DISSERTATION

SIGNIFICANCE OF STEROLS IN SEDIMENTS AS ORGANIC GEOCHEMICAL PROXIES FOR PALEO-ENVIRONMENTAL STUDY

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We certify that we have read this dissertation and that, in our opininon, it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Enginnering

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Chapter 1
General Introduction

1.1. Biological sterols

Sterols are important components in lipids, which are incorporated into phospholipid bilayer of organisms (e.g., Simons and Ikonen, 1997, Figs. 1-1 and 1-2). These compounds are present in almost all eukaryotes including zoo- and phytoplankton, terrestrial plants, animals, and fungi (e.g., Nelson et al., 2000; Volkman et al., 1998; Weete, 1989). Sterols in vivo play as membrane stabilizer for fluidity and phase inversion of temperature in membranes (Demel and De Kruyff, 1976; Bloch, 1983). In the history of life, the first appearance of sterols is thought to be after the formation of an oxidative environment on the earth. In fact, sterols are biosynthesized through squalene, and it is known that oxygen is necessary for this synthetic system (Ourisson et al., 1987). Before the production of oxygen in the earth's history, it is suggested that hopanoids were used as a membrane stabilizer instead of sterols (Sáenz et al., 2012), and the evolution of these lipid compositions has also been studied as an interesting research subjects (e.g., Brocks and Banfield, 2009).

Sterols have a variety of structures with number of carbon side chains and differences in position of double bonds and others, and those structural features vary within organisms. For example, campesterol, β-sitosterol, and stigmasterol are known as major sterol compositions in terrestrial plants. Meanwhile, in animal sterols, cholesterol is found widely in various organisms. In addition, phytoplankton species contain diatomsterol (brassicasterol), 24-methylencholesterol.

1.2. Sterols in sediments as geochemical tracer

Sterols are also widely detected in sediments including marine and lacustrine environments. Sterols in sediments have been used as tracers because of their structural features. Volkman (1986) examined characteristics of sterol compositions in

sediments and tried to identify their origins. In this examination, the sterol compositions in sediments were concluded to be mainly composed of terrestrial plants and/or phytoplankton origin. Therefore, in general, in the field of organic geochemistry, sterols have been used as tracers of phytoplankton and/or terrestrial inputs. Huang and Meinschein (1979) proposed that plant and plankton origins of sterols can be divided using C_{27}, C_{28} and C_{29} sterols. Sterol compositions in terrestrial plants are composed of C_{29} sterol such as β-sitosterol. Plankton species contain rich C_{27} sterols such as cholesterol. Therefore, plots of C_{27}, C_{28}, and C_{29} sterols can indicate different distributions between plants and plankton (Fig. 1-3). This application has been used for various studies as a method for easily identifying sterol origin in sediments. On the other hand, as studies of sterols in sediments as bio-tracer progressed, the identification of specific sterol origins was required, and analysis of biological sterols in species level has also been actively carried out. These studies have been revised with the recognition of sterol biomarkers such as the fact that phytoplankton having β-sitosterol and campestanol is found and diatomsterol that has been thought to originate from diatoms is found in a wide range of phytoplankton species. Among these studies, characteristics of sterol compositions in diatoms compared with phylogenetic trees have been investigated (Rampen et al., 2010), and by such studies, sterol compositions in specific species have been gradually grasped.

Structures of sterol vary not only by the structural features in organisms but also by geochemical factors in the deposition process. Diagenesis means chemical changes occurring geological time scale during deposition, and diagenetic reaction in sterols is documented well. Diagenetic reactions occurring in steroids are shown in Fig 1-4. Sterols change to steren, diastererene, stellane, aromatic steroids, and others by diagenetic alteration. Ultimately, steroids undergo a cracking reaction and become a

polycyclic aromatic hydrocarbon (e.g., Mackenzie et al., 1982). As an example of the oldest steroid, eukaryotic biomarkers (steranes) have been detected from organic matter extracted from shale collected from the Pilbara Craton about 2.7 billion years ago (Brocks et al., 1999). However, this finding was shown to be contaminated by younger organic matter after about 2.2 billion years ago (Rasummusen et al., 2008). In any case, it is a unique study as an example of long-term preservation of sterols.

In shorter time scale, sterols change composition by biotic (bacterial reduction of sterols) and abiotic (photo- and autoxidation) processes corresponding to their surrounding environmental conditions (Fig. 1-5). In abiotic processes, Δ^4-3β,6α/β-dihydroxysterols and 3β,5α,6β-trihydroxysterols is produced by photooxidation and autoxidation, respectively. Therefore, these compounds are useful tracers for estimation of phoooxidation and autoxidation (e.g., Rontani et al., 2014). Meanwhile, as biotic processes, Δ^5-sterols are reduceded to 5α(H)-stanols under anoxic conditions by bacterial reaction. Thus, ration of 5α(H)-stanol / Δ^5-sterol have been used for redox tracer (e.g., Zheng et al., 2015). These diagenetic, biotic, and abiotic alternation processes indicate that sterols in sediments are applicable as various environmental tracers recording sedimentary conditions.

1.3. Methods of analysis for sterols

As for sterol analysis methods, regardless of the sediment and biological samples, solvent extraction method by solvent has mainly been used. Regarding the steps of the method, at first, a solvent such as methanol is added to sample, and the solvent-soluble organic compounds are dissolved using an ultrasonic vibration or a soxhlet extractor. The extracted compounds are performed to hydrolysis by potassium hydroxide as necessary. The extracted compounds are divided into acidic and neutral fractions by

liquid-liquid partitioning. Then, the neutral fraction is divided mainly into four fractions using silica gel column chromatography. The polar fraction containing the sterol is derivatized with a trimethylsilyl (TMS) reagent or the likes and analyzed by gas chromatography (GC). This analytical method has been widely used in the most traditional way, but the pre-treatment steps to analysis take time as described above. Therefore, since it is necessary to analyze many specimens of sediment samples, it takes much labor to analyze sterols in sediment samples by this method.

On the other hand, thermochemosis using tetramethyl ammonium hydroxide (TMAH) reagent has attracted attention as a method that can analyse various organic compounds in shorter time. In this method, with the assistance of TMAH, the organic compound in the sample is hydrolyzed and derivatized in simultaneously. Furthermore, the TMAH method is characterized by being able to analyze organic matter with a smaller amount of sample than solvent extraction method. Hatcher and Clifford (1994) applied the TMAH method to the soil sample, and showed that it is possible to analyze many organic compounds including fatty acids and phenol, which is suggested to be useful for understanding constituents of humic substances. To the best of my knowledge, this research is the first application to a geochemical sample of the TMAH method. Also, in the same age, Martin et al (1994) applied to fulvic acid. Clifford et al (1995) showed that the TMAH method is applicable to analyze lignin compounds. This method is recognized as analyzing for lignin and fatty acids. As other examples, analysis of organic substances such as cutin acids, sugars, and amino acids using the TMAH method have also been reported (e.g., del Rio and Hatcher, 1998; Schwarzinger et al., 2002; Zang et al., 2001). Utilizing the characteristics that can analyze numerous organic matters in a short time, this method has been used not only on geological samples but also on a wide range of samples. As one of interesting case study, in order to investigate the paint

ingredients used in Vermeer's art, some of the art were scraped and examined by the TMAH method (Pastorova et al., 1997).

Meanwhile, as far as I know there is no application of analysis of sterols in sediments using the TMAH method. Asperger et al (1999b) analyzed lipid components in three natural waxes (bleached beeswax, lanolin, yellow carnauba wax) using the TMAH method, and reported that sterols can be obtained by the method as sterol methyl ester. Methylation efficiencies by TMAH method were observed to be in the range > 90% for sterols, and this result indicates that sterol analysis by the TMAH method can be applicable with stable methylation. If this method is applied for sterol analysis in sediments, it is possible to treat many samples in a shorter time. Therefore, in this study, I evaluate significance of sterol analysis in sediment as bio-tracer and sterol analysis with TMAH method. Especially, I focus Δ^5-sterols and 5α(H)-sterols as redox tracer for paleo-environmental studies.

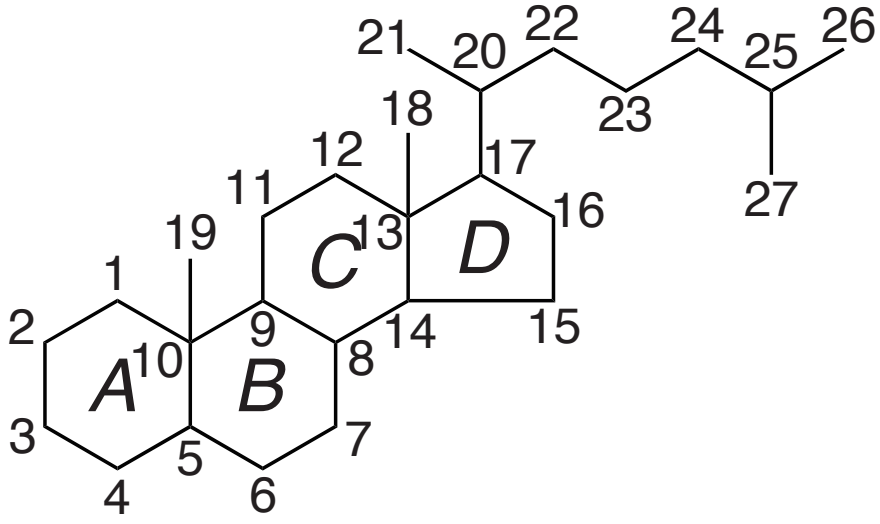

Fig. 1-1 Structure of sterols

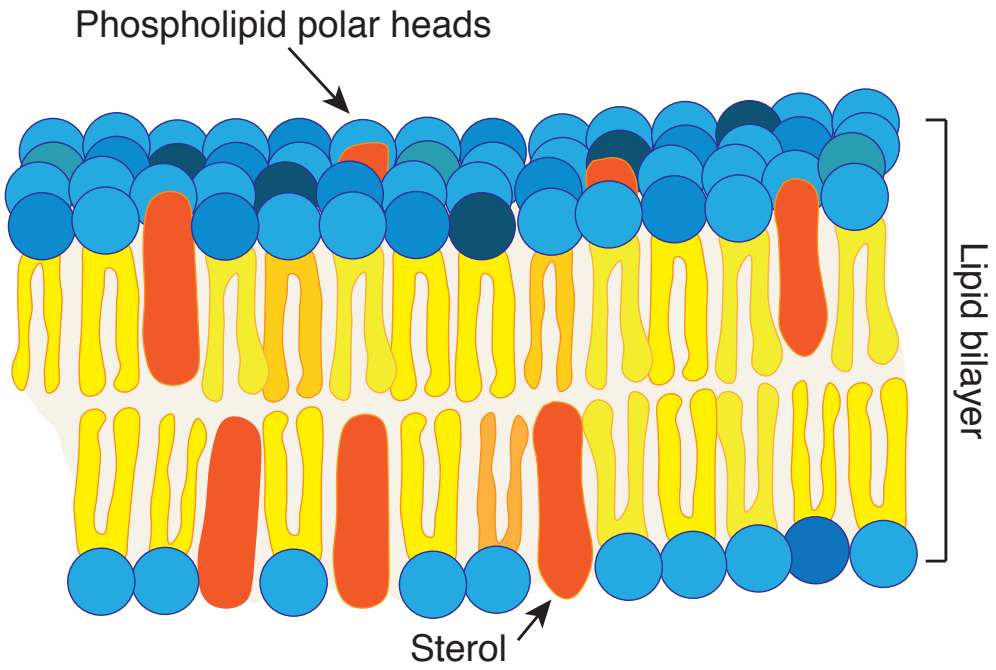

Fig. 1-2 Illustration of membrane structure

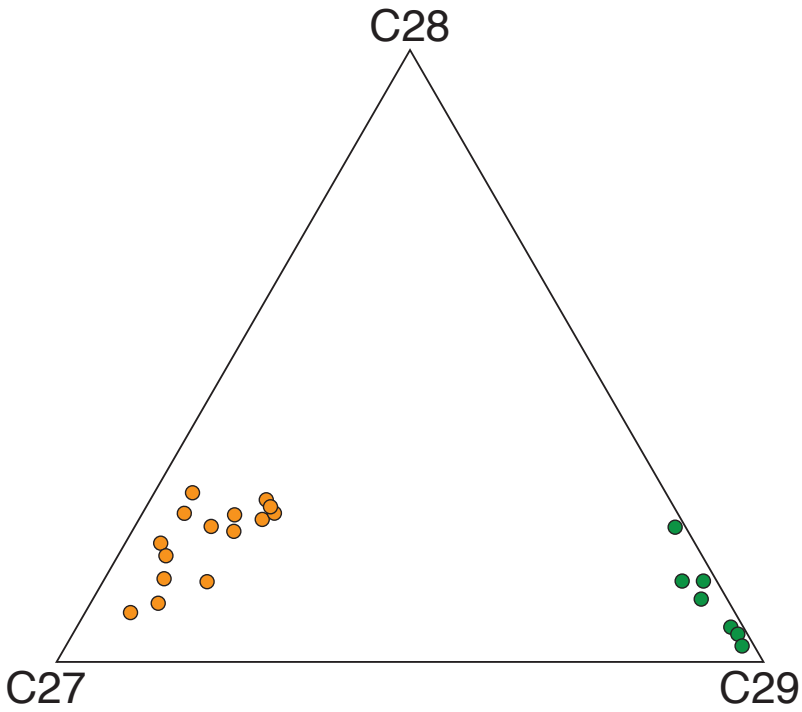

Fig. 1-3 Distribution of C_{27}, C_{28}, and C_{29} sterols in marine plankton and higher plants (adapted from Huang and Meinschein, 1979). Orange and green circles denote marine plankton and higher plants, respectively.

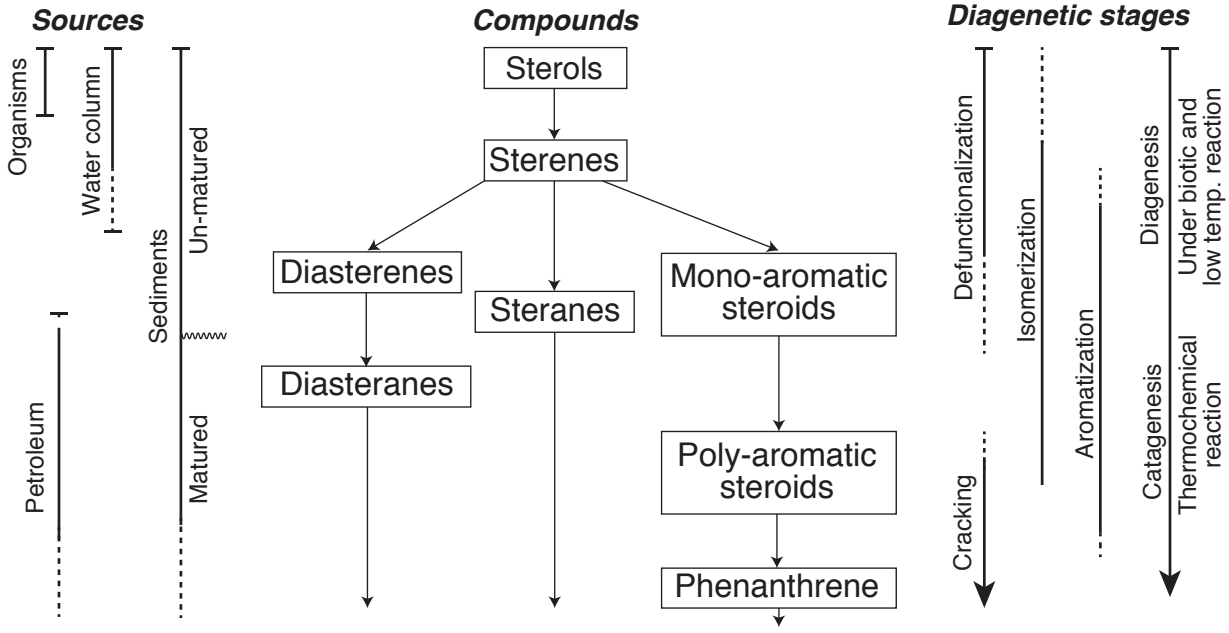

Fig. 1-4 Diagenesis processes of steroid. The figure was modified from Ishiwatari and Yamamoto (2004).

