

Carbon and hydrogen isotopic compositions of sterols from riverine and marine sediments

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Abstract

The sources (marine algae, terrestrial C3 and C4 plants) of sterols deposited to sediments along a riverine–marine transect from Ohtsuchi River in Iwate Prefecture, Japan, to the northwestern Pacific Ocean were estimated using carbon ($\delta^{13}\text{C}$) and hydrogen isotopic compositions (δD) in surface sediments. In marine sediments, algal sterols such as 24-methylcholesta-5,22-dien-3 β -ol had $\delta^{13}\text{C}$ values of $-22.7 \pm 0.4\text{‰}$ and δD values of $-292 \pm 3\text{‰}$. In contrast, sterols derived from multiple potential sources, such as 24-methylcholest-5-en-3 β -ol and 24-ethylcholest-5-en-3 β -ol, were gradually enriched in ^{13}C from riverine (-30.7‰ and -30.5‰ , respectively) to marine sediments (-22.3‰ and -24.4‰ , respectively), but showed little variation in δD values ($-262 \pm 1\text{‰}$). These isotopic signatures suggest that 24-methylcholest-5-en-3 β -ol and 24-ethylcholest-5-en-3 β -ol are derived from C3 plants ($\sim 90\%$) and C4 plants ($\sim 10\%$) in the riverine sediments, and marine algae ($\sim 30\%$), C3 plants ($\sim 30\%$), and C4 plants ($\sim 40\%$) in the open marine sediments. Thus, dual isotopic compositions ($\delta^{13}\text{C}$ - δD) of sterols allow determinations of the proportions of three biological sources, in which the relative contribution of C3 plants decreases while that of marine algae and C4 plants increases from riverine to marine sediments. These results indicate that $\delta^{13}\text{C}$ - δD signatures of sterols provide a useful tool for interpreting the sources of sedimentary organic matter and for understanding the transport and mixing processes of distinct biological sources in riverine–marine environments.

Quantitative assessment of organic matter inputs from marine algae and terrestrial plants to riverine and marine sediments is important for understanding the burial and mixing processes affecting organic matter at continental margins and for estimating marine primary productivity and carbon flux associated with biogeochemical cycles in surface environments. Marine algae and terrestrial C3 and C4 plants are major primary producers and potential sources for sedimentary organic matter in river-dominated coastal environments. Molecular and isotopic compositions of sedimentary organic materials should reflect both variations in the input of marine algae relative to terrestrial plants and in the proportions of C3 and C4 terrestrial plant inputs. Many previous studies have estimated the relative contribution between two biological sources (marine algae vs. terrestrial plants or terrestrial C3 vs. C4 plants) for sedimentary organic materials from riverine (or coastal) to marine environments based on biomarker assessments (e.g., Laureillard and Saliot 1993; Mannino and Harvey 1999). Carbon isotopic compositions of bulk organic carbon (e.g., Wada et al. 1987; Carreira et al. 2002) and specific biomarkers including lignin-derived com-

pounds (e.g., Hedges and Parker 1976; Goñi et al. 1997) and long-chain *n*-alkyl molecules (e.g., Huang et al. 2000; Naraoka and Ishiwatari 2000) have been also employed. For example, Goñi et al. (1997) reported ^{13}C -enriched lignin-derived compounds, specific to terrestrial vascular plants, in open-marine sediments relative to coastal sediments from the Gulf of Mexico, and suggested that the contribution of C4 plants relative to C3 plants increased seaward as a result of hydrodynamic particle sorting during transport. Generally, bulk organic carbon and specific compounds become gradually enriched in ^{13}C from riverine to marine sediments, which has been explained by the change of contribution from terrestrial C3 plants to marine algae or from terrestrial C3 plants to C4 plants (e.g., Wada et al. 1987; Goñi et al. 1997; Carreira et al. 2002). However, a simultaneous estimation of relative source contribution among three biological sources (marine algae, terrestrial C3 and C4 plants) has not yet been reported. In particular, contributions from marine algae and terrestrial C4 plants to the ^{13}C -enriched bulk organic matter in open marine sediments has been difficult to assess because carbon isotopic signatures cannot readily distinguish between mixtures of marine algae and terrestrial C3 plants and mixtures of terrestrial C3 and C4 plants.

Sterols are widely distributed in eukaryotic organisms and have been frequently used as algal and vascular plant biomarkers (e.g., Huang and Meinschein 1979; Harvey 1994; Pearson et al. 2000). They are well preserved in recent sediments, where specific patterns of alkyl side-chains, nuclear methylation, and positions of double bonds are diagnostic of their specific biological sources (e.g., Volkman 1986, 2003), as summarized in Table 1. In addition to the structural characteristics, compound-specific carbon (Hayes et al. 1990) and hydrogen isotope analyses (Burgoyne and Hayes 1998; Hilkert et al. 1999) of sterols can be a potentially useful tool in resolving sources of the sterols produced by different organisms (e.g., Canuel et al. 1997; Matsumoto et al. 2001;

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Table 1. Names, abbreviations, and biological sources of sterols.

