) Stable carbon isotope studies on organic matter in the marine environment. *Handbook of Environmental Isotope Geochemistry Vol. 3* (Fritz, P. and Fontes, J. Ch., eds.), 139–170, Elsevier, New York.
- Sackett, W. M. and Tompson, R. R. (1963) Isotopical organic carbon composition of recent continental derived clastic sediments of the eastern Gulf of Mexico. *Am. Assoc. Pet. Geol. Bull.* **47**, 525–528.
- Simoneit, B. R. T. (1977a) The Black Sea, a sink for terrigenous lipids. *Deep-Sea Res.* **24**, 813–830.
- Simoneit, B. R. T. (1977b) Organic matter in eolian dusts over the Atlantic Ocean. *Mar. Chem.* **5**, 443–464.
- Simoneit, B. R. T. (1978) The organic chemistry of marine sediments. *Chemical Oceanography Vol. 7* (Riley, J. P. and Chester, R., eds.), 233–311, Academic Press, London.
- Simoneit, B. R. T. (1997) Compound-specific carbon isotope analyses of individual long-chain alkanes and alkanolic acids in Harmattan aerosols. *Atm. Env.* **31**, 2225–2233.
- Vogler, E. A. and Hayes, J. M. (1980) Carbon isotopic compositions of carboxyl groups of biosynthesized fatty acids. *Advances in Organic Geochemistry 1979* (Douglas, A. G. and Maxwell, J. R., eds.), 697–704, Pergamon, London.
- Volkman, J. K., Johns, R. B., Gillan, F. T., Perry, G. J. and Bavor, H. J., Jr. (1980) Microbial lipids of an intertidal sediment—I. Fatty acids and hydrocarbons. *Geochim. Cosmochim. Acta* **44**, 1133–1143.
- Wada, E., Minagawa, M., Mizutani, H., Tsuji, T., Imaizumi, R. and Karasawa, K. (1987) Biogeochemical studies on the transport of organic matter along the Otsuchi River watershed, Japan. *Estuar. Coast. Shelf Sci.* **25**, 321–336.
- Walker, K. A. and Harwood, J. L. (1986) Evidence for separate elongation enzymes for very-long-chain-fatty-acid synthesis in potato (*Solanum tuberosum*). *Biochem. J.* **237**, 41–46.
- Wettstein-Knowles, P. von (1979) Genetics and biosynthesis of plant epicuticular waxes. *Advances in the Biochemistry and Physiology of Plant Lipids* (Appelqvist, L.-A. and Liljenberg, C., eds.), 1–26, Elsevier, Amsterdam.
- Yamada, K., Uzaki, M., Kon, M., Naraoka, H. and Ishiwatari, R. (1994) Molecular sieve isolation technique for use in stable carbon isotope analysis of individual long-chain *n*-alkanes in crude oil. *J. Mass Spectrom. Soc. Jpn.* **42**, 237–246 (in Japanese with an English abstract).