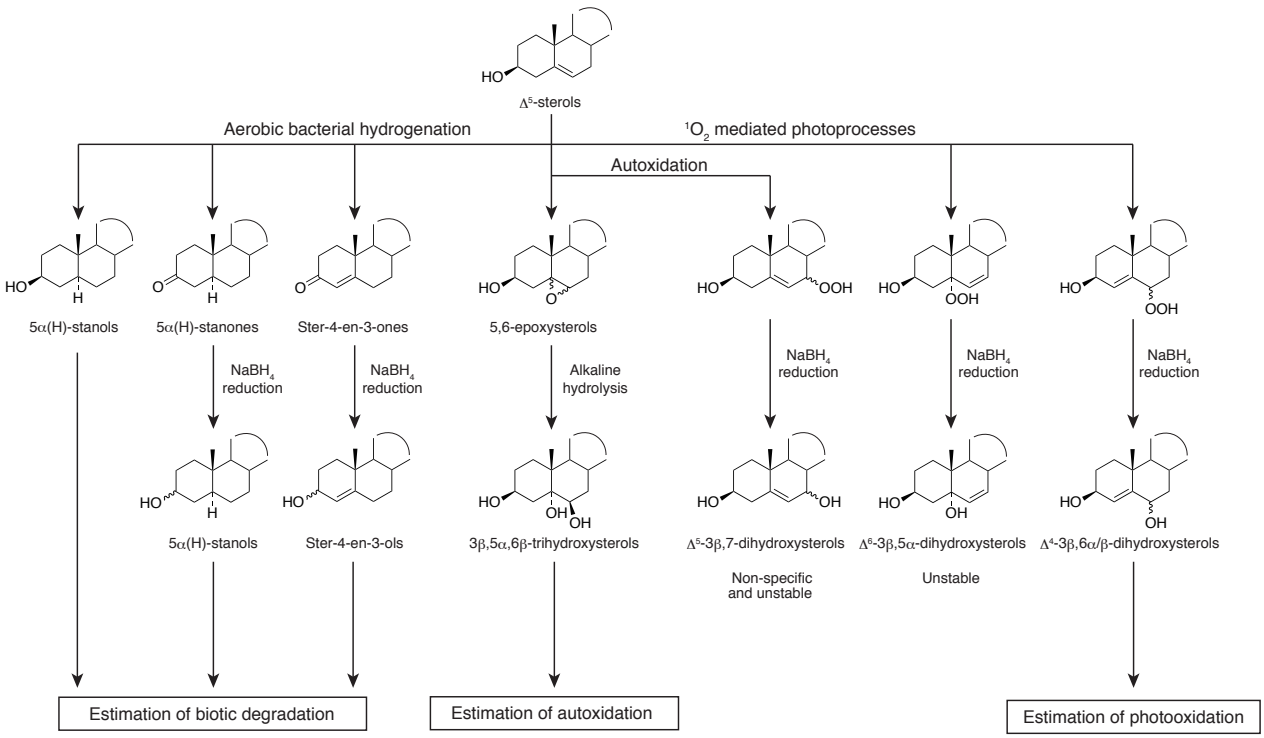

Fig. 1-5 Degradation proccees of Δ^5-sterols by aerobic bacterial degradation, autoxidation, and photooxidation. The figure was taken from Christodoulou et al. (2009), Rontani et al. (2009), and Rontani et al. (2014) with partial modification.

Chapter 2

Sterols by Using Gas Chromatography–mass Spectrometry of the Methyl Ether Derivatives Found in The TMAH Method

2.1. Introduction

Gas chromatography–mass spectrometry (GC–MS) analysis of sterols in sediments is performed by acetyl derivatization (e.g., Chikaraishi et al., 2005) or trimethylsilyl derivatization (TMS, e.g., Kondo et al., 1991a, 1991b; Yamamoto et al., 2015) via organic solvent extraction process. TMS derivatization can also be used for acids and alcohols with carboxyl and hydroxyl groups. The derivatization is a simple and quantitative method of reaction for about 30 minutes at 75℃. However, TMS derivatized products are vulnerable to water and acids and have drawbacks that are susceptible to hydrolysis. Acetyl derivatization is also a simple and quantitative method that can be derivatized by mixing an extracted sample with the derivatization reagent and reacting it for several hours at room temperature. Especially, Acetylation is useful for isotopic compositions of hydroxy compounds at molecular level because it does not contain non-flammable elements such as silicon (Chikaraishi et al., 2005). However, this method has disadvantages such that it can be used only for alcohols, or when ester solvent is used, the substituent is transesterified.

On the other hand, because of necessity to analyze many samples for paleoenvironmental studies, a method that can analyze many compounds with a small amount of required sample and is simple is desired. Therefore, the TMAH-GCMS method is used for geological samples (e.g., Challinor, 2001; Hatcher and Clifford, 1994; Ishiwatari et al., 2006; Yamamoto et al., 2007). When sample is heated with the TMAH reagent, the ester bond and the ether bond are hydrolyzed, and the obtained products become compounds having a functional group of a carboxyl group or a hydroxyl group, and these are methyl-derivatized. Although analyses of sterols using the TMAH–GC–MS method have been applied in previous studies (Asperger et al., 1999a, 1999b; Asperger et al., 2001; Gonzalez-Vila et al., 2001; Spaccini and Piccolo, 2007), no mass

spectrums of sterol are reported. Thus, in this section, mass spectrum of methyl derivative of sterol analyzed by TMAH–GC–MS method of marine sediment sample and these characteristics and fragmentation patterns are described.

2.2. Material and methods

2.2.1. Sampling location

The sediment core used in this study was collected by the Ocean Drilling Program (ODP) Leg 167 (Hole 1017E) from the Santa Lucia Slope (34°32.099'N, 121°6.430'W; Fig. 2-1).

2.2.2. Offline TMAH–GC–MS method

Sterols and stanols were analyzed using the offline TMAH–GC–MS method. Finely powdered (< 106 μm) and dried sediment samples (~100 mg) were added to a glass ampoule with the TMAH reagent (97%, Sigma-Aldrich Co., 25 wt.% methanol; 150 μL) and with nonadecanoic-d_{37} acid (99.1%, CDN isotopes Co., 100 ng/μL in methanol; 50 μL) as an internal standard. The sample was left for 30 min in a vacuum desiccator and then dried under a stream of N_2 on a hot plate at 40 °C. The ampoule was sealed under vacuumed conditions before being placed in an oven (SS TS-13K, Isuzu Seisakusho Co.) at 300 °C for 30 min. After cooling to room temperature, the yield sample was washed four times with 300 μL of ethyl acetate. The combined extracts were dried under a vacuum desiccator and dissolved in 50 μL of ethyl acetate. Finally, 2 μL of the dissolved sample was injected and analyzed by CG–MS (6890N GC– 5973 MS; Agilent Technologies Co.). The conditions of the GC–MS were as follows: (a) 30 m long DB-5MS capillary column with a 0.25 mm internal diameter (i.d.) and 0.25 μm film thickness (Agilent Technologies Co.); (b) splitless injection type with a temperature of

300 °C; helium carrier gas at 1.0 mL/min; an oven temperature of 60 °C (2 min) to 310 °C (6 °C /min) to 310 °C (20 min); MS ionization using an electron ionization (EI) mode MS ion source with a temperature or 230 °C; an electron impact spectra to 70 eV; a quadrupole temperature of 150 °C; and a mass spectrometer in full scan ion monitoring mode (50–650 Dalton) and with an MS scanning interval of 0.5 s.

Fragmentation patterns of sterols were interpreted by comparing with previous studies of Idler et al. (1970) for that of methyl ether sterols and Henderson et al. (1972), Huang and Meinschein (1978), Smith et al. (1982 and 1983), Volkman et al. (1990), Kondo et al. (1991a, 1991b) and Yamamoto et al. (2015) for that of trimethylsilyl ether sterols.

2.3. Results and discussion

2.3.1. Detected sterols and fundamental fragment patterns

Total ion chromatogram with detected sterols is shown in Fig. 2-1. The detected sterols in the sediment are identified ten type of sterol groups (thirty-six sterols): four Δ^5-sterols, four 5α-stanols, five Δ^{22}-sterols, six $\Delta^{5,22}$-sterols, four $\Delta^{24(28)}$-sterols, five $\Delta^{5,24(28)}$-sterols, two Δ^7-sterols, four 4α-methylsterols, and two 4α-methly-Δ^{22}-sterols (Table. 2-1 and Fig. 2-2).

Basic fragmentation patterns of sterols have seven fragmentation as shown in Fig. 2-3, which are the same fragmentation patterns with the case of TMS derivatives sterols.

2.3.2. Δ^5-Sterols

As Δ^5-sterols, cholest-5-en-3β-ol (cholesterol) 24-methyl-cholest-5-en-3β-ol (campesterol), 23,24-dimethylcholest-5-en-3β-ol, and 24-ethylcholest-5-en-3β-ol (β-sito-

sterol) were identified (Fig. 2-4). Fragments at *m/z* 255, *m/z* 247, *m/z* 229, *m/z* 213, *m/z* 71, and *m/z* 82 were found for Δ^5-sterols, corresponding to loss of the side chain (Fig. 2-4).

2.3.3. 5α(H)-Stanols

As 5α(H)-stanols, 5α(H)-cholestan-3β-ol (cholestanol), 24-methyl-5α(H)-cholestan-3β-ol, 23,24-dimethyl-5α(H)-cholestan-3β-ol, 24-ethyl-5α(H)-cholestan-3β-ol (stigmastanol) were identified (Fig. 2-5). The mass spectrums of methyl ether 5α(H)-stanols were remarkably different from that of corresponding Δ^5-sterols. For 5α(H)-stanols, fragments at *m/z* 257, *m/z* 248, *m/z* 230, and *m/z* 215 were found, resulting from loss of the side chain (Fig. 2-5).

2.3.4. Δ^{22}-Sterols

As Δ^{22}-sterols, 24-norcholest-22-en-3β-ol (24-nordehydro-cholestanol), 5α(H)-cholest-22-en-3β-ol (22-dehydrocholestanol), 24-methyl-5α(H)-cholest-22-en-3β-ol (brassicastanol), 23,24-dimethyl-5a(H)-cholest-22-en-3β-ol, 24-ethyl-5α(H)-cholest-22-en-3β-ol were identified (Figs. 2-6 and 2-7). Fragments at *m/z* 316, *m/z* 301, *m/z* 287, *m/z* 257, *m/z* 229, *m/z* 215, and *m/z* 201 for Δ^{22}-Sterols were found, corresponding to loss of the side chain (Figs. 2-6 and 2-7).

2.3.5. $\Delta^{5,22}$-Sterols

As $\Delta^{5,22}$-sterols, 24-norcholesta-5, 22-dien-3β-ol (24-nor-dehydrocholesterol), 27-nor-24-methylcholesta-5, 22-dien-3β-ol, cholesta-5,22-dien-3β-ol (22-dehydro cholesterol), 24-methyl-cholesta-5, 22-dien-3β-ol (diatomsterol), 23,24-dimethyl

cholesta-5, 22dien-3β-ol, 24-ethylcholest-5, 22-dien-3β-ol (stigmasterol) were identified (Figs. 2-7 and 2-8). Fragments at *m/z* 314, *m/z* 299, *m/z* 285, *m/z* 255, *m/z* 229, *m/z* 213, and *m/z* 199 were found from $\Delta^{5,22}$-sterols, corresponding to loss of the side chain (Figs. 2-7 and 2-8).

2.3.6. $\Delta^{24(28)}$-Sterols

As $\Delta^{24(28)}$-sterols, 24-methyl-5α(H)-cholest-24(28)-en-3β-ol, 24-ethyl-5α(H)-cholest-24(28)E-en-3β-ol (fucostanol), 24-ethyl-5α(H)-cholest-24(28)Z-en-3β-ol (isofucostanol), and 24(E)-propylidene-5α(H)-cholestan-3β-ol were identified (Fig. 2-9) However, since clear spectrum of 24-ethyl-5α(H)-cholest-24(28)E-en-3β-ol was not obtained, the spectrum is not described. Fragments, resulting from loss of the side chain, at *m/z* 330, *m/z* 315, *m/z* 301, *m/z* 287, *m/z* 247, *m/z* 231, and *m/z* 215 were found for $\Delta^{24(28)}$-sterols (Fig. 2-9).

2.3.7. $\Delta^{5,24(28)}$-Sterols

As $\Delta^{5,24(28)}$-sterols, 24-methylcholesta-5,24(28)-dien-3β-ol (24- methylene-cholesterol), 24-ethylcholesta-5,24(28)E-dien-3β-ol (fucosterol), 24-ethyl-cholesta-5,24(28)Z-dien-3β-ol (isofucostero1), 24(E)- propylidene-cholest-5-en-3β-ol, and 24(Z)-propylidene-cholest-5-en-3β-ol were identified (Figs. 2-10 and 2-11). However, since clear spectrum of 24(Z)-propylidene cholest-5-en-3β-ol was not obtained, the spectrum is not described. Fragments at *m/z* 328, *m/z* 313, *m/z* 296, *m/z* 285, *m/z* 281, *m/z* 255, *m/z* 253, *m/z* 243, *m/z* 229, *m/z* 213, and others were found for $\Delta^{5,24(28)}$-sterols, as results of loss of the side chain (Figs. 2-10 and 2-11).

2.3.8. Δ⁷-Sterols

In Δ⁷-sterols, two compounds of 5α(H)-cholest-7-en-3β-ol (lathosterol) and 24-ethyl-5α(H)-cholest-7-en-3β-ol were identified (Fig. 2-11). For Δ⁷-sterols, fragments at *m/z* 255, *m/z* 229, and *m/z* 213, and ions of [M]+, [M−15]+, [M−32]+, and [M−(32+15)]+ were found, as the same pattern with that of Δ⁵-sterols having a double bond in sterol backbone.

2.3.9. 4α-Me-stanol

As 4α-Me-stanol which is 5α(H)-stanol with a methyl group at the 4-position, 4α-methyl-5 α(H)-cholestan-3β-ol, 4α,24-dimethyl-5α(H)-cholestan-3β-ol, 4α,23,24-trimethyl-5α(H)-cholestan-3β-ol (dinostanol), and 4α-methyl,24-ethyl- 5α(H)-cholestan-3β-ol were identified (Fig. 2-12). For 4α-Me-stanols, ions of [M]+, [M−15]+, [M−32]+, [M−(32+15)]+ were found which are the same pattern with Δ⁵-sterols. Additionally, fragments at *m/z* 262, *m/z* 245, and *m/z* 229 were observed, corresponding to addition of a methyl group at 4-position of 5α(H)-stanol backbone (+14 daltons, Fig. 2-12). Ions of *m/z* 71 and [M-71]+, which are characteristic ions of Δ⁵-sterol, were also found (Fig. 2-12).

2.3.10. 4α-Me-Δ²²-sterols

4α-Me-Δ²²-stanols are Δ²²-sterol with a methyl group at the 4-position, and 4α,24-dimethyl-5α(H)-cholest-22E-en-3β-ol and 4α,23,24-trimethyl-5α(H)-cholest-22-en-3β-ol (dinosterol) were identified as 4α-Me-Δ²²-stanols (Fig. 2-13). For 4α-Me-Δ22-sterols, ions of *m/z* 330, *m/z* 315, *m/z* 301, *m/z* 271, *m/z* 245, *m/z* 229, [M]+, and [M−15]+ were found (Fig. 2-13). These ions can be interpreted as fragment patterns

corresponding to Δ^{22}-sterols having a methyl function at 4-position (Fig. 2-13).

Table. 2-1 Sterols identified in the Southern California sediment (Hole 1017E-1H).