Systematic name	Trivial name	Abbreviation	Source
24-Nor-cholesta-5,22-dien-3 β -ol		26 Δ ^{5,22}	Algae*
24-Nor-cholest-22-en-3 β -ol		26 Δ ²²	Algae*
Cholesta-5,24-dien-3 β -ol	Desmosterol	27 Δ ^{5,24}	Algae
Cholesta-5,22-dien-3 β -ol	22-Dehydrocholesterol	27 Δ ^{5,22}	Algae*
Cholest-22-en-3 β -ol	22-Dehydrocholestanol	27 Δ ²²	Algae*
Cholest-5-en-3 β -ol	Cholesterol	27 Δ ⁵	Algae*
24-Methylcholesta-5,22-dien-3 β -ol	Brassicasterol	28 Δ ^{5,22}	Algae
24-Methylcholest-22-en-3 β -ol		28 Δ ²²	Algae*
24-Methylcholesta-5,24(28)-dien-3 β -ol	Methylenecholesterol	28 Δ ^{5,24(28)}	Algae
24-Methylcholest-5-en-3 β -ol	Campesterol	28 Δ ⁵	Algae, terrestrial C3 and C4 plants
24-Ethylcholesta-5,22-dien-3 β -ol	Stigmasterol	29 Δ ^{5,22}	Algae, terrestrial C3 and C4 plants
24-Ethylcholesta-5,24(28) <i>E</i> -dien-3 β -ol	Fucosterol	29 Δ ^{5,24(28)<i>E</i>}	Algae
24-Ethylcholest-5-en-3 β -ol	β -Sitosterol	29 Δ ⁵	Algae, terrestrial C3 and C4 plants
24-Ethylcholesta-5,24(28) <i>Z</i> -dien-3 β -ol	Isofucosterol	29 Δ ^{5,24(28)<i>Z</i>}	Algae
4,23,24-Trimethylcholest-22-en-3 β -ol	Dinosterol	d Δ ²²	Algae

* This algal sterol is also produced and/or biotransformed from algal sterols by heterotrophic organisms such as zooplankton.

Chikaraishi and Naroka, 2005). For example, Matsumoto et al. (2001) estimated the proportion of biological sources of 29 Δ ⁵ sterol in marine sediments assuming that marine algae and terrestrial C3 plants have distinct carbon isotopic compositions. Distinctive carbon and hydrogen isotopic compositions were also observed for sterols derived from C3 and C4 terrestrial plants (Chikaraishi et al. 2004b). Thus, sterols are isotopically heterogeneous depending on primary producers, and therefore, isotope analysis of sedimentary sterols is of use in assessing relative contributions among marine algae and terrestrial C3 and C4 plants. This study describes the use of dual isotope ($\delta^{13}\text{C}$ and δD) measurements to identify contributions from three biological sources (marine algae and terrestrial C3 and C4 plants) for the various sterols in surface sediments across a river-to-marine transition. This information should further our understanding of the fate of

organic matter derived from terrestrial C3 and C4 plants as well as phytoplankton that is supplied to marine sediments.

Materials and methods

Riverine (OR-1 and OR-5), bay (OB-6 and OB-7), coastal (SOB-3), and open-marine (LM-10) surface sediment samples (0–2 cm in depth for OR-1, OR-5, OB-6, OB-7, and OB-3; 2–6 cm in depth for LM-10) were analyzed in this study (Fig. 1, Table 2). The study area is located from Ohtsuchi River in Iwate Prefecture, Japan, to the northwestern Pacific Ocean. The carbon isotopic compositions of bulk organic carbon, individual *n*-alkyl molecules, and hopanoids using the same series of surface sediments were reported previously (e.g., Naraoka and Ishiwatari 2000; Naraoka et al. 2000). All sediments were freeze dried and crushed to a fine powder before analysis.

After HCl treatment to remove carbonate, total organic carbon (TOC) contents of dry sediments were determined by an elemental analyzer (EA) using a FISON NA-1500 EA. Carbon isotopic compositions of TOC were determined by EA/conflo/isotope ratio mass spectrometry (EA/conflo/IRMS) using a FISON NA-1500 EA coupled to a Finnigan Delta S via Finnigan Conflo II interface. $\delta^{13}\text{C}$ values are given in per mil (‰) relative to Pee Dee Belemnite (PDB), and standard deviations of the isotopic measurements were generally better than $\pm 0.1\text{‰}$.

Preparation methods and compound-specific carbon and hydrogen isotope analyses of sterols followed Chikaraishi et al. (2004a,b). Briefly, the dried sediments were saponified by 0.5 mol L⁻¹ KOH in CH₃OH/H₂O (95/5, w/w) by refluxing for 4.5 h and extracted with CH₂Cl₂/CH₃OH (2/1, v/v) by sonication in order to isolate lipids. The combined solution (KOH/CH₃OH/H₂O + CH₂Cl₂/CH₃OH) was extracted with hexane/diethylether (9/1, v/v) to partition out the neutral lipids. The mono-alcohol fraction (including *n*-alkanols and sterols) was obtained from the neutral lipids by silica gel column chromatography (using silica gel deactivated by 5 wt% water; 4 cm in length and 6 mm in diameter) with hexane/ethyl acetate (4 ml, 4/1, v/v) after elution of hydro-