Retention Time	Symbol	Systematic Name	Trivial name	Formula	MW
Δ5α-stanol (m/z 215)					
41.340	1	5α(H)-cholestan-3β-ol	cholestanol	$C_{27}H_{48}O$	388
42.181	2	24-methyl-5α(H)-cholestan-3β-ol	campestanol	$C_{28}H_{50}O$	402
42.754	3	4α,24-dimethyl-5α(H)-cholestan-3β-ol		$C_{29}H_{52}O$	416
43.100	4	24-ethyl-5α(H)-cholestan-3β-ol	sitostanol	$C_{29}H_{52}O$	416
4α-Me-stanol (m/z 229)					
41.639	5	4α-methyl-5α(H)-cholestan-3β-ol		$C_{28}H_{50}O$	402
42.754	6	4α,24-dimethyl-5α(H)-cholestan-3β-ol		$C_{29}H_{52}O$	416
43.563	7	4α,23,24-trimethyl-5α(H)-cholestan-3β-ol	dinostanol	$C_{30}H_{54}O$	430
43.657	8	4α-methyl-24-ethyl-5α(H)-cholestan-3β-ol		$C_{30}H_{54}O$	430
Δ^5-sterol, $\Delta^{5,22}$-sterol, $\Delta^{5,24(28)}$-sterol, and Δ^7-sterol (m/z 255)					
38.881	9	24-norcholesta-5,22-dien-3β-ol	24-nordehydrocholesterol	$C_{26}H_{42}O$	370
40.256	10	27-nor-24-methylcholesta-5,22-dien-3β-ol	occelasterol	$C_{27}H_{44}O$	384
40.468	11	cholesta-5,22-dien-3β-ol	22-dehydrocholesterol	$C_{27}H_{44}O$	384
40.900	12	cholest-5-en-3β-ol	cholesterol	$C_{27}H_{46}O$	386
41.340	13	24-methylcholesta-5,22-dien-3β-ol	diatomsterol	$C_{28}H_{46}O$	398
41.568	14	5α(H)-cholest-7-en-3β-ol	lathosterol	$C_{27}H_{46}O$	386
42.023	15	24-methylcholest-5-en-3β-ol	campesterol	$C_{28}H_{48}O$	400
42.173	16	23,24-dimethylcholesta-5,22-dien-3β-ol		$C_{29}H_{48}O$	412
42.306	17	24-ethylcholest-5,22-dien-3β-ol	stigmasterol	$C_{29}H_{48}O$	412
42.833	18	23,24-dimethylcholest-5-en-3β-ol		$C_{29}H_{50}O$	414
42.935	19	24-ethylcholest-5-en-3β-ol	β-sitosterol	$C_{29}H_{50}O$	414
43.595	20	24-ethyl-5α(H)-cholest-7-en-3β-ol		$C_{29}H_{50}O$	414
Δ^{22}-sterol (m/z 257)					
39.054	21	24-norcholest-22-en-3β-ol	24-nordehydrocholestanol	$C_{26}H_{44}O$	372
40.633	22	5α(H)-cholest-22-en-3β-ol	22-dehydrocholestanol	$C_{27}H_{46}O$	386
41.505	23	24-methyl-5α(H)-cholest-22-en-3β-ol	diatomstanol	$C_{28}H_{48}O$	400
42.353	24	23,24-dimethyl-5α(H)-cholest-22-en-3β-ol		$C_{28}H_{50}O$	414
42.479	25	24-ethyl-5α(H)-cholest-22-en-3β-ol		$C_{29}H_{50}O$	414
$\Delta^{5,24(28)}$-sterol (m/z 328)					
41.945	26	24-methylcholesta-5,24(28)-dien-3β-ol	24-methylenecholesterol	$C_{28}H_{46}O$	398
42.911	27	24-ethylcholesta-5,24(28)*E*-dien-3β-ol	fucosterol	$C_{29}H_{48}O$	412
43.092	28	24-ethylcholesta-5,24(28)*Z*-dien-3β-ol	isofucosterol	$C_{29}H_{48}O$	412
43.414	29	24(*E*)-propylidenecholest-5-en-3β-ol		$C_{30}H_{50}O$	426
43.720	30	24(*Z*)-propylidenecholest-5-en-3β-ol		$C_{30}H_{50}O$	426
$\Delta^{24(28)}$-sterol, 4α-Me-Δ^{22}-sterol (m/z 330)					
42.086	31	4α,24-dimethyl-5α(H)-cholest-22-en-3β-ol		$C_{29}H_{50}O$	414
42.110	32	24-methyl-5α(H)-cholest-24(28)-en-3β-ol		$C_{28}H_{48}O$	400
42.927	33	4α,23,24-trimethyl-5α(H)-cholest-22-en-3β-ol	dinosterol	$C_{30}H_{52}O$	428
43.084	34	24-ethyl-5α(H)-cholest-24(28)*E*-en-3β-ol	fucostanol	$C_{29}H_{50}O$	414
43.257	35	24-ethyl-5α(H)-cholest-24(28)*Z*-en-3β-ol	isofucostanol	$C_{29}H_{50}O$	414
43.893	36	24*(E)*-propylidene-5α(H)-cholestan-3β-ol		$C_{30}H_{52}O$	428

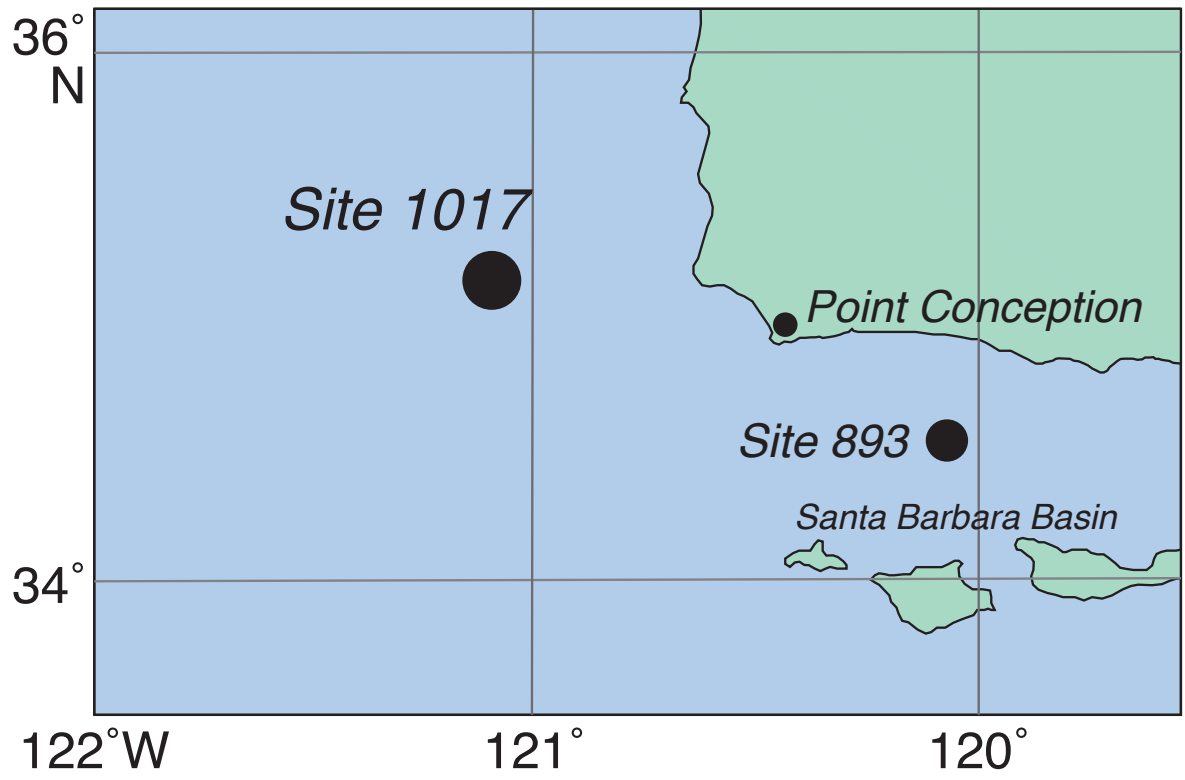

Fig. 2-1 Location map for Site 1017 and Site 893.

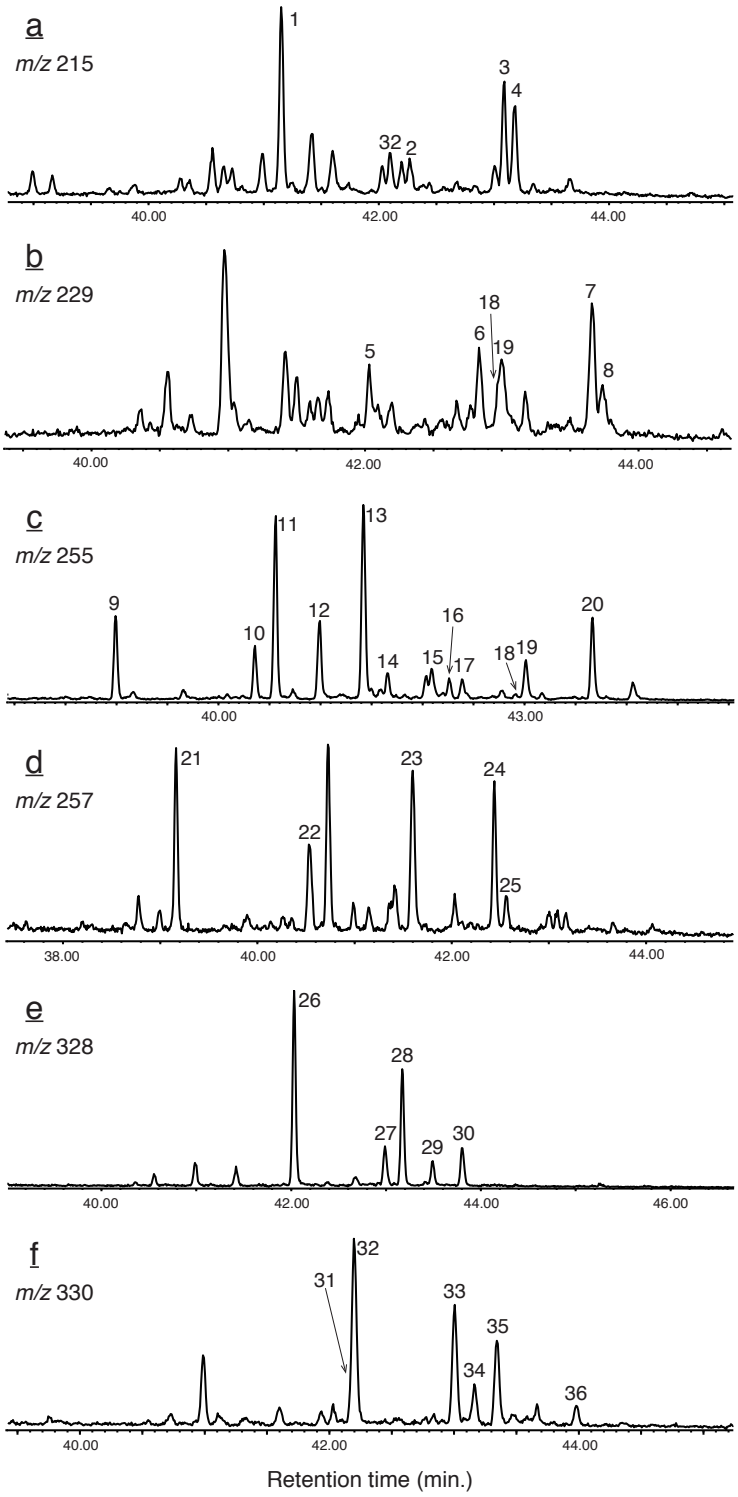

Fig. 2-2. Mass chromatograms of sterols (methyl ethers) from the sediment of ODP Leg167 Hole 1017E-1H at 19.5 cm. (a): m/z 215 mass chromatogram. (b): m/z 229 mass chromatogram. (c): m/z 255 mass chromatogram. (d): m/z 257 mass chromatogram. (e): m/z 328 mass chromatogram. (f): m/z 330 mass chromatogram. See Table 2-1 for a list of identified compounds.

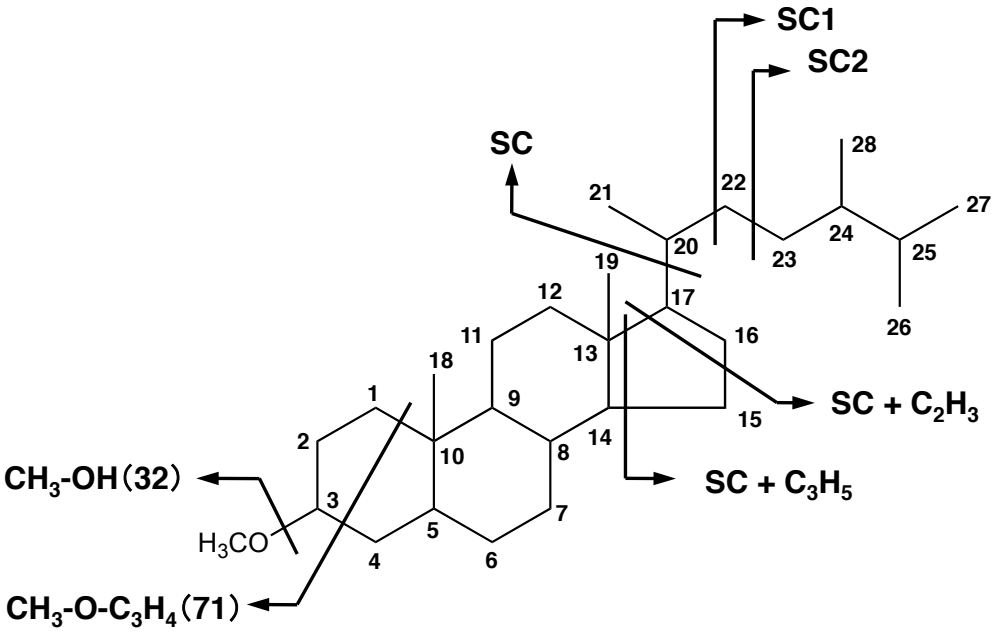

Fig. 2-3. Diagnostic fragmentation of sterols (methyl ethers).

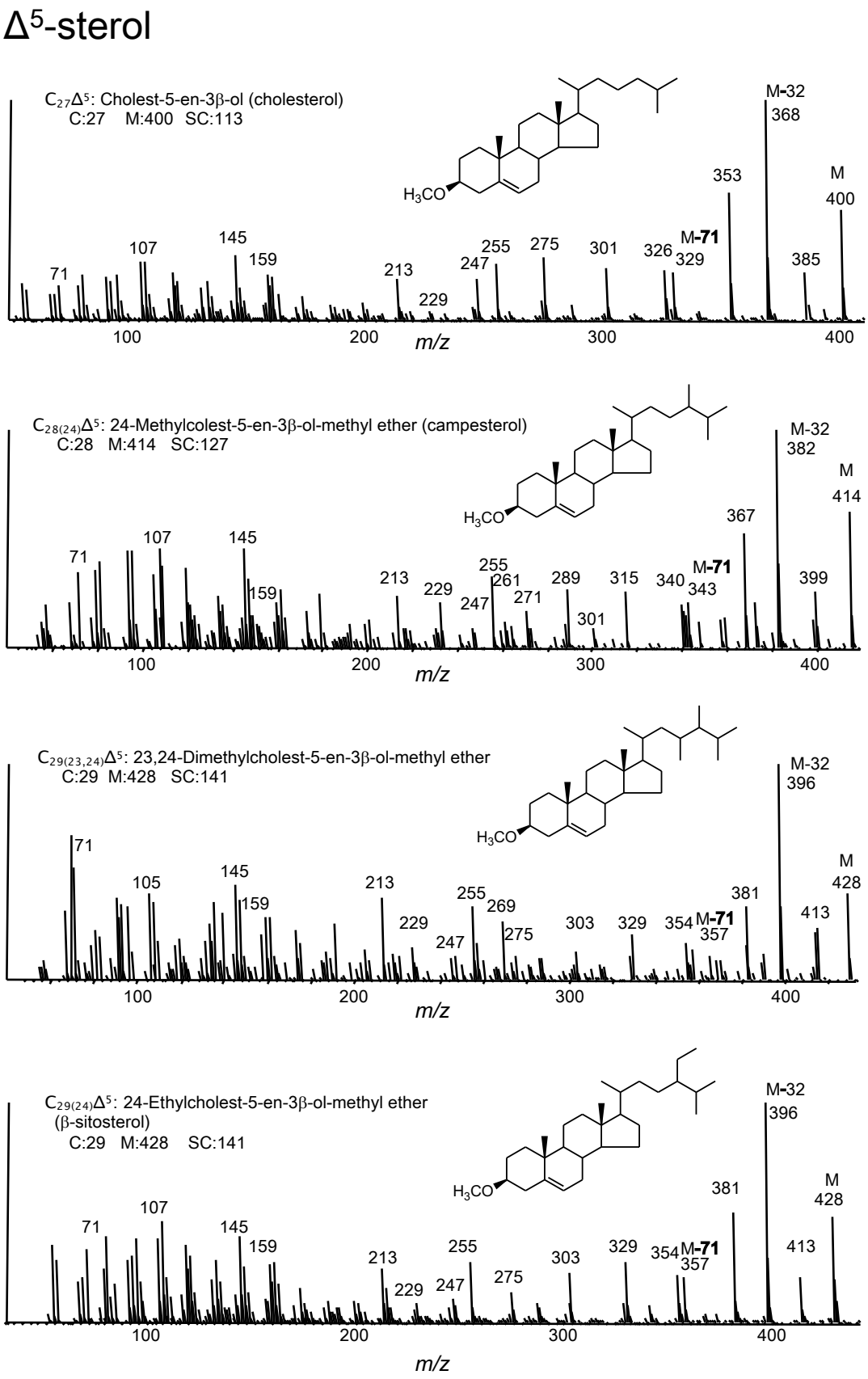

Fig. 2-4 Mass spectra of Δ^5-sterols.