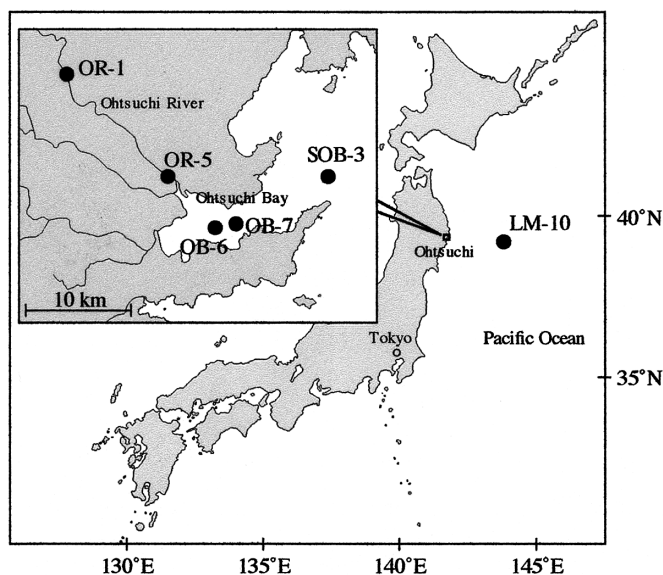


Fig. 1. Maps showing location of sampling station (circles) along a transect from the Ohtsuchi river in Iwate Prefecture, Japan, into the northwestern Pacific Ocean.

Table 2. Chemical characteristics of riverine-marine sediments used in this study.*

Sample	Environment (see Fig. 1)	TOC (wt %/dry sediment)	TN (wt %/dry sediment)	TOC/TN (by atomic)	$\delta^{13}\text{C}_{\text{TOC}}$ (‰, relative to PDB)
OR-1	Riverine	3.20	0.22	17.0	-27.2 ± 0.0
OR-5	Riverine	2.37	0.19	14.6	-26.4 ± 0.0
OB-6	Bay	3.93	0.27	17.0	-25.9 ± 0.1
OB-7	Bay	2.87	0.23	14.6	-24.8 ± 0.1
SOB-3	Coastal	1.10	0.12	10.7	-22.8 ± 0.1
LM-10	Open marine	1.48	0.25	6.9	-21.5 ± 0.0

* TOC, total organic carbon; TN, total nitrogen.

carbons with hexane (2 ml). The mono-alcohol fraction was acetylated with acetic anhydride/pyridine (1/1, v/v), followed by separation into *n*-alkanol and sterol fractions by urea adduction.

Acetylated sterol mixtures of natural samples are gener-

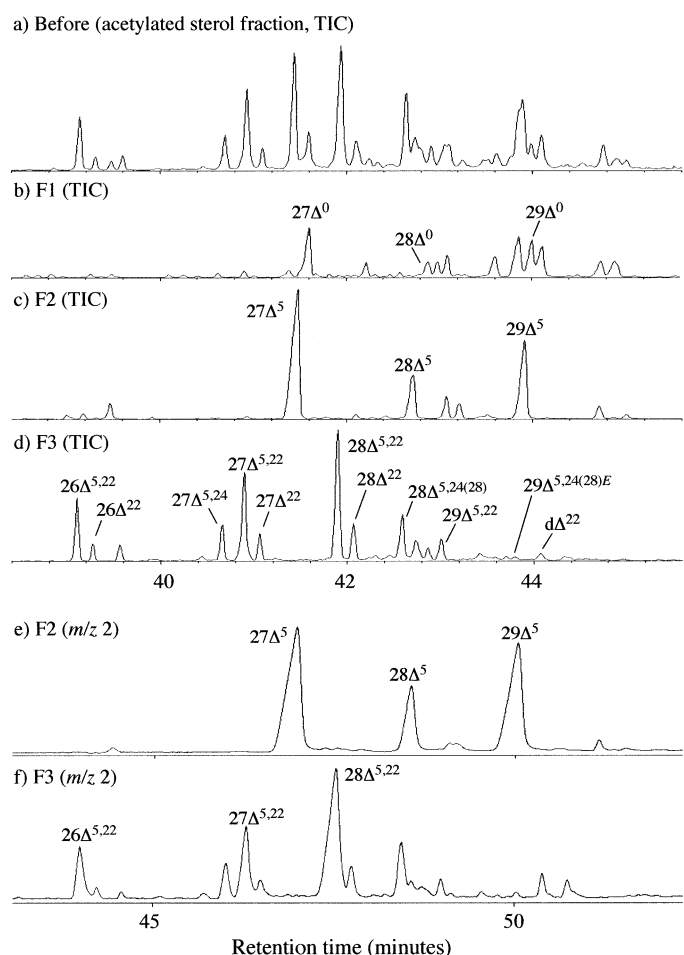


Fig. 2. Representative chromatograms of (a–d) total ion chromatogram (TIC) on GC/MS analysis and (e and f) *m/z* 2 Chromatograms on GC/pyrolysis/IRMS analysis before and after separation of acetylated sterols (in marine sediment, LM-10). Acetylated sterols were separated into three fractions: (b) F1 (stanols), (c) F2 (Δ^5 sterols), and (d) F3 (other sterols) by silver nitrate (10 wt%) -impregnated silica gel column chromatography (see text). Sterol abbreviations are provided in Table 1.

ally complex, with coeluting occurring during gas chromatographic separations (Fig. 2a), which does not allow for accurate compound-specific isotopic determinations. Therefore, the acetylated sterol fraction was further separated into three fractions: F1 (stanols), F2 (Δ^5 sterols), and F3 (other sterols, including $\Delta^{5,22}$, $\Delta^{5,24}$, and Δ^{22} sterols) using silver nitrate (10 wt%) -impregnated silica gel column chromatography (2.5 cm in length, 6 mm in diameter) (Chikaraishi and Naroka, 2005). The F1 was eluted with 16 ml of *n*-hexane/ CH_2Cl_2 (9/1, v/v), and F2 was subsequently eluted with 12 ml of *n*-hexane/ CH_2Cl_2 (9/1, v/v). Subsequently, the F3 fraction was recovered by elution with 4 ml of CH_2Cl_2 (100%). Representative chromatograms before and after separation are shown in Fig. 2a–d. Each fraction was completely separated with a recovery of more than ~95% for replicate analyses.

Stanols and sterols were identified by gas chromatography/mass spectrometry (GC/MS) using an HP 6890 GC interfaced to an HP MSD 5972A. The concentrations of individual sterols were quantified using an HP 6890 GC with a flame-ionization detector (FID) compared with the peak area of external *n*-alkane standards (mixture of 16 *n*-alkanes ranging from C_{18} to C_{36}).

Compound-specific carbon ($\delta^{13}\text{C}$) and hydrogen (δD) isotope analyses were carried out by GC/combustion/IRMS using a Finnigan Delta S interfaced with an HP 5890II GC and by GC/pyrolysis/IRMS using a Finnigan Delta plus XL interfaced with an HP 6890 GC, respectively. Combustion was performed in a microvolume ceramic tube with CuO and Pt wires at 840°C (Hayes et al. 1990). Pyrolysis was performed in a microvolume ceramic tube with graphite at 1,440°C (Hilkert et al. 1999). $\delta^{13}\text{C}$ and δD values were given in per mil (‰) relative to PDB and standard mean ocean water (SMOW), respectively. Standard deviations of carbon and hydrogen isotope measurements were generally better than $\pm 0.5\text{‰}$ ($\pm 0.3\text{‰}$ on average) and $\pm 7\text{‰}$ ($\pm 3\text{‰}$ on average), respectively. The measured $\delta^{13}\text{C}$ and δD values of acetylated sterols were corrected by isotopic mass balance for contributions of carbon and hydrogen incorporated during acetylation (Chikaraishi et al. 2004a,b).

Results and discussion

Isotopic compositions of TOC and sterols—Carbon isotopic compositions of TOC ($\delta^{13}\text{C}_{\text{TOC}}$) gradually increase from riverine (-27.2‰) to marine (-21.5‰) sediments (Table 2).

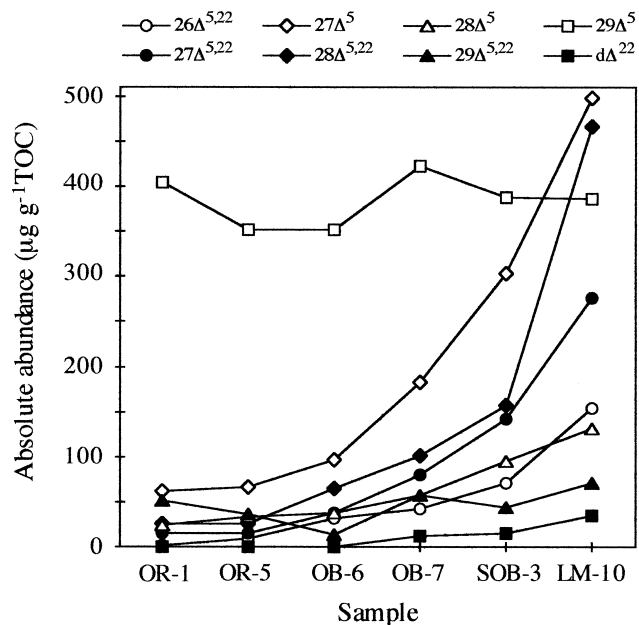


Fig. 3. Concentration of major sterols normalized to TOC content from riverine and marine sediments. Sterol abbreviations are provided in Table 1.

This ^{13}C enrichment is consistent with previous isotopic studies in this region (Wada et al. 1987; Naraoka and Ishiwatari 2000; Naraoka et al. 2000) as well as many riverine-marine transects (e.g., Canuel et al. 1995; Carreira et al. 2002), suggesting a consistent geochemical gradient. Although the $\delta^{13}\text{C}$ variations reflect changes in organic matter sources depending on geographical location, relative contribution from marine algae and terrestrial C_3 and C_4 plants cannot be achieved solely using $\delta^{13}\text{C}_{\text{TOC}}$. This led us to focus on dual isotopic measurements on source-specific biomarkers.