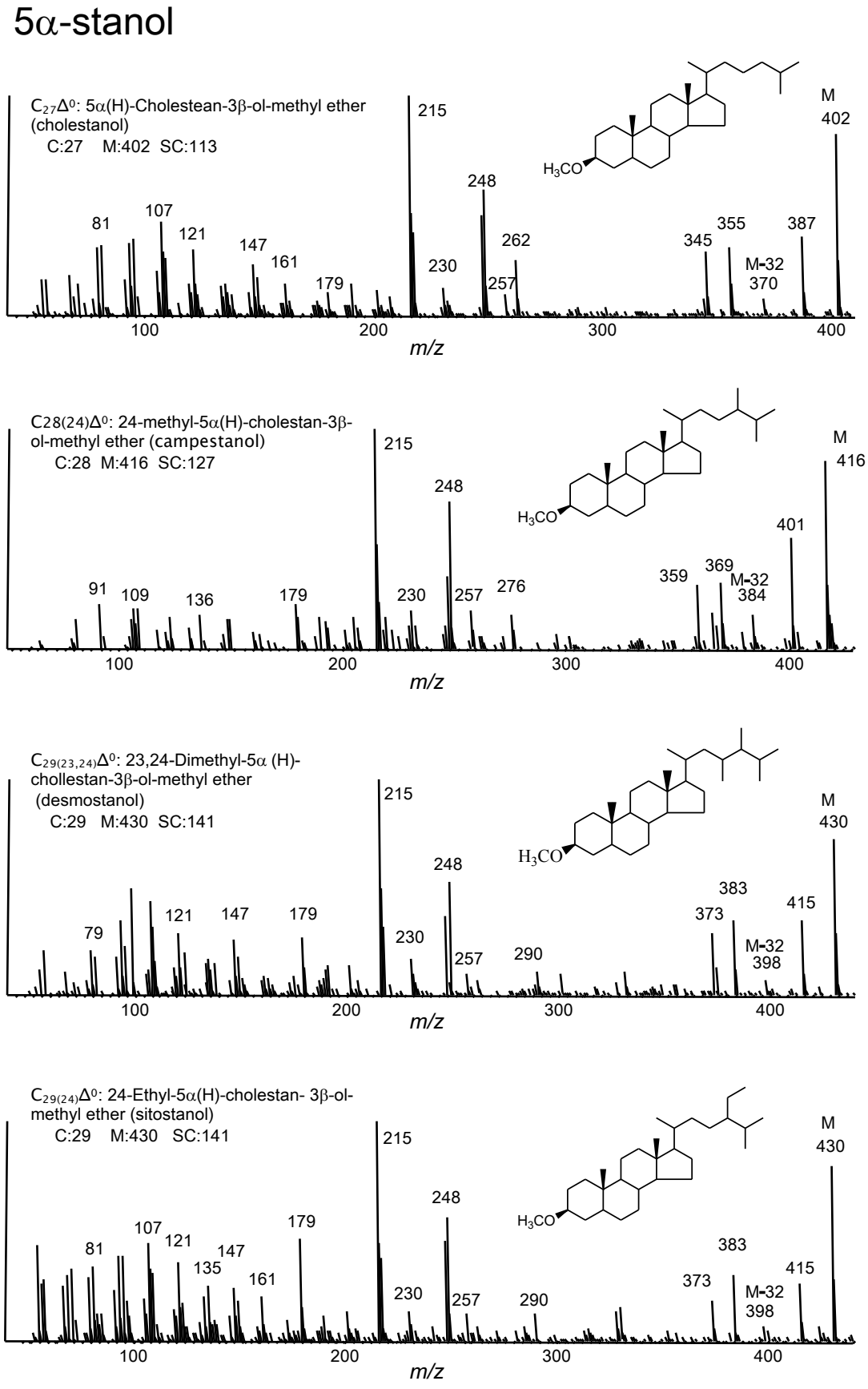

Fig. 2-5 Mass spectra of 5α(H)-stanols.

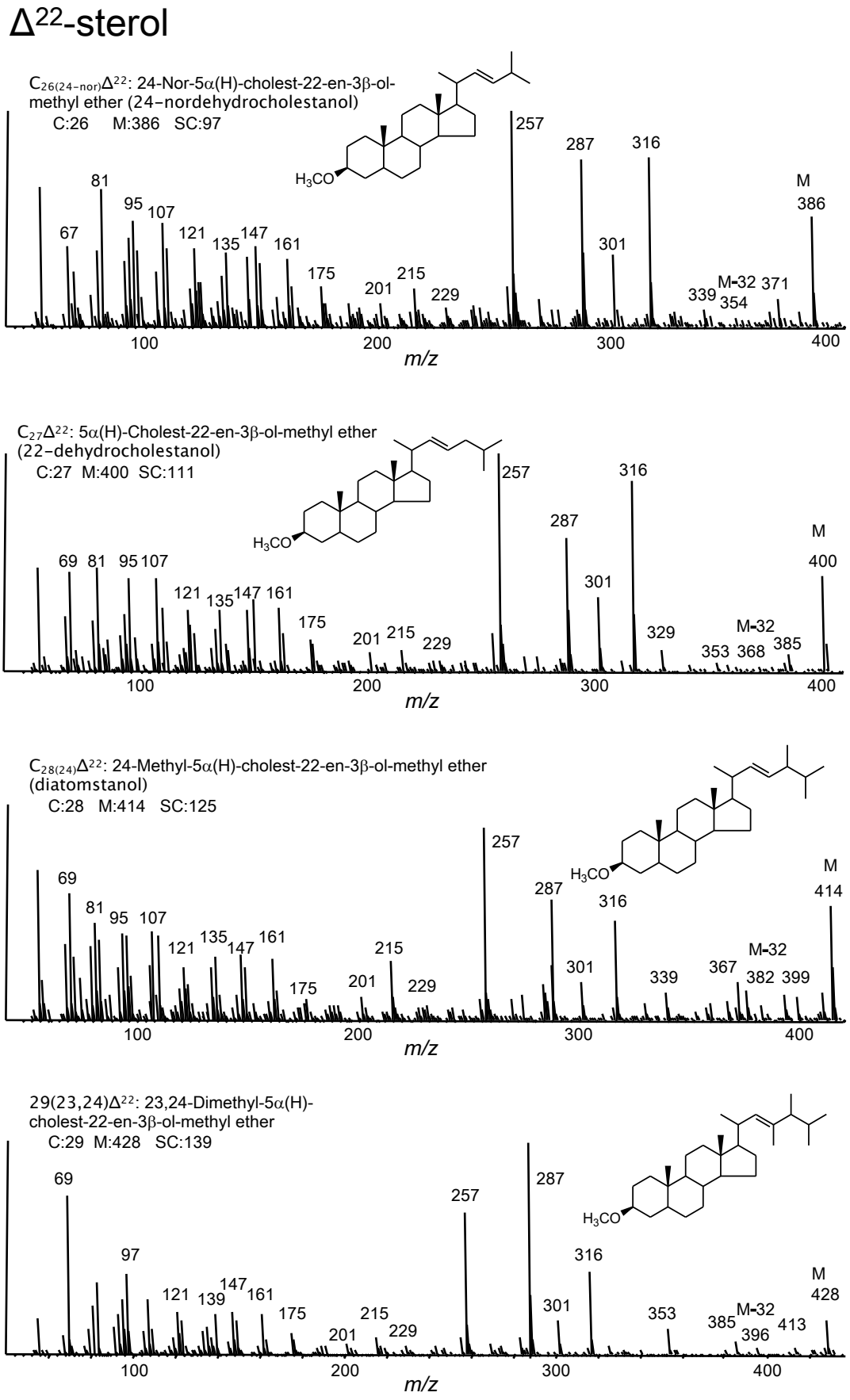

Fig. 2-6 Mass spectra of Δ^{22}-sterols.

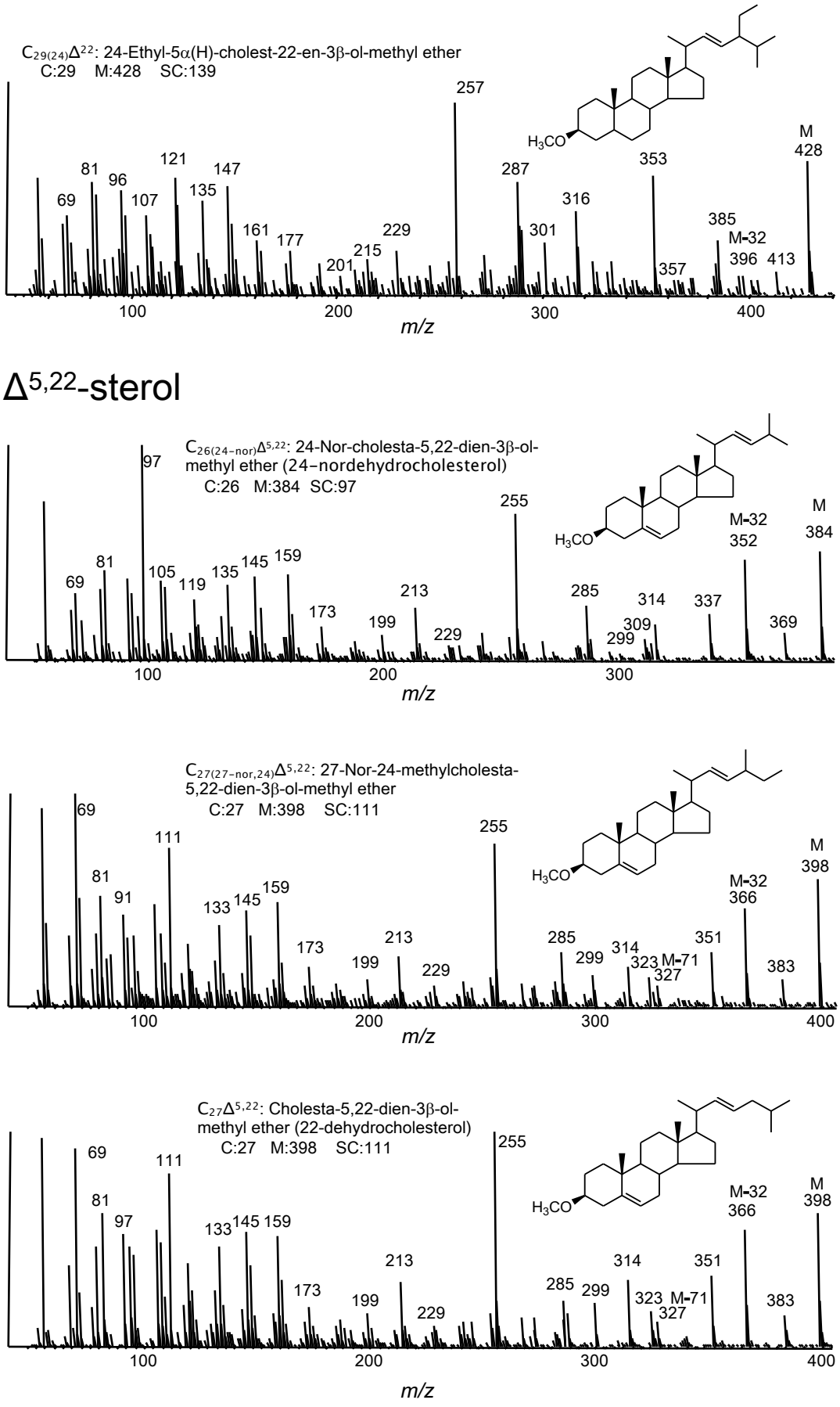

Fig. 2-7 Mass spectra of Δ^{22}-sterol and $\Delta^{5,22}$-sterols.

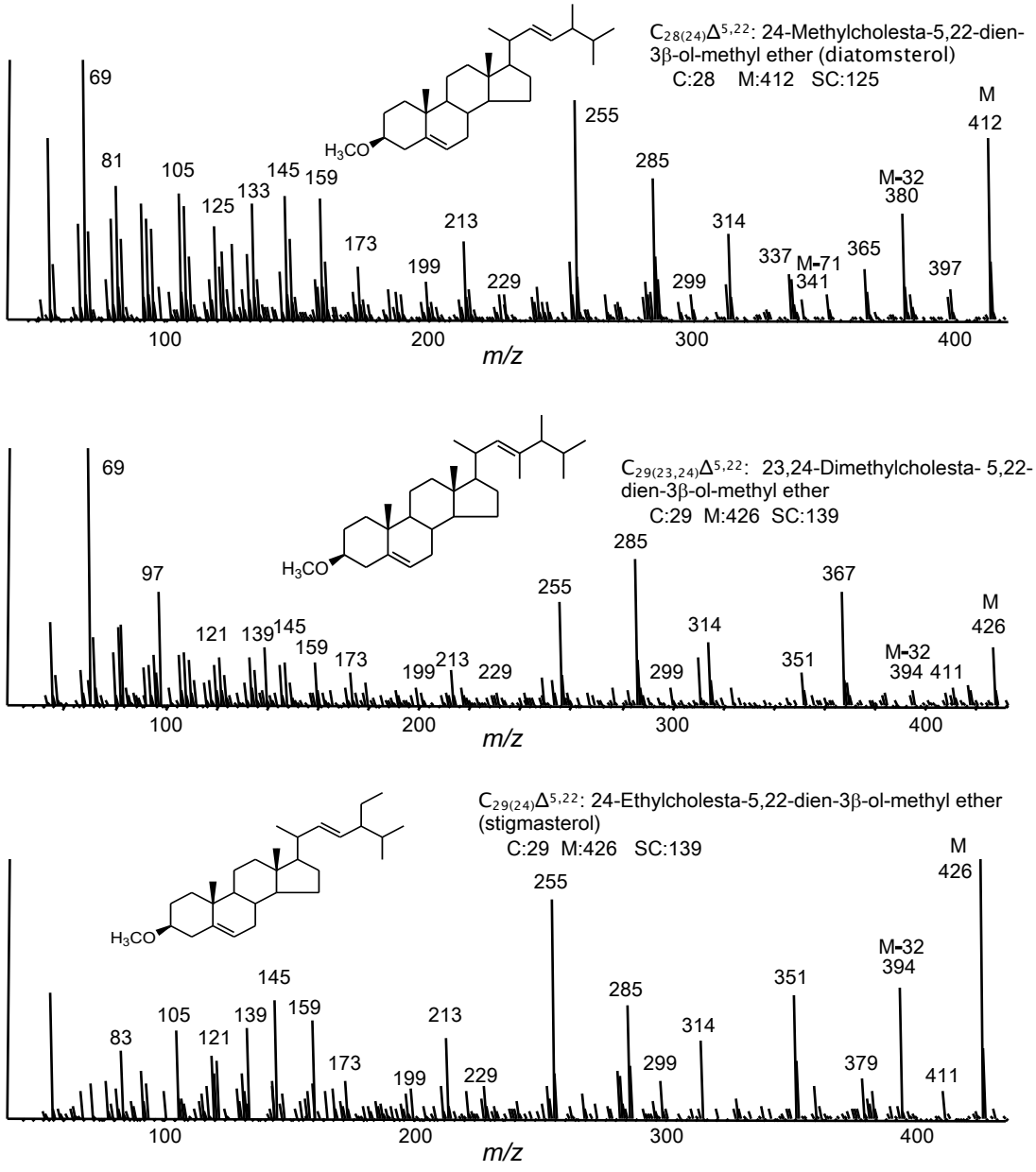

Fig. 2-8 Mass spectra of $\Delta^{5,22}$-sterols.