Various sterols were identified in riverine and marine sediment samples (Table 1). The absolute abundance of the algal sterols ($26\Delta^{5,22}$, $27\Delta^{5,22}$, and $28\Delta^{5,22}$) normalized to the TOC

content gradually increases from riverine to marine sediments (Fig. 3), indicating the seaward increase of algal contributions. On the other hand, the abundance of sterols potentially derived from both marine algae and terrestrial plants, such as $28\Delta^5$, $29\Delta^{5,22}$, and $29\Delta^5$ sterols, is relatively constant from riverine to marine sediments, implying positive correlations between these sterols and TOC content (e.g., abundance of $29\Delta^5$ sterol is $384 \pm 28 \mu\text{g g}^{-1}\text{TOC}$, correlation coefficient: $R^2 = 0.95$). This may suggest that TOC content is well reflected by the population of potential sources producing these sterols. The variation in the molecular abundance of different stations is generally consistent with previous studies (e.g., Laureillard and Saliot 1993), indicating enrichments in algal organic matter and depletions in terrigenous contribution to sedimentary organic matter from riverine to marine environments.

Carbon and hydrogen isotopic compositions of various sterols from the riverine and marine sediment samples are summarized in Table 3. Isotopic compositions of algal sterols are reported as a combined value of $\Delta^{5,22}$ sterols with 5,24-diene ($\Delta^{5,24}$) and/or 22-ene (Δ^{22}) sterols of the corresponding carbon number because these sterols could not be significantly well resolved for separate isotope determination (Fig. 2f). These algal sterols have $\delta^{13}\text{C}$ values of -22.2‰ to -23.2‰ and δD values of -287‰ to -295‰ , showing little variation among not only different carbon numbers but also different station. These $\delta^{13}\text{C}$ values of algal sterols are similar to typical isotope values of marine algal sterols (e.g., Pancost et al. 1999; Matsumoto et al. 2001). For example, Pancost et al. (1999) reported $\delta^{13}\text{C}$ values of -18.1‰ to -24.3‰ for $27\Delta^{5,22}$ sterol and -19.2‰ to -26.3‰ for $28\Delta^{5,22}$ ($+28\Delta^{22}$) sterol isolated from suspended particle organic matter (SPM) in Peru coastal waters. On the other hand, $28\Delta^5$ and $29\Delta^5$ sterols become enriched in ^{13}C in riverine sediments to marine sediments ($28\Delta^5$ is enriched from -30.7‰ to -22.3‰ and $29\Delta^5$ is enriched from -30.5‰ to -24.4‰). The ^{13}C enrichment of $29\Delta^5$ is similar to that of TOC, and a positive correlation ($R^2 = 0.93$) is observed between $\delta^{13}\text{C}$ values of $29\Delta^5$ and TOC (Fig. 4). In contrast,

Table 3. $\delta^{13}\text{C}$ and δD values of sterols (sterol abbreviations are provide in Table 1).

	Riverine		Bay		Coastal	Open marine
	OR-1	OR-5	OB-6	OB-7	SOB-3	LM-10
$\delta^{13}\text{C}$ values (‰, relative to PDB)						
$26\Delta^{5,22} + 26\Delta^{22}$					-22.2 ± 0.4	-22.7 ± 0.4
$27\Delta^{5,24} + 27\Delta^{5,22} + 27\Delta^{22}$					-23.1 ± 1.0	-23.2 ± 0.4
$27\Delta^5$	-27.8 ± 0.7	-27.3 ± 0.1	-23.8 ± 0.1	-23.9 ± 0.4	-24.1 ± 0.2	-22.7 ± 0.2
$28\Delta^{5,22} + 28\Delta^{22}$					-22.6 ± 0.2	-22.6 ± 0.1
$28\Delta^5$	-30.7 ± 0.7	-30.1 ± 0.1	-26.1 ± 0.5	-25.3 ± 0.4	-23.6 ± 1.0	-22.3 ± 0.4
$29\Delta^5$	-30.5 ± 0.5	-30.2 ± 0.3	-28.2 ± 0.2	-27.7 ± 0.3	-26.6 ± 0.3	-24.4 ± 0.3
δD values (‰, relative to SMOW)						
$26\Delta^{5,22} + 26\Delta^{22}$					-292 ± 7	-287 ± 1
$27\Delta^{5,24} + 27\Delta^{5,22} + 27\Delta^{22}$					-291 ± 7	-291 ± 13
$27\Delta^5$	-271 ± 3	-288 ± 4	-297 ± 11	-286 ± 2	-288 ± 12	-292 ± 1
$28\Delta^{5,22} + 28\Delta^{22}$					-295 ± 0	-294 ± 2
$28\Delta^5$	-259 ± 4	-260 ± 4	-261 ± 6	-263 ± 7	-261 ± 5	-260 ± 6
$29\Delta^5$	-259 ± 8	-260 ± 1	-258 ± 11	-261 ± 4	-262 ± 8	-262 ± 7

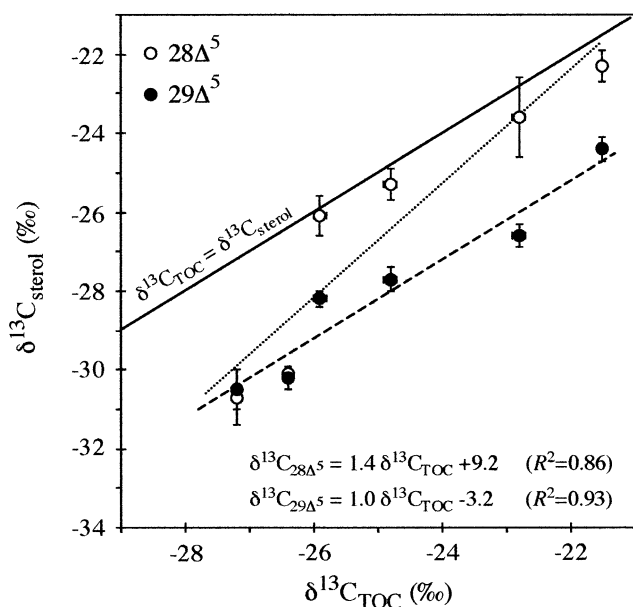


Fig. 4. $\delta^{13}\text{C}$ relationships between TOC and sterols ($28\Delta^5$ and $29\Delta^5$) in riverine-marine sediments. Dashed lines indicate correlations between TOC and sterols.

the δD values of these sterols show little variation ($-262 \pm 1\text{‰}$) regardless of difference in carbon number of sterols and sediment locations. Moreover, δD values of these sterols are distinct from those considered exclusively of algal origin, the former being enriched in D by $\sim 30\text{‰}$. This D enrichment of sedimentary $29\Delta^5$ is consistent with the finding of Sauer et al. (2001), in which $29\Delta^5$ from terrestrial sources was enriched in D by 30‰ relative to algal sterols.

An estimate of relative source contribution of sterols—Similar to $\delta^{13}\text{C}$ values for bulk organic carbon, $\delta^{13}\text{C}$ and δD values of sterols reflect variation in the biological sources of sedimentary organic matter by geographical location. Therefore, the relative source contribution of sedimentary sterols

can be estimated by isotopic mass balance calculations with a four end-member model using Eqs. 1–3:

$$\delta^{13}\text{C}_{\text{sed}} = R_m \times \delta^{13}\text{C}_m + R_{3a} \times \delta^{13}\text{C}_{3a} + R_{3g} \times \delta^{13}\text{C}_{3g} + R_4 \times \delta^{13}\text{C}_4 \quad (1)$$

$$\delta\text{D}_{\text{sed}} = R_m \times \delta\text{D}_m + R_{3a} \times \delta\text{D}_{3a} + R_{3g} \times \delta\text{D}_{3g} + R_4 \times \delta\text{D}_4 \quad (2)$$

$$R_m + R_{3a} + R_{3g} + R_4 = 1 \quad (3)$$

where the subscripts indicate sediment (sed) and each potential source: marine algae (m), terrestrial C3 angiosperms (3a), C3 gymnosperms (3g), and C4 plants (4). R indicates the relative contribution of each potential source. In addition, we make two assumptions in order to solve for four unknowns (i.e., R_m , R_{3a} , R_{3g} , and R_4) from the three equations:

1. Marine algae do not contribute to riverine sediments ($R_m = 0$).
2. Contributions of C3 angiosperms relative to C3 gymnosperms are constant through all sediment samples, which is equal to the ratio of riverine sediment (OR-1, $R_{3a}/R_{3g} = \sim 1.23$) calculated using assumption 1.

We also assume that $\delta^{13}\text{C}$ and δD values of $28\Delta^5$ and $29\Delta^5$ sterols in marine algae are similar to those of other algal sterols observed in the marine sediments (SOB-3 and LM-10; i.e., $\delta^{13}\text{C} = -22.7 \pm 0.4\text{‰}$, $\delta\text{D} = -292 \pm 3\text{‰}$). $\delta^{13}\text{C}$ and δD values of $28\Delta^5$ and $29\Delta^5$ sterols in terrestrial plants are listed in Table 4, based on the isotopic values of sterols from terrestrial plants in Japan (Chikaraishi et al. 2004a,b). In addition to marine algae and terrestrial plants, riverine algae might comprise an additional source of sedimentary $28\Delta^5$ and $29\Delta^5$ sterols (Volkman et al. 1999). However, both $\delta^{13}\text{C}$ and δD values of these two sterols do not differ substantially between upper (OR-1) and lower (OR-5) reaches of the Ohtsuchi River (Table 3). Moreover, δD values of these two sterols are similar to those of terrestrial C3 plants (Fig. 5). Sterols of freshwater algae should be more depleted in D relative to those of marine algae (Sauer et al. 2001;

Table 4. Carbon and hydrogen isotopic compositions of reference materials.

Reference materials	$\delta^{13}\text{C}$ (‰, relative to PDB)	$^2\epsilon$ (‰, relative to rainwater)	δD (‰, relative to SMOW)
Terrestrial sterol			
C3 angiosperms (13 species)	$-34.7 \pm 2.0^*$	$-211 \pm 15^\dagger$	$-260 \pm 15^\ddagger$
C3 gymnosperms (2 species)	$-29.2 \pm 1.6^*$	$-216 \pm 5^\dagger$	$-264 \pm 5^\ddagger$
C4 plants (2 species)	$-16.9 \pm 1.3^*$	$-186 \pm 13^\dagger$	$-236 \pm 13^\ddagger$
Rainwater			
1983–1986 (141.5°E, 39.02°N)§			-61 ± 20

* Average of carbon isotopic compositions of plant sterols, reported in Chikaraishi et al. (2004a, b).

† Average of hydrogen isotopic fractionations of plant sterols relative to rainwater, reported in Chikaraishi et al. (2004a, b).