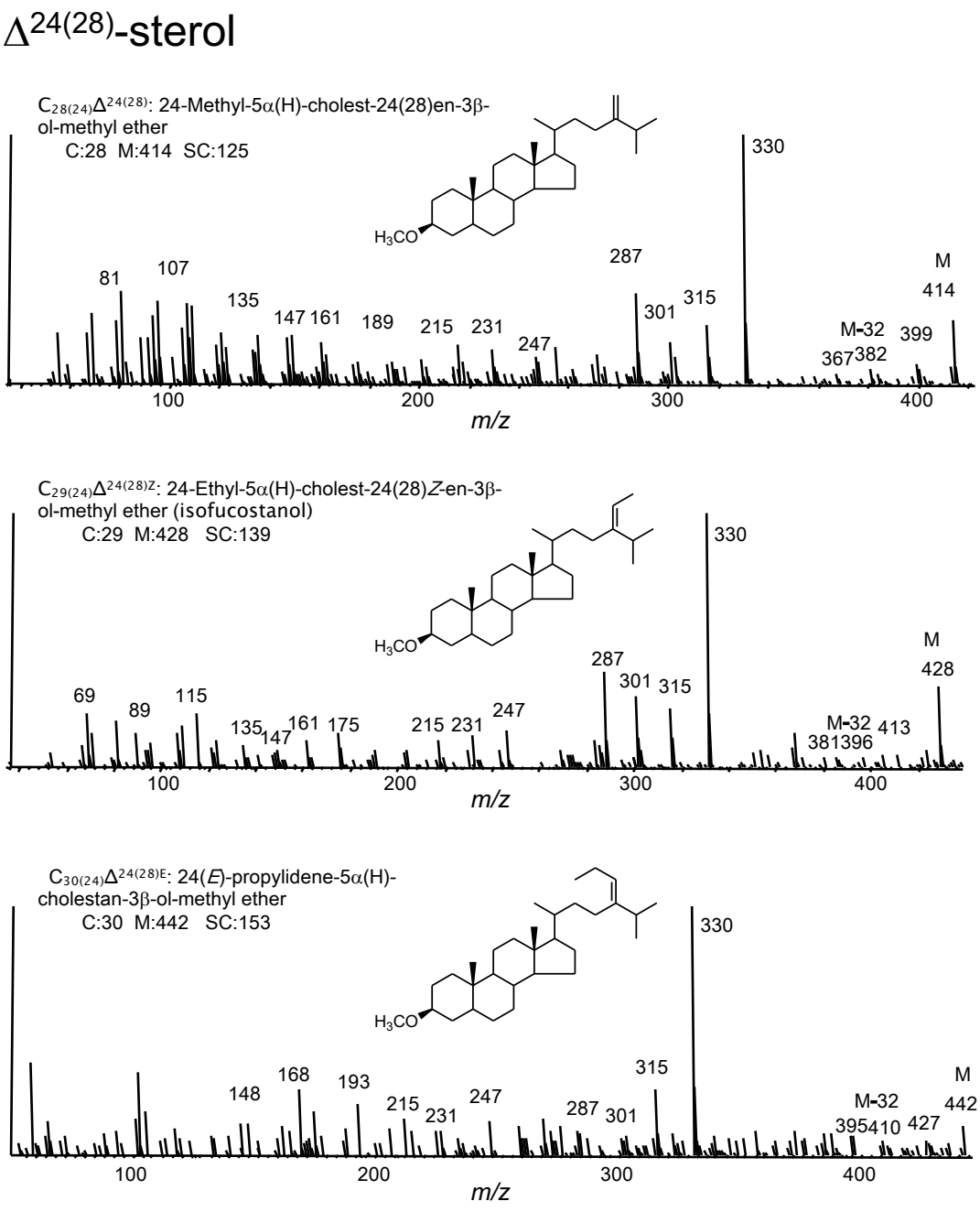

Fig. 2-9 Mass spectra of $\Delta^{24(28)}$-sterols.

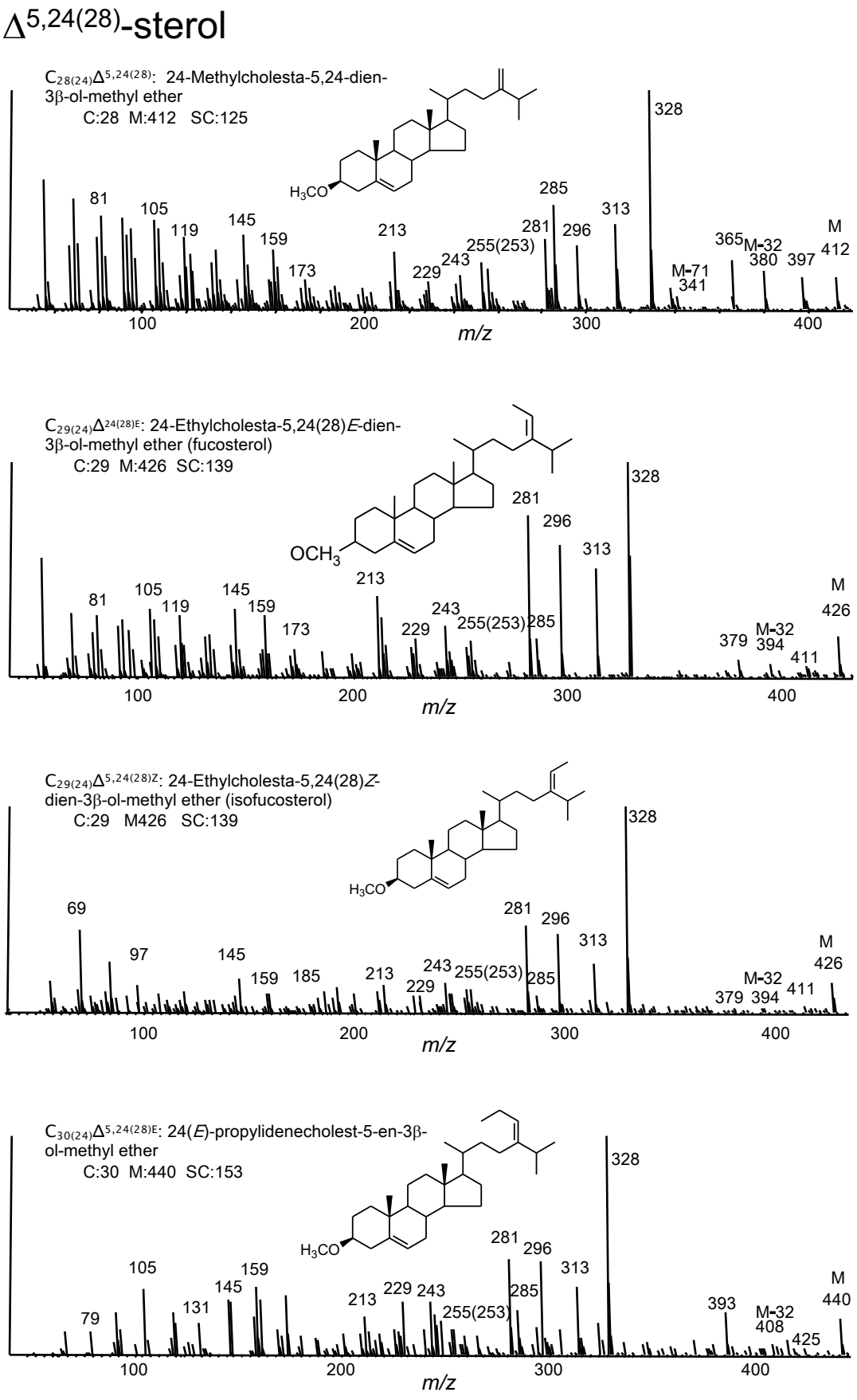

Fig. 2-10 Mass spectra of $\Delta^{5,24(28)}$-sterols.

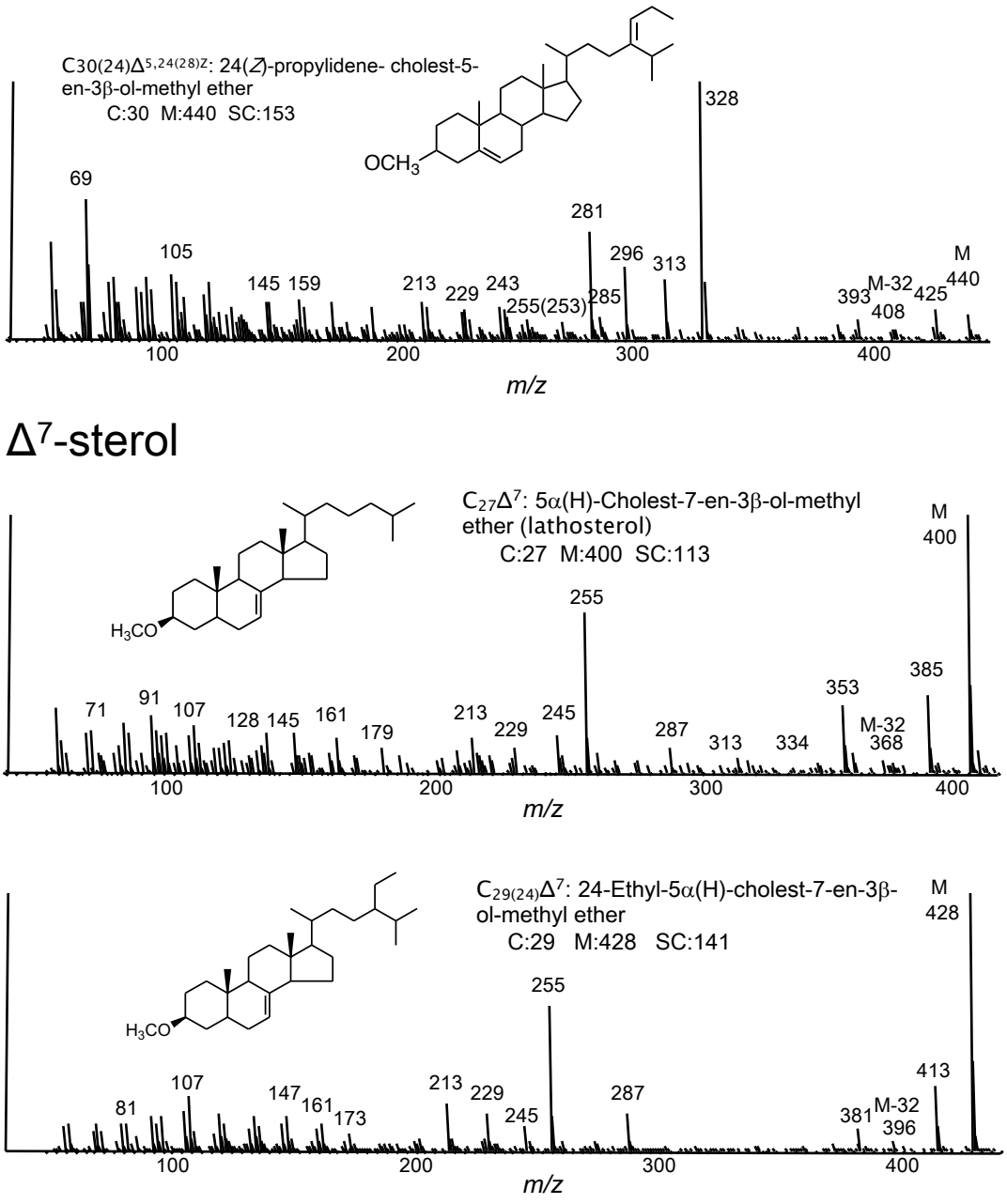

Fig. 2-11 Mass spectra of $\Delta^{5,24(28)}$-sterol and Δ^{7}-sterols.

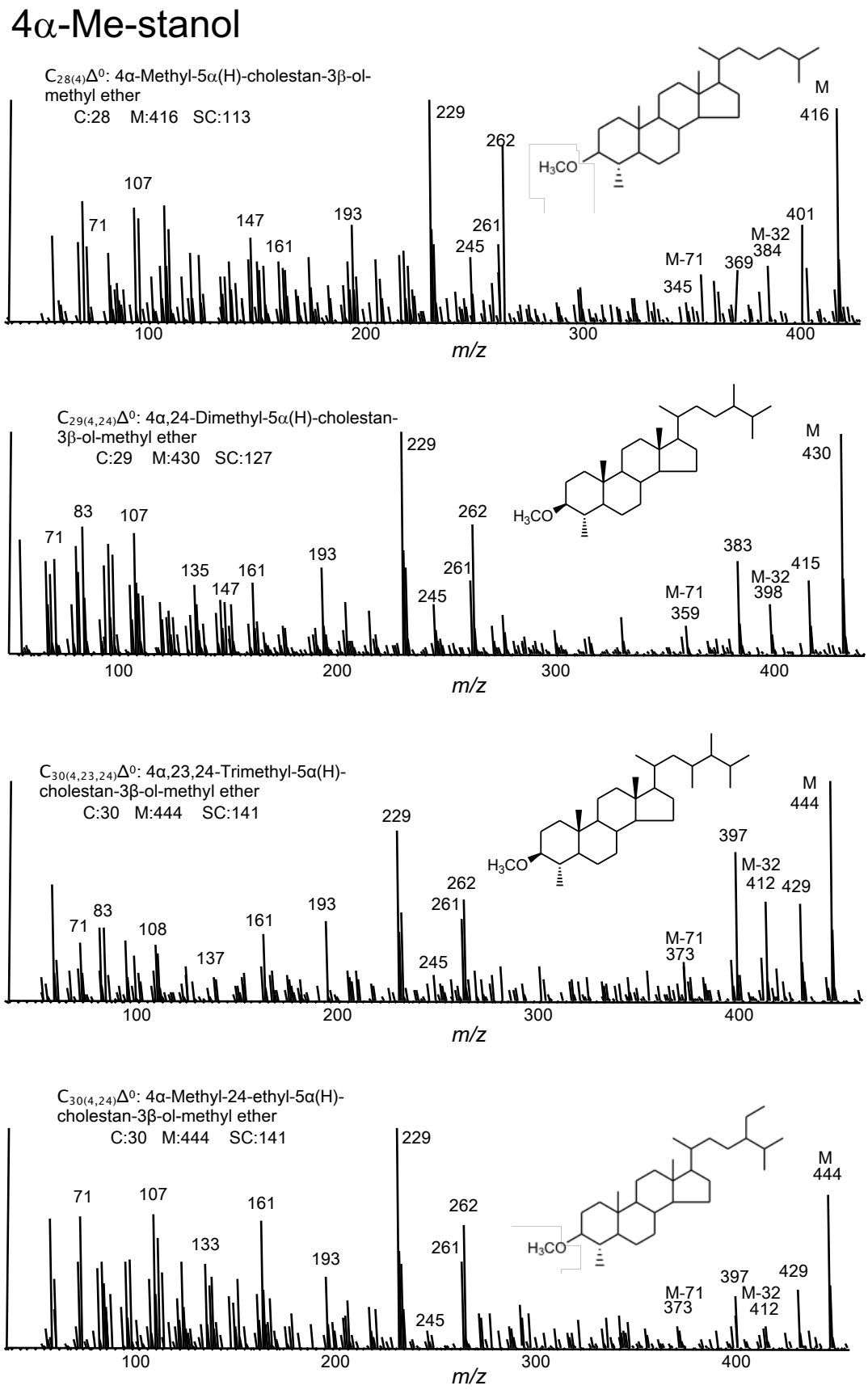

Fig. 2-12 Mass spectra of 4α-Me-stanols.

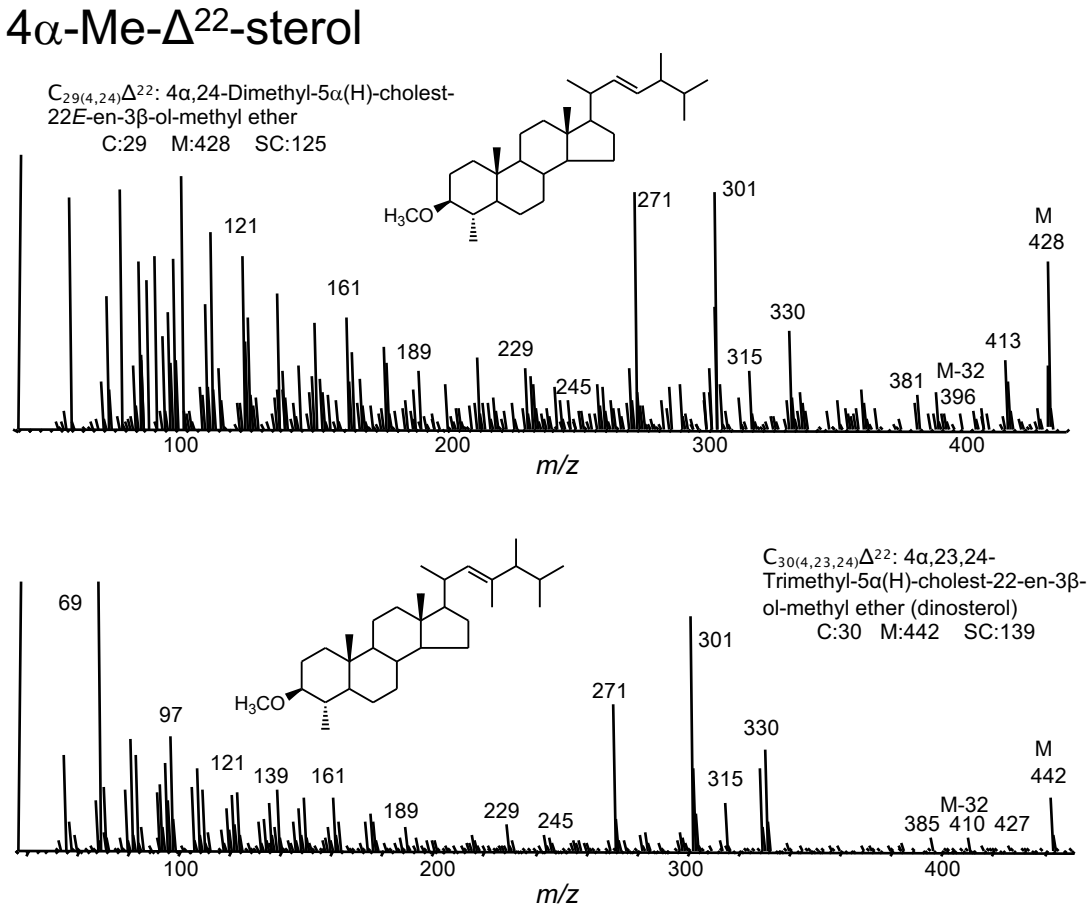

Fig. 2-13 Mass spectra of 4α-Me-Δ22-sterols.

Chapter 3

Sterol Compositions and Stanol/sterol Ratios in Late Quaternary Sediments from Southern California

3.1. Introduction

Redox events in marine or lake environments are important for understanding paleo-environmental systems. To trace paleo-anoxic conditions, various indicators have been used, including biomarkers (e.g., isorenieratene), levels of bioturbation, anoxic foraminifer, iron oxide ratios, and others (e.g., Sinninghe Damsté et al., 1993; Koopmans et al., 1996; Cannariato and Kennett, 1999; Cannariato et al., 1999; Rouxel et al., 2005). These tracers all have the advantages of being easy to analyze and of yielding robust results.

Sterol Δ^5 double bonds reduce to 5α(H)-stanols through microbiological conversion under anoxic condition (Rosenfeld and Hellman, 1971; Eyssen et al., 1973; Fig. 3-1); therefore, 5α(H)-stanol / Δ^5-sterol ratios increase significantly under anoxic conditions in the water column and surface sediments (Nishimura and Koyama, 1977; Wakeham, 1989). On this basis, 5α(H)-stanol / Δ^5-sterol ratios have been used as tracers for redox conditions. Canuel and Martens (1993) showed significant seasonal variability in the 5α(H)-stanol / Δ^5-sterol ratio recorded in surface sediments from Cape Lookout Bight, North Carolina (USA), indicating that the 5α(H)-stanol / Δ^5-sterol ratio can record environmental changes over short timescales. Furthermore, since the stanol to sterol conversion occurs in the water column and surface sediments, this tracer should be suitable for identifying paleo-environmental redox events in continual sediments sequences, allowing for the analysis of longer timescales.

To analyze Δ^5-sterol and 5α(H)-stanol, the compounds need to be extracted and derivatized for gas chromatography; however, these methods traditionally require the time-consuming extraction of a relatively large volume of organic compounds. Recently, tetramethylammonium hydroxide (TMAH) thermochemolysis, which simultaneously performs sample hydrolysis and derivatizes the produced compounds under heated

condition, has been used for analyzing organic compounds (Hatcher and Clifford, 1994; Pulchan et al., 2003; Ishiwatari et al., 2006, 2009a, 2009b). The TMAH method requires a relatively lower volume of sample material and provides high throughput; therefore, the method is suitable for high time resolution analysis that has many samples (e.g., sediment cores). Using this method on marine sediments from southern California, numerous Δ^5-sterols and 5α(H)-stanols have been detected in Chapter 2. The use of 5α(H)-stanol / Δ^5-sterol ratios as an oxic/anoxic tracer is complicated by the fact that stanols are not only derived from the microbiological reduction of Δ^5-sterols. Some organisms produce stanols directly (Fig. 3-1); for example, Robinson et al. (1984) reported freshwater-field dinoflagellate that contain 5α(H)-stanols such as cholestanol, stigmastanol and dinostanol. Furthermore, Volkman et al. (1990) reported prymnesiophyte algae of the genus Pavlova that contain significant amounts of stanols. On this basis, the use of 5α(H)-stanol / Δ^5-sterol ratio as a tracer for oxic/anoxic requires caution. In particular, ratios need to be evaluated by testing absolute compound concentrations in sediments, and should be compared with other records of anoxic events.

Along the southern California margin, the oxygen minimum zone (OMZ) could be controlled by the strength and location of Intermediate Pacific Water production and surface productivity (Kennett and Ingram, 1995; van Geen et al., 1996), which has varied with glacial-interglacial fluctuations during the late Quaternary (Cannariato and Kennett, 1999; Cannariato et al., 1999). The OMZ was strengthened in the warm periods, and was weakened in the cool periods during millennial-scale global climate changes. It is possible that 5α(H)-stanol / Δ^5-sterol ratios record this fluctuation. In this study, I attempted to evaluate specific 5α(H)-stanol / Δ^5-sterol ratios obtained using the TMAH GC–MS method for use as a proxy for oxic/anoxic events in late Quaternary

southern California sediments by comparison with OMZ proxies.

3.2. Materials and methods

3.2.1. Sampling location

The sediment core used the same with Chapter 2 (Fig. 2-1). Hole 1017E is a high-resolution upper Pliocene-to-Holocene sediment section. In ordinary core sampling, collecting the upper part of sediment is unsuitable because coring of water-sediment surface is not designed. However, since Hole 1017E was collected using Advanced Piston Coring (APC), this hole successfully captured the uppermost part of the sediment column without any hiatus (Lyle et al., 1997). Therefore, high-resolution analysis of upper Pliocene-to-Holocene is possible. At this location, the OMZ is located between ~525 and 1000 m (Cannariato and Kennett, 1999), with Site 1017 (Water depth, 955.5m) located at the bottom of the present-day OMZ. Late Quaternary OMZ variability has been extensively studied using samples from Hole 1017E (Cannariato and Kennett, 1999); therefore, the core is known to be suitable for estimating and comparing redox records. The core is cut at 3 cm intervals, with a time resolution of ~150 years per sample. After cutting, samples were freeze-dried and preserved in a refrigerator. The age model was estimated using AMS ^{14}C dating of planktonic foraminifera shells (Kennett et al., 2000). Seki et al. (2002) estimated sea surface temperature (SST) from alkenone unsaturation ratios.

3.2.2. Offline TMAH–GC–MS method

Δ^5-Sterols and 5α(H)-stanols were analyzed using the same method as described in Chapter 2. Concentrations of sterols were calculated by comparing peak areas of total ion chromatogram with that of the internal standard (nonadecanoic-d_{37}

acid). For quantifying concentrations, the total ion chromatogram areas of sterols were calculated from areas of characteristic mass fragments of each sterol using a correction factor for each of the sterols. The correction factors were calculated as ratios of total ions of each sterol to the characteristic mass fragments. For characteristic mass fragments, *m*/*z* 215 was used for cholestanol, campestanol, and sitostanol; *m*/*z* 255 was used for 24-nordehydrocholesterol, 22-dehydrocholesterol, cholesterol, diatomsterol, campesterol, and β-sitosterol; and *m*/*z* 257 was used for 24-nordehydrocholestanol, 22-dehydrocholestanol, and diatomstanol.

Significance was analyzed using the Student's T-test. Differences of $p < 0.01$ were considered to be significant.

3.2.3. Analytical reproducibility of the 5α(H)-stanol / Δ⁵-sterol ratio results

Effect of the TMAH process on the stanol-to-sterol ratio was confirmed using cholesterol (analytical grade reagent, Wako) and cholestanol (> 95%, Sigma) reagents. Then, methylated cholesterol and cholestanol were detected, and no change in stanol-to-stanol ratio and other compounds was confirmed under the TMAH reaction conditions. This implied that the TMAH process does not alter stanol-to-sterol ratios.

In this study, each sample was analyzed once. However, two samples (41.7 cm; 1.84 ka and 254.3 cm; 11.39 ka) were analyzed three times to determine the analytical reproducibility of the 5α(H)-stanol / Δ^5-sterol ratio results using the offline TMAH–GC–MS method. The average coefficients of variations for 24-nordehydrocholestanol / 24-nordehydrocholesterol, diatomstanol / diatomsterol, cholestanol / cholesterol, campestanol / campesterol, sitostanol / β-sitosterol, and 22-dehydrocholestanol / 22-dehydrocholesterol were ±5% (41.7 cm, ratio; 0.66 ± 0.03) and ±9% (254.3 cm, 0.53 ± 0.05), ±9% (0.32 ± 0.03) and ±10% (0.21 ± 0.02), ±8% (0.25 ± 0.02) and ±10% (0.21 ± 0.02),

±5% (0.38 ± 0.02) and ±4% (0.46 ± 0.02), ±3% (0.78 ± 0.02) and ±9% (0.87 ± 0.08), and ±8% (0.13 ± 0.01) and ±7% (0.15 ± 0.01), respectively. Hence, the analytical error of 5α(H)-stanol / Δ^5-sterol ratios was < 10%.

3.3. Results

3.3.1. Sterols and stanols in southern California marine sediments

Based on previous cahpetr on the mass fragment interpretation of sterols and stanols in TMAH reaction from the sediment, I chose six pairs of 5α(H)-stanols and Δ^5-sterols with relatively high abundance for analysis. Absolute sterol concentrations vary between pairs; however, temporal trends are similar (Fig. 3-2). The mean concentrations of Δ^5-sterol and 5α(H)-stanol are shown in Table 3-1. The concentrations of 24-methylcholesta-5,22E-dien-3β-ol (diatomsterol or brassicasterol), cholest-5-en-3β-ol (cholesterol) and cholesta-5,22E-dien-3β-ol (22-dehydrocholesterol) were relatively high (diatomsterol; 1.98 ± 1.40 μg/g-ds [dey sediment], cholesterol; 1.99 ± 1.53 μg/g-ds, 22-dehydrocholesterol; 1.36 ± 1.18 μg/g-ds). Among stanols, 24-methyl-5α(H)-cholest-22E-en-3β-ol (diatomstanol or brassicastanol) and 5α(H)-cholestan-3β-ol (cholestanol) showed a relatively high concentration (diatomstanol; 0.48 ± 0.40 μg/g-ds, cholestanol; 0.46 ± 0.35 μg/g-ds). I found that 24-ethylcholest-5-en-3β-ol (β-sitosterol) and 24-ethyl-5α(H)-cholestan-3β-ol (sitostanol) varied with almost the same range (β-sitosterol; 0.52 ± 0.46 μg/g-ds, sitostanol; 0.40 ± 0.38 μg/g-ds). All Δ^5-sterol and 5α(H)-stanol concentrations increased from the Holocene warming interval onward.

3.3.2. 5α(H)-stanol / Δ^5-sterol variability

Mean 5α(H)-stanol / Δ^5-sterol ratios are shown in Table 3-2. All Δ^5-sterol

concentrations were higher than 5α(H)-stanol concentrations, with the exception of β-sitosterol to sitostanol. The sitostanol / β-sitosterol ratio varied from 0.20 to 1.09, and was over "one" for some periods (Fig. 3-3e). The ratios of 24-norcholest-22E-en-3β-ol (24-nordehydrocholestanol) to 24-norcholesta-5,22E-dien-3β-ol (24-nordehydrocholesterol) and 24-methyl-5α(H)-cholestan-3β-ol (campestanol) to 24-methylcholest-5-en-3β-ol (campesterol) showed similar ranges (24-nordehydrocholestanol / 24-nordehydrocholesterol; 0.49 ± 0.09, campestanol / campesterol; 0.39 ± 0.12). The diatomstanol / diatomsterol and cholestanol / cholesterol ratios also varied over the same range (diatomstanol / diatomsterol; 0.23 ± 0.03, cholestanol / cholesterol; 0.23 ± 0.03). The 24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios increased during warming periods of the Dansgaard-Oeschger (D/O) cycle and from the Bølling-Ållerød (B/A) interval onwards, and decreased during cooling periods of the D/O cycle and Heinrich events (Fig. 3-3a–b). In contrast, although the cholestanol / cholesterol and campestanol / campesterol ratios increased during warming intervals of the D/O cycle during MIS3, they did not increase from the B/A interval onward (Fig. 3-4c–d). The ratios of sitostanol / β-sitosterol and 5α(H)-cholest-22E-en-3β-ol (22-dehydrocholestanol) / 22-dehydrocholesterol showed no trend with warming and cooling intervals (Fig. 3-3e–f).

3.4. Discussion

3.4.1. Records of 5α(H)-stanol / Δ^5-sterol

Bioturbation-level and benthic foraminiferal record OMZ variability during the late Quaternary, and strongly imply that the OMZ varied with warm and cool intervals (Behl and Kennett, 1996; Cannariato and Kennett, 1999; Cannariato et al., 1999). The OMZ fluctuations at Site 1017E reconstructed using benthic foraminiferal records

(Cannariato and Kennett, 1999) have been roughly divided into three sections: (1) "suboxic" conditions at 0.6–0.8 km water depth during modern times, interstadial periods, and during the last glacial maximum (suboxic in Fig. 3-3h); (2) a "dysoxic" zone at 0.4–1.1 km water depth during interstadial periods 14, 11, 8, B/A, and the earliest Holocene (dysoxic in Fig. 3-3h); and (3) an "oxic" zone at 0.75–0.8 km water depth during stadial periods (oxic in Fig. 3-3h). These fluctuations were significant during MIS3 and exhibited a millennial-scale cycle (D/O cycle). Cholestanol / cholesterol, 24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios increased during some intervals of MIS3 interstadial periods (e.g., interstadial periods 4–11) and decreased during MIS3 cooling events (especially Heinrich events 3 and 4; Fig. 3-3). Student T-test results showed significant differences between the 5α(H)-stanol/Δ^5-sterol ratios of interstadial and stadial periods of MIS3 ($p < 0.001$; Fig. 3-4a–b); although both show an increasing trend from the B/A interval onward during stable anoxic condition (Fig. 3-3a–b). These results confirm that the 24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios record oxic/anoxic conditions. The significant relationship between the two ratios (R = 0.667; Fig. 3-5) implies that both recorded the same event. I speculate that two ratios did not show unity because diatomsterol might be less affected by redox conditions than 24-nordehydrocholesterol or because of differences in conversion rates from Δ^5-sterol to 5α(H)-stanol caused by a difference in habitat depth of origins for each sterol source.

In contrast, the other 5α(H)-stanol/Δ^5-sterol ratios considered (cholestanol / cholesterol, campestanol / campesterol, sitostanol / β-sitosterol, and 22-dehydrocholestanol / 22-dehydrocholesterol) showed no significant correlations (p = 0.073, p = 0.14, p = 0.89, and p = 0.48, respectively) during MIS3 (Fig. 3-4c–f). Although

the cholestanol / cholesterol ratio varied with oxic/anoxic events during MIS3 (Fig. 3-3c), the fluctuations are not significant, and the ratio did not increase from the B/A interval onward. These results indicate that these 5α(H)-stanol / Δ^5-sterol ratios are not useful as tracers for redox events in the southern California region.

3.4.2. Detailed comparison between stanol/sterol ratio and other redox records

In order to increase the credibility of the 5α(H)-stanol / Δ^5-sterol ratios (24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios) as a redox indicator, I performed a detailed comparison with other redox records from Hole 1017E (Fig. 3-6). Since 24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios showed similar trends (Fig. 3-5), only the 24-nordehydrocholestanol / 24-nordehydrocholesterol ratio was chosen for comparison in Fig. 3-7.