‡ Putative hydrogen isotopic compositions of sterols in terrestrial plants growing up around Ohtsuchi river, which is calculated by the $^2\epsilon$ of plant sterols with δD of rainwater at this location. $\delta\text{D}_{\text{sterol}} = ^2\epsilon_{\text{sterol}} + \delta\text{D}_{\text{rainwater}} + (^2\epsilon_{\text{sterol}} \times \delta\text{D}_{\text{rainwater}})/1,000$.

§ The Global Network of Isotopes in Precipitation (GNIP) Database, Release 3, October 1999, from International Atomic Energy Agency/World Meteorological Organization (IAEA/WMO).

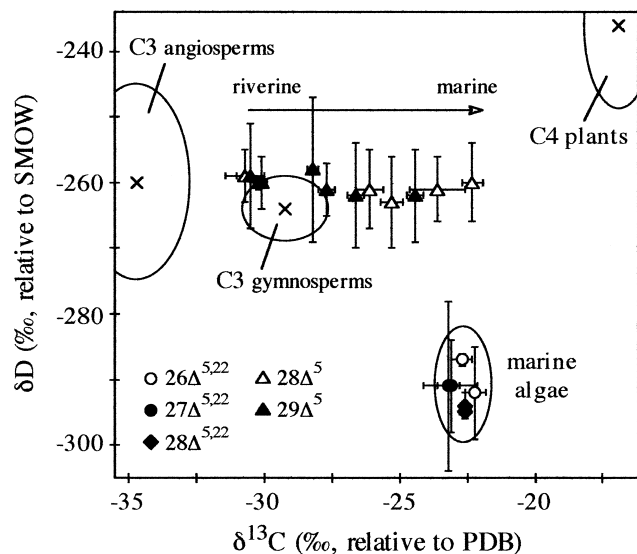


Fig. 5. $\delta^{13}\text{C}$ - δD cross plot of various sterols in riverine-marine sediments, with putative $\delta^{13}\text{C}$ and δD values of sterols for the potential source organisms. Sterol abbreviations are provided in Table 1.

Chikaraishi and Naroka, 2005) because river water ($\delta\text{D} \sim -60\text{‰}$) is depleted in D relative to seawater ($\delta\text{D} \sim 0\text{‰}$). Therefore, we assume riverine algae as a minor source in this riverine-marine environment.

Using the above assumptions, we calculate that the proportion of $28\Delta^5$ sterol derived from C3 angiosperms, C3 gymnosperms, and C4 plants in river sediment (OR-1) is 49.7%, 40.3%, and 10.0%, respectively (Fig. 6a). From riverine to marine sediments, the relative contribution of C3 plants (angiosperms and gymnosperms) decreases, while that of marine algae and C4 plants increases. In the case of the open marine sediment (LM-10), marine algae and C4 plants contribute 31.7% and 45.0% of $28\Delta^5$ sterol, respectively, with the remaining 23.3% attributed to C3 plants. Similarly, for $29\Delta^5$ sterol, C3 angiosperms, C3 gymnosperms, and C4 plants account for 49.7%, 39.0%, and 11.3% of this sterol in riverine sediment (OR-1), respectively (Fig. 6b). From riverine to marine sediments, the relative contribution of C3 plants decreases, while that of marine algae and C4 plants increases. In the case of open marine sediment (LM-10), marine algae and C4 plants contribute 29.3% and 32.9% of $29\Delta^5$ sterol, respectively, and the remaining 37.8% is attributed to C3 plants.

Transport and mixing of sterols derived from different biological sources in riverine-marine environments—As described above, the $\delta^{13}\text{C}$ and δD signatures of sedimentary $28\Delta^5$ and $29\Delta^5$ sterols demonstrate that these sterols derive predominantly from terrestrial C3 plants in riverine sediments, while contributions from marine algae and terrestrial C4 plants increase in marine sediments (Fig. 6). A seaward increase in the terrestrial C4 plant contributions is consistent with a previous study based on $\delta^{13}\text{C}$ analysis of lignin-derived compounds in the Gulf of Mexico (Goñi et al. 1997). Although $28\Delta^5$ and $29\Delta^5$ sterols are only a small fraction of

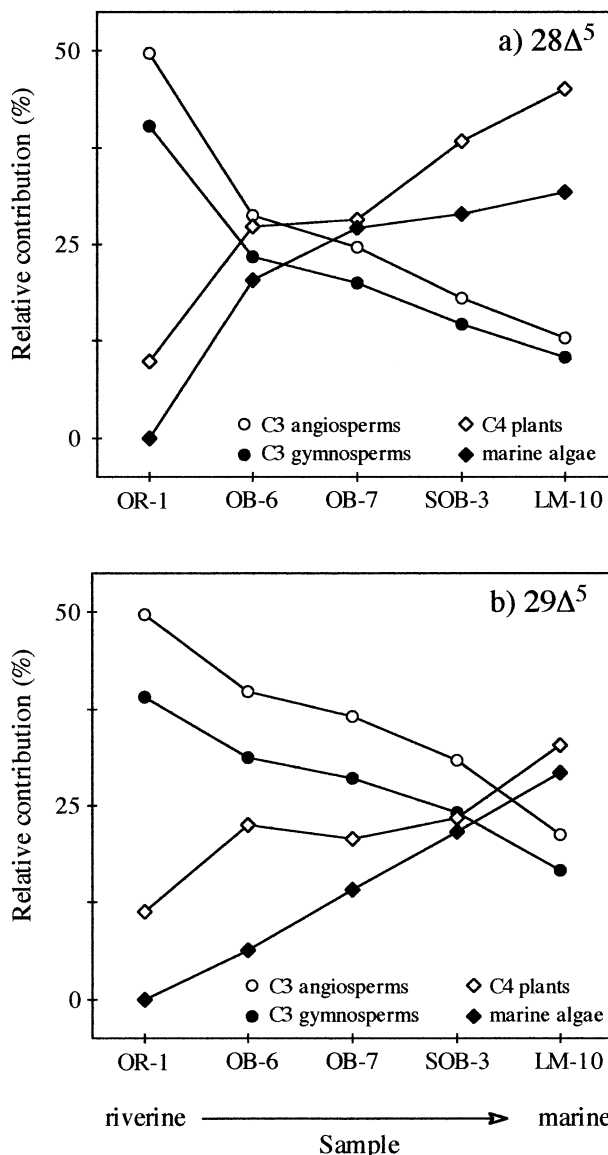


Fig. 6. Source variation of relative contributions for (a) $28\Delta^5$ and (b) $29\Delta^5$ sterols from riverine to marine sediments.

the whole organic matter in sediments (Fig. 3), their ^{13}C enrichments with distance seaward follows the trend with TOC (Fig. 4). Moreover, the strong correlation of $\delta^{13}\text{C}$ values between $29\Delta^5$ sterol and TOC (slope = 1.0, $R^2 = 0.93$) may suggest a similar source.

Offshore differences in the contributions of C3 and C4 plant-derived organic matter are probably related to the particle associations of organic matter derived from different types of vegetation. Bird and Pousai (1997) reported that C3 plant-derived organic matter is incorporated into the coarse size fractions compared with C4 plant-derived organic matter. Moreover, around Ohtsuchi River, terrestrial C3 plants such as oaks (angiosperms) and cedars (gymnosperms) grow on the coarse soils in wet forests, while terrestrial C4 plants, such as pampas grass, grow on the fine soils in dry fields. Generally, the fine size fractions of soils can be carried greater distances relative to the coarse size fractions during trans-

port by river and oceanic water. Therefore, the preferential transport of the fine-grained particles can disperse C4 plant-derived organic matter over longer distances from the river to offshore regions. In contrast, C3 plant-derived organic matter is transported shorter distances in association with the coarse size particles and deposited within the river and/or the adjacent bay and inner coast. Thus, hydrodynamic particle sorting during transport may induce the geochemical gradients between terrestrial inputs from riverine to marine sediments (Keil et al. 1994).

In many previous studies, the ^{13}C enrichment of organic materials from riverine to marine sediments has been explained by changes in the dominance of marine versus terrestrial sources, under an assumption of the two end-member model that ^{13}C -depleted organic materials are primarily derived from terrestrial C3 plants while ^{13}C -enriched organic materials are derived from autochthonous marine algae (e.g., Wada et al. 1987; Hedges et al. 1988). However, such an assumption does not recognize the contribution of terrestrial C4 plants to the sedimentary organic matter, even though terrestrial C4 plants may contribute significantly to marine environments (e.g., Goñi et al. 1997; Huang et al. 2000). This study is the first attempt to deconvolute the contributions of marine algae and terrestrial C4 plants into ^{13}C -enriched organic materials in open marine sediments. Although our findings only directly apply to sources of sedimentary sterols, the significance of identifying contributions from terrestrial C4 plants is that previous studies using a two end-member model may have overestimated the contribution of marine algae and underestimated terrigenous contributions. In such studies, a realistic understanding of marine primary productivity and associated organic carbon flux cannot be achieved. In the case of this study, $\delta^{13}\text{C}$ values of the open marine sediment (LM-10) is estimated to be due to approximately equal contributions between marine algae and terrestrial C4 plants, and therefore the relative contribution of total terrigenous inputs is more than 70% (Fig. 6b). Thus, in certain geographical settings where the supply of C4-derived terrestrial organic carbon is potentially significant, the sources of sedimentary organic matter as well as marine primary productivity and associated organic carbon fluxes should be reevaluated using a three biological source (marine algae and terrestrial C3 and C4 plants) model. In such studies, dual isotope ($\delta^{13}\text{C}$ - δD) analysis of sterols provides a useful means for estimating the relative contribution from different biological sources to sedimentary organic matter.

Preliminary findings based on this study indicate that several assumptions must be confirmed in future studies. In particular, no information is available on the molecular and isotopic compositions of sterols in natural biological sources from the study area. Previous studies indicate wide $\delta^{13}\text{C}$ and δD variations in sterols from different biological sources (e.g., Canuel et al. 1997; Sessions et al. 1999; Chikaraishi and Naroka, 2005), which may depend partly on growth conditions. In addition, eolian transport of terrestrial organic matter by global wind systems, such as westerlies, may also contribute to terrestrial organic matter deposited in marine environments (e.g., Huang et al. 2000). In such cases, δD values of potential terrestrial sources may be more variable because δD values of rainwater vary in relation to the hy-

drological cycle in surface environments. Combined with further information on biological and geological settings, dual isotope ($\delta^{13}\text{C}$ - δD) analysis promises to provide more precise and reliable information about the relative source contributions of sedimentary sterols.

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