Tada et al (2000) reconstructed redox conditions of bottom-water using changes in degree of pyritization (ratio of pyrite Fe within total Fe; DOP_T) from Hole 1017E (Fig. 3-6c). This tracer (DOP_T) was used to perform a relative evaluation of bottom-water oxygenation. High DOP_T suggests a less oxic condition (Berner, 1984; Calvert and Karlin, 1991; Tada et al., 1992; Tada et al., 1999). Variations in DOP_T tended to increase during the B/A interval and MIS3 interglacial periods and during the Last Glacial Maximum (LGM). These trends were similar to the 5α(H)-stanol / Δ^5-sterol ratios. On the other hand, the tendency of DOP_T in the Holocene period lower than the B/A interval was different from the relatively stable 5α(H)-stanol / Δ^5-sterol ratios from the B/A interval onward (Fig. 3-6b–c). In another research finding, Cannariato and Kennett (1999) reconstructed redox conditions using benthic foraminiferal assemblages from Hole 1017E (Fig. 3-6d). Benthic foraminiferal species were classified into dysoxic,

suboxic, and oxic, according to differences in bottom-water oxygenation (Cannariato and Kennett, 1999). Interglacial periods were characterized by a high rates of dysoxic and suboxic I species, indicating less oxic conditions during interglacial periods. These species also increased in the LGM. On the other hand, during glacial periods, increases in suboxic II and oxic species were observed, indicating that glacial periods had relatively oxic conditions. These trends were consistent with the trend of the 5α(H)-stanol / Δ^5-sterol ratios. In particular, the two records of OMZ fluctuations in the interstadials and stadials of MIS 3 were in good agreement (Fig. 3-6b and d). On the other hand, dysoxic and suboxic I species increased from the B/A interval to the earliest Holocene, implying a less strong oxic condition. However, after that, those species showed a gradual decrease to the present, and this trend was similar to the trend of DOP_T. These trends were not consistent with the 5α(H)-stanol / Δ^5-sterol ratios. As a whole, although there was a different tendency, the 5α(H)-stanol / Δ^5-sterol ratios show a tendency similar to other records, and the 5α(H)-stanol / Δ^5-sterol ratios recorded redox events in southern California as an independent indicator.

The discrepancy between the 5α(H)-stanol / Δ^5-sterol ratios and other records around the B/A interval and the Holocene is probably due to the different characteristics of each record. In surface sediments, sulfate ions present in water bodies are reduced to hydrogen sulfide by bacterial action, and then pyrite is produced from reaction of iron with hydrogen sulfide (Berner, 1984). Therefore, the DOP_T records redox conditions at the surface sediment. In addition, the benthic foraminiferal assemblage records were classified according to differences in bottom-water oxygenation. On the other hand, since conversion from 5α(H)-sterol to Δ^5-stanol occurs in water column and surface sediments (Nishimura and Koyama, 1977; Wakeham, 1989), the 5α(H)-stanol / Δ^5-sterol ratios record not only the sediment surface condition but also the redox condition of

water column. Therefore, the differences between the 5α(H)-stanol / Δ^5-sterol ratios and the other two tracers were probably due to the difference in this redox state recording range. Benthic foraminiferal assemblage records of Site 893 (more shallow than Site 1017; water depth 576.5 m) show a stable high rate of dysoxic species, suggesting that the shallow water column was stable under less oxic conditions during the Holocene in southern California (Cannariato et al., 1999). This trend is consistent with the relatively stable trend of the 5α(H)-stanol / Δ^5-sterol ratios in the Holocene. It seems that this characteristics of 5α(H)-stanol / Δ^5-sterol ratio should be considered when applied to other samples. Therefore, the 5α(H)-stanol / Δ^5-sterol ratio can be used as an independent index for a redox record, but redox condition is preferably interpreted by comparing with other records to reconstruct detailed paleo-environment analysis.

3.4.3. Origin of 5α(H)-stanol and Δ⁵-sterol

Before Δ^5-sterols and 5α(H)-stanols ratios can be used to reconstruct redox conditions, their formation by planktonic organisms must be discounted. Diatomsterol is found in various phytoplankton, including dinoflagellates (Teshima et al., 1980; Goad and Withers, 1982), diatoms (Kanazawa et al., 1971; Orcutt and Patterson, 1975; Ballantine et al., 1979; Gillan et al., 1981), and prymnesiophyte algae (Berenberg and Patterson, 1981; Volkman et al., 1981; Lin et al., 1982; Marlowe et al., 1984). In addition, 24-nordehydrocholesterol can be derived from phytoplankton, and especially from diatoms (Smith et al., 1982), and has also been identified in zooplankton such as jellyfish and amphipods (Nelson et al., 2000; 2001). It is reported that the reduction of Δ^5-sterol to 5α(H)-stanol under anoxic conditions by bacteria occurs at the Δ^5 double bond, but not at the Δ^{22} and Δ^7 double bonds (Eyssen et al., 1973); therefore, diatomsterol and 24-nordehydrocholesterol can be converted to diatomstanol and 24-nordehydrocholestanol,

respectively, under anoxic condition by bacterial reduction. In organisms that create brassicastanol and 24-nordehydrocholestanol directly (e.g., zooplanktons such as jellyfish), the ratios of diatomstanol / diatomsterol and 24-nordehydrocholestanol / 24-nordehydrocholesterol are relatively high, ~28 and ~19, respectively (Nelson et al., 2000; 2001). However, there is a low possibility that these zooplankton could be the main source of organic compounds. Although there are some cases where sterols (cholesterol and 22-dehydrocolesterol) make significant contributions to sediment from molts and fecal pellets of zooplankton (e.g., copepods and euphausiids; Gagosian et al., 1983), most sedimentary sterols are contributed by phytoplanktonic and terrigenous sources (e.g., Huang and Meinschein, 1976; Gagosian et al., 1983; Volkman, 1986). Furthermore, sedimentary sterols (brassicasterol, cholesterol, β-sitosterol) and stable carbon isotope values in core 1017E have been shown to have a phytoplanktonic origin (Matsumoto et al., 2000). The low biomass and content of the two stanols suggest that the contribution from red algae is low (Chardon-Loriaux et al., 1976). *Gymnodinium sanguineum* (a species of dinoflagellate) has a high diatomstanol content (~30% of total sterols; Mansour et al., 1999); therefore, where it is present in abundance, 5α(H)-stanol / Δ^5-sterol ratios cannot be used as a tracer for oxic/anoxic conditions. However, *Gymnodinium sanguineum* is not the primary phytoplankton taxon in southern California (Goodman et al., 1984); therefore, its contribution was not taken into account in this study. In summary, there is little or no input of stanols from living organisms in the sediment, and the main sources of diatomstanol and 24-nordehydrocholestanol are the reduction of bacteria under anoxic conditions. In particular, because organisms that create 24-nordehydrocholestanol are restricted to just a few low-abundance marine plankton species (Chardon-Loriaux et al., 1976), the 24-nordehydrocholestanol / 24-nordehydrocholesterol ratio provides the best tracer for anoxic conditions in late

Quaternary sediments. However, it should be noted that the results gained using this method should be considered with caution. While their full characterization was beyond the scope of this study, sterol diagenetic and degradation processes can influence 5α(H)-stanol / Δ^5-sterol ratios. Sterols convert to sterene by dehydration, and it is reported that this reaction can occur in marine surface sediment (Gagosian and Farrington, 1978). Volkman (1986) showed that marine sterols are relatively easier to degrade than terrestrial sterols (Volkman, 1986). It has also been suggested that Δ^5-sterols more easily decompose under oxic condition relative to 5α(H)-stanols (Arzayus and Canuel, 2005; Bogus et al., 2012).

Previous studies into the stanols and sterols in organisms and sediments have shown parallel distributions, which suggests that the contribution of living organisms to recent sediments cannot always be ignored (Nishimura and Koyama, 1976; Nishimura and Koyama, 1977). This is consistent with our results, where the other 5α(H)-stanol / Δ^5-sterol (cholestanol / cholesterol, campestanol / campesterol, sitostanol / β-sitosterol, and 22-dehydrocholestanol / 22-dehydrocholesterol) ratios were found to be unsuitable as redox tracers (Fig. 3-4). This likely reflects the contribution of organisms that produce stanols directly (Table 1); for example, cholestanol is observed in dinoflagellates, haptophyte, and red algae (Chardon-Loriaux et al., 1976; Volkman et al., 1990; Mansour et al., 1999). In particular, the Scrippsiella species of dinoflagellate has high content of cholestanol (cholestanol/cholesterol ratio, <122). Campestanol is observed in dinoflagellates (Volkman et al., 1990; Mansour et al., 1999), where campestanol / campesterol ratios are <3.8. Fresh water *Peridinium lomnickii* has significant cholestanol and campestanol contents (cholestanol / cholesterol ratio = ~100; campestanol / campesterol ratio = ~1.8). Finally, despite its relatively low concentrations, sitostanol was obtained from *Pavlova lutheri* (sitostanol / β-sitosterol

ratio, <0.09) (Volkman et al., 1990).

In this study, the sitostanol / β-sitosterol ratio in the sediment was over "one" for some periods, likely reflecting a contribution of converted sitostanol from terrestrial-derived β-sitosterol during transport by degradation, reduction, and/or contribution from an unreported rich source of organism-derived sitostanol. Regarding 22-dehydrocholestanol / 22-dehydrocholesterol, it has previously been reported that sources of 22-hydrochorestanol are confined to a small number of organisms (e.g., red algae; Chardon-Lariaux et al., 1976); therefore, it was thought this ratio would be a useful tracer for redox conditions. However, no significant trend was observed, suggesting the existence of unreported sources of organism-derived-22-hydrochorestanol and/or different decomposition process from other sterols.

3.5. Conclusions

The results of this study show that some, but not all, 5α(H)-stanol / Δ^5-sterol ratios are useable as anoxic tracers. In particular, 24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios recorded variability based on anoxic/oxic event related to glacial-interglacial cycles in marine sediments from southern California. The results of this study have also confirmed the suitability organic compound analysis using the TMAH GC–MS method for reconstructing redox conditions. Data from this study show that 24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios increased during warming intervals of MIS3 and from the B/A interval onwards, but decreased during stadial periods of MIS3 and during Heinrich events. On the other hand, other 5α(H)-stanol / Δ^5-sterol ratios did not record redox events because sources of stanol were not only derived from the reduction products of bacteria, but were also derived from stanol-creating organisms. Based on

this result, the use of 5α(H)-stanol / Δ^5-sterol ratios as a redox tracer should be taken with caution, ensuring that appropriate selection of Δ^5-sterols and 5α(H)-stanols.

Table 3-1. Mean sterol and stanol concentrations (mean ± standard deviation: μg/g-ds [dry sediment]) and presumed sources

Compounds	Mean concentration	Presumed sources	References
Δ^5-Sterols			
24-Nordehydrocholesterol	0.60 ± 0.33	Phytoplankton (diatom), zooplankton (jellyfish, amphipods)	Smith et al., 1982; Nelson et al., 2000; 2001
Diatomsterol	1.98 ± 1.40	Phytoplankton (diatoms, dinoflagellates, prymnesiophyceae algae)	Kanazawa et al., 1971; Orcutt and Patterson, 1975; Ballantine et al., 1979; Teshima et al., 1980; Berenberg and Patterson, 1981; Gillan et al., 1981; Volkman et al., 1981; Goad and Withers, 1982; Lin et al., 1982; Marlowe et al., 1984
Cholesterol	1.99 ± 1.53	Phytoplankton, zooplankton	Volkman, 1986; Volkman et al., 1998
Campesterol	0.35 ± 0.23	Phytoplankton (cyanobacteria, diatom, dinoflagellate, green algae), terrestrial plant	Volkman, 1986; Volkman et al., 1990; Mansour et al., 1999; Killops and Killops, 2013
β-Sitosterol	0.52 ± 0.46	Phytoplankton, terrestrial plant	Paoletti et al., 1976; Matsumoto et al., 1982; Killops and Killops, 2013
22-Dehydrocholesterol	1.36 ± 1.18	Phytoplankton (diatom, dinoflagellate, bangiophyceae)	Beastall et al., 1971, 1974; Orcutt and Patterson, 1975; Ballantine et al., 1979; Volkman et al., 1980; Nichols et al., 1984; Volkman et al., 1984
5α(H)-Stanols			
24-Nordehydrocholestanol	0.30 ± 0.20	Red alage, zoopkankton (jellyfish), reduction by bacteria	Chardon-Loriaux et al., 1976; Nelson et al., 2000; 2001
Diatomstanol	0.48 ± 0.40	Dinoflagellate, red alage, zoopkankton (jellyfish), reduction by bacteria	Chardon-Loriaux et al., 1976; Mansour et al., 1999; Nelson et al., 2000; 2001
Cholestanol	0.46 ± 0.35	Dinoflagellates, haptophyte, red algae, reduction by bacteria	Chardon-Loriaux et al., 1976; Volkman et al., 1990; Mansour et al., 1999
Campestanol	0.13 ± 0.10	Dinoflagellates, reduction by bacteria	Volkman et al., 1990; Mansour et al., 1999
Sitostanol	0.40 ± 0.38	Haptophyte, reduction by bacteria	Volkman et al., 1990
22-Dehydrocholestanol	0.21 ± 0.14	Reduction by bacteria	

Table 3-2. Mean 5α(H)-stanol / Δ^5-sterol ratios (mean ± standard deviation).

5α(H)-stanol/Δ^5-sterol ratio	
24-Nordehydrocholestanol/24-nordehydrocholesterol	0.49 ± 0.09
Diatomstanol/diatomsterol	0.23 ± 0.03
Cholestanol/cholesterol	0.23 ± 0.03
Campestanol/camplesterol	0.39 ± 0.12
Sitostanol/β-sitosterol	0.76 ± 0.12
22-Dehydrocholestanol /22-dehydrocholesterol	0.17 ± 0.03

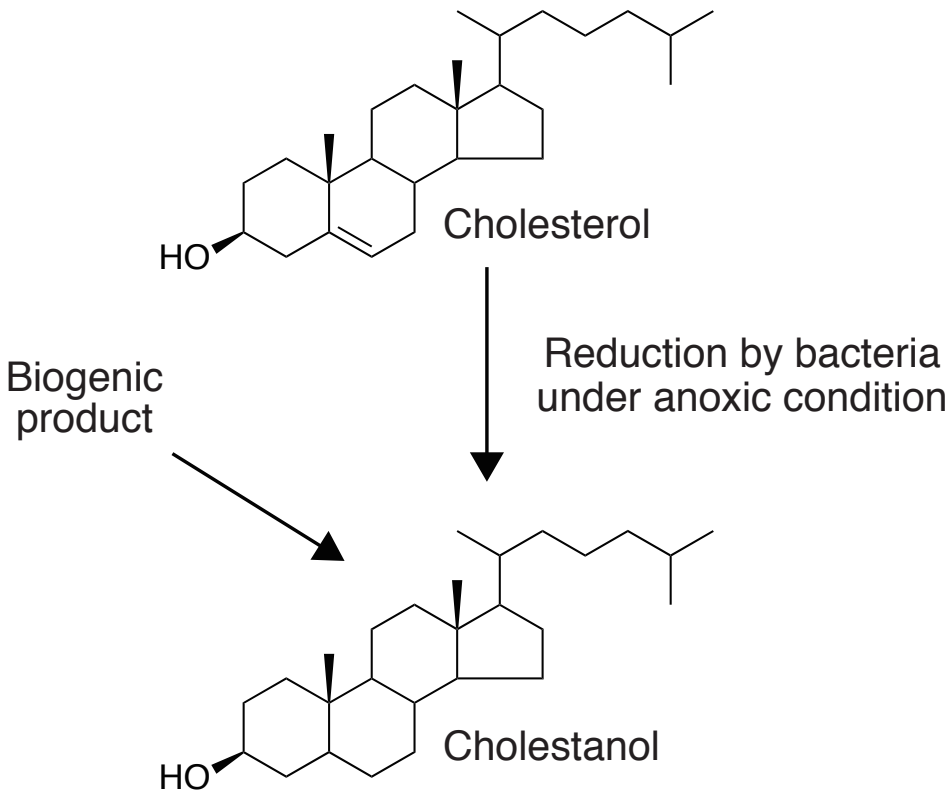

Fig. 3-1 Stanol generation processes through bacterial biogenic and a dehydrogenate reactions.

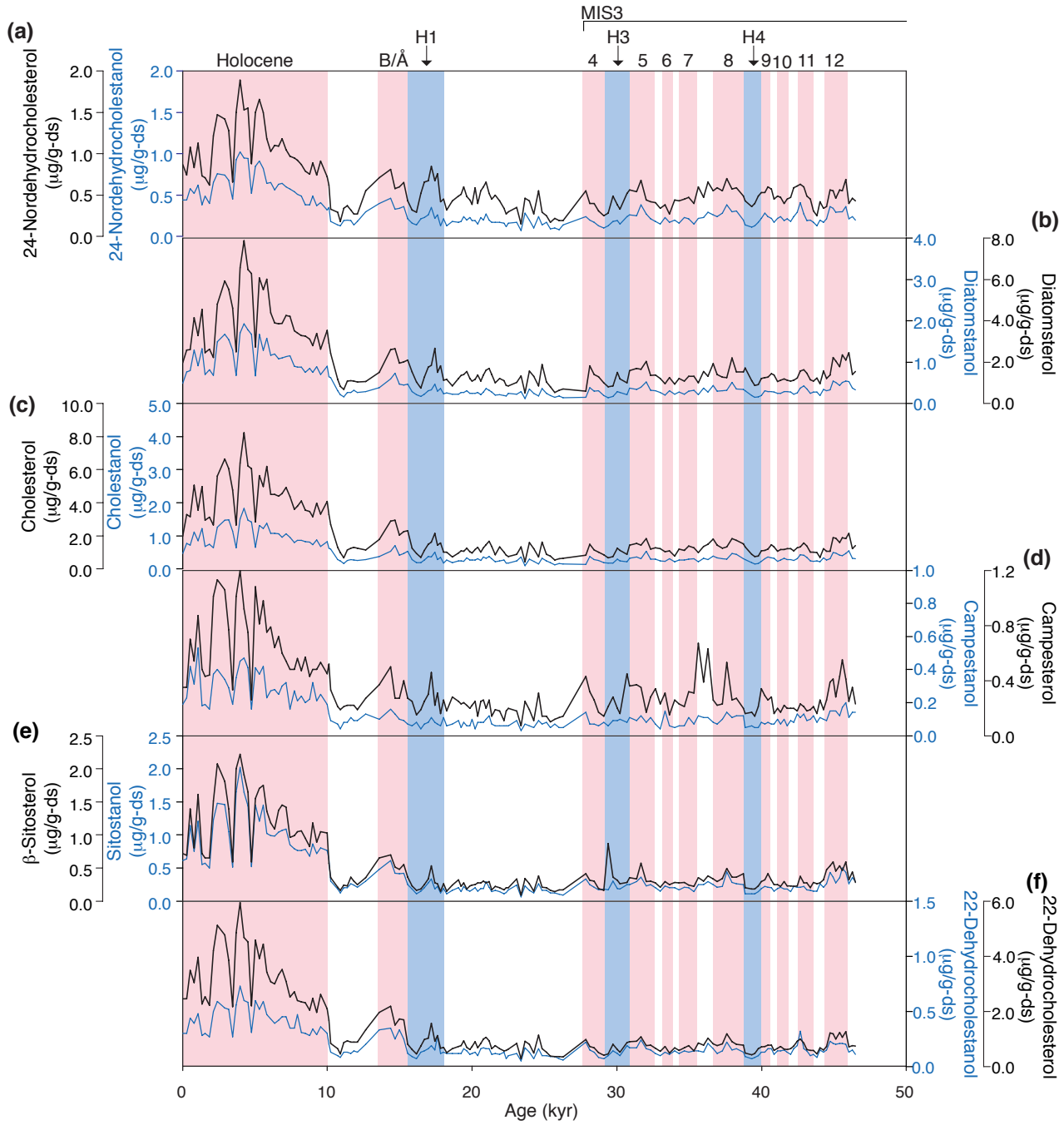

Fig. 3-2 Vertical profiles of Δ^5-sterol and 5α(H)-stanol concentration. (a) 24-Nordehydrocholesterol and 24-nordehydrocholestanol concentrations (µg/g-ds [dry sediment]), (b) diatomsterol and diatomstanol concentrations (µg/g-ds), (c) cholesterol and cholestanol concentrations (µg/g-ds), (d) campesterol and campestanol concentrations (µg/g-ds), (e) β-sitosterol and sitostanol concentrations (µg/g-ds), and (f) 22-dehydrocholesterol and 22-dehydrocholesanol concentrations (µg/g-ds). Black lines denote sterols and blue lines denote stanols. MIS3 = marine isotope stage 3. Numbers 4–12 denote interstadial periods. H1, H3, and H4 = Heinrich events 1, 3, and 4. B/Å = Bølling-Ållerød. The timings of those events are taken from Seki et al., 2002.

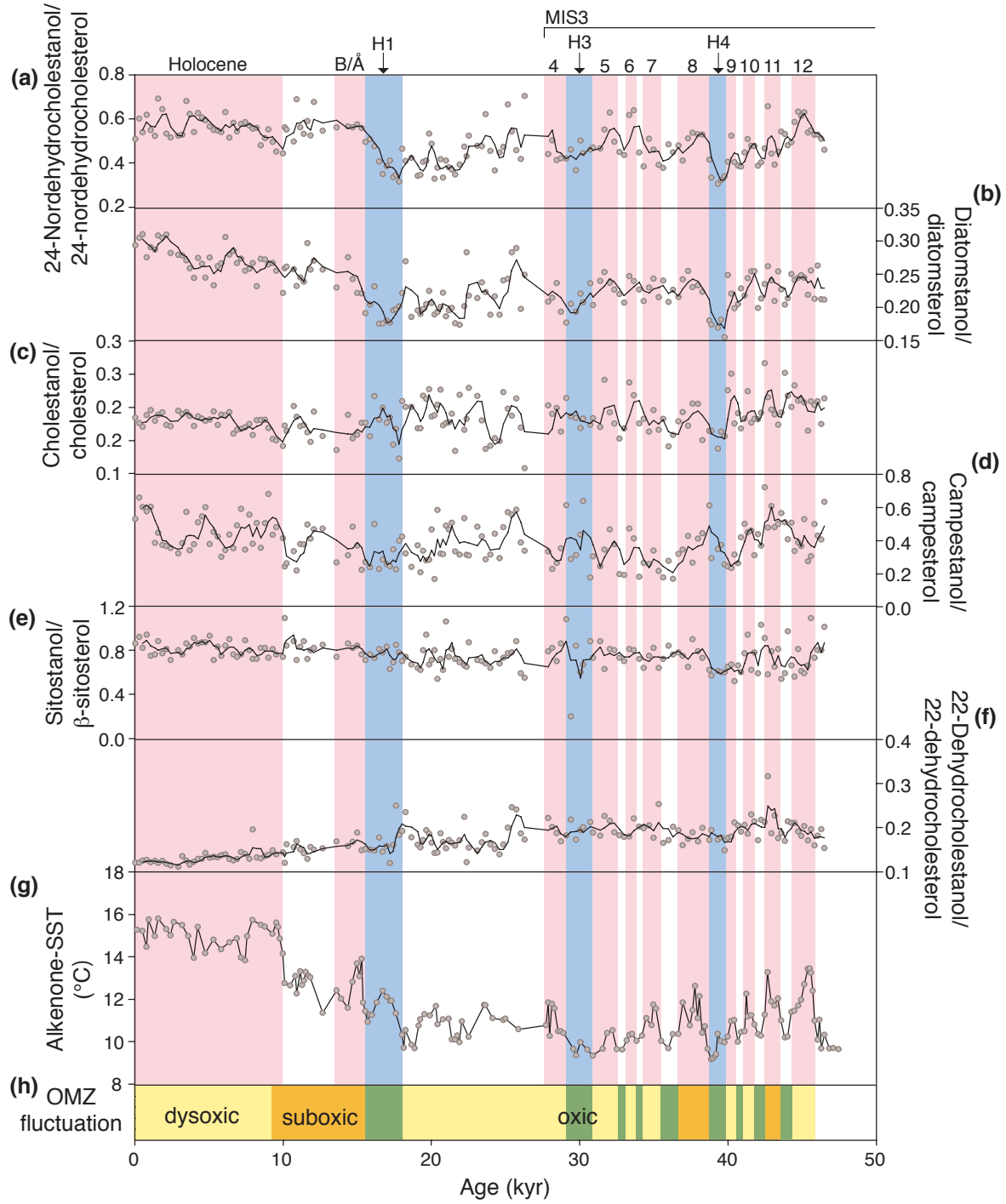

Fig. 3-3 Vertical profiles of 5α(H)-stanol / Δ^5-sterol and geo-records. (a) 24-Norhydrocholestanol / 24-norhydrocholesterol ratio, (b) diatomstanol / diatomsterol ratio, (c) cholestanol / cholesterol ratio, (d) campestanol / campesterol ratio, (e) sitostanol / β-sitosterol ratio, (f) 22-hydrocholestanol / 22-hydrocholesterol ratio, (g) alkenone-SST record of Hole 1017E (ODP Leg167), and (h) reconstructed oxygen level at Site 1017. Black lines in (a)–(f) denote three point running average. MIS3 = marine isotope stage 3. Numbers 4–12 denote interstadial periods. H1, H3 and H4 = Heinrich events 1, 3 and 4. B/Å = Bølling-Ållerød. The timings of those events and the alkenone-SST record are taken from Seki et al., 2002. Reconstructed oxygen levels at Site 1017 are taken from Cannariato and Kennett, 1999. Relationships between 5α(H)-stanol/Δ^5-sterols and alkenone-SST record are given in Fig 3-7.

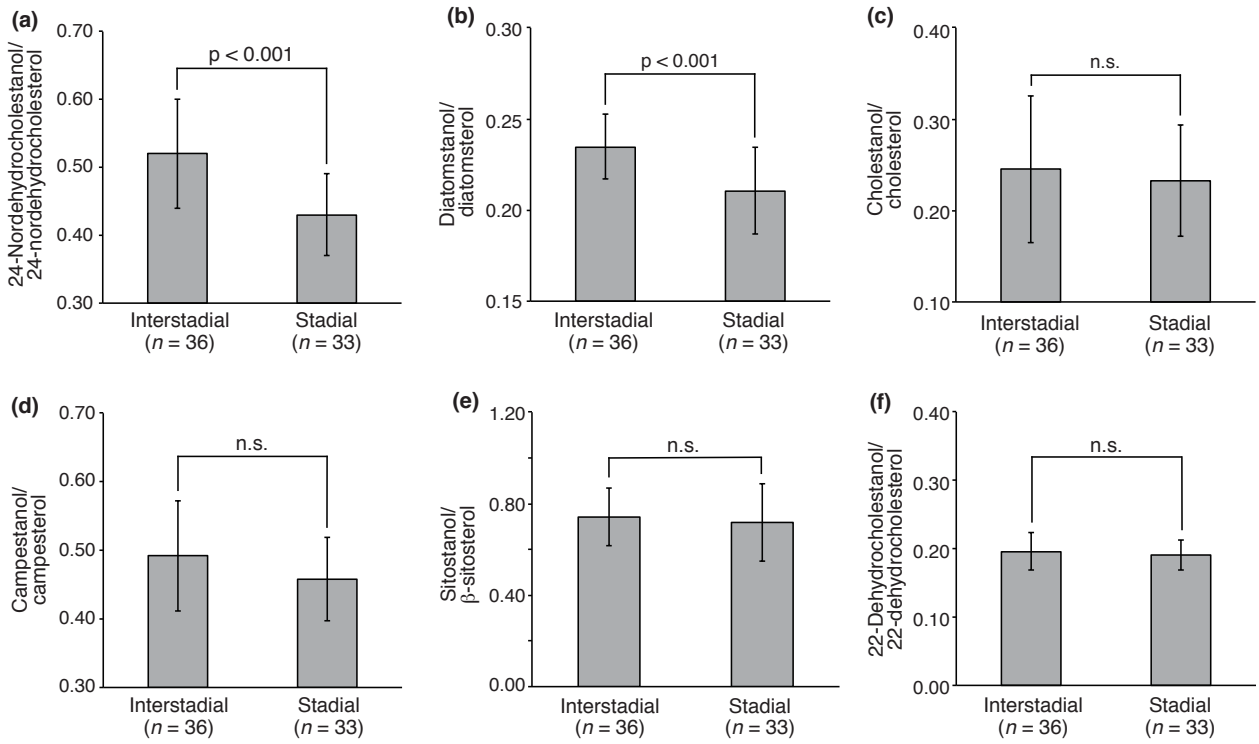

Fig. 3-4. Comparisons of 5α(H)-stanol / Δ^5-sterol ratios between interstadial and stadial periods during marine isotope stage 3 (27.7–44.5 ka). (a) 24-Norhydrocholestanol / 24-norhydrocholesterol ratio, (b) diatomstanol / diatomsterol ratio, (c) cholestanol / cholesterol ratio, (d) campestanol / campesterol ratio, (e) sitostanol / β-sitosterol ratio, and (f) 22-hydrocholestanol / 22-hydrocoresterol ratio. Values are means ± standard deviation Interstadial; n = 36, Stadial; n = 33. n.s. = no significance ($p > 0.01$).

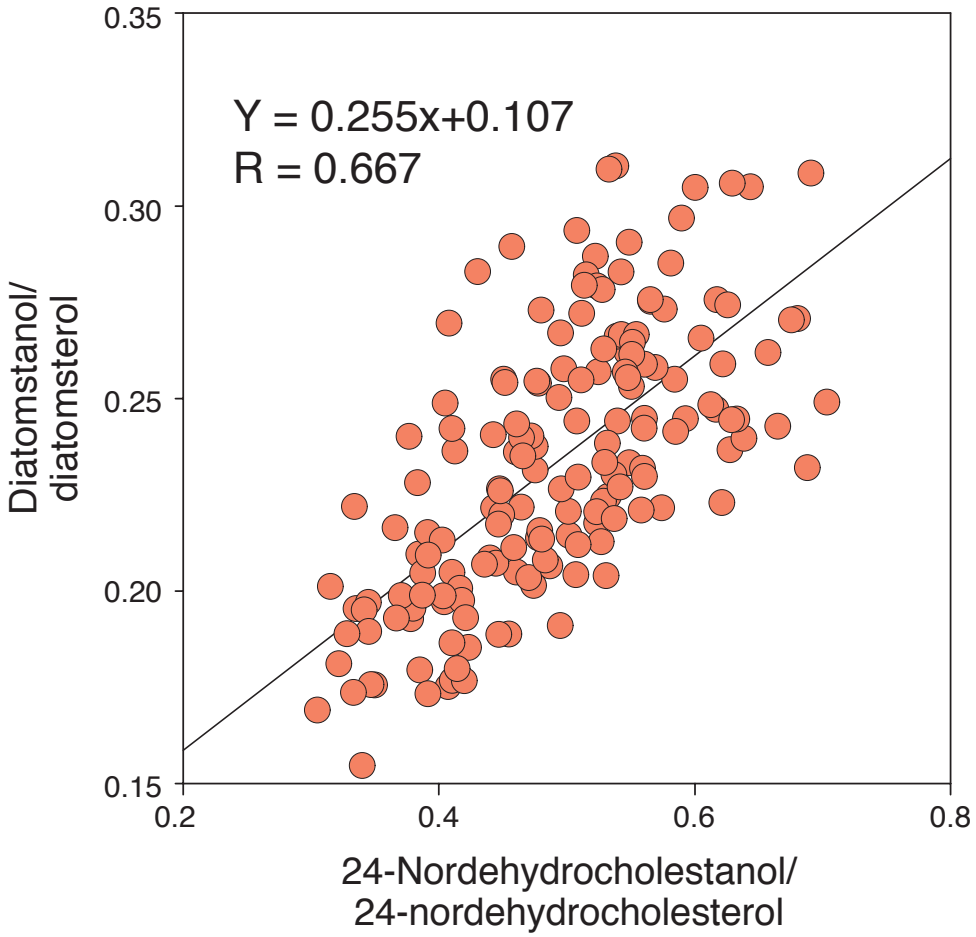

Fig. 3-5. Relationship between the 24-norhydrocholestanol / 24-norhydrocholestanol ratio and the diatomstanol / diatomsterol ratio in sediments using the tetramethylammonium hydroxide thermochemolysis (TMAH) method (Y = 0.255x + 0.107, n = 163, R = 0.667). Relationships among other 5α(H)-stanol / Δ^5-sterol ratios show no significant correlation (See Fig. 3-8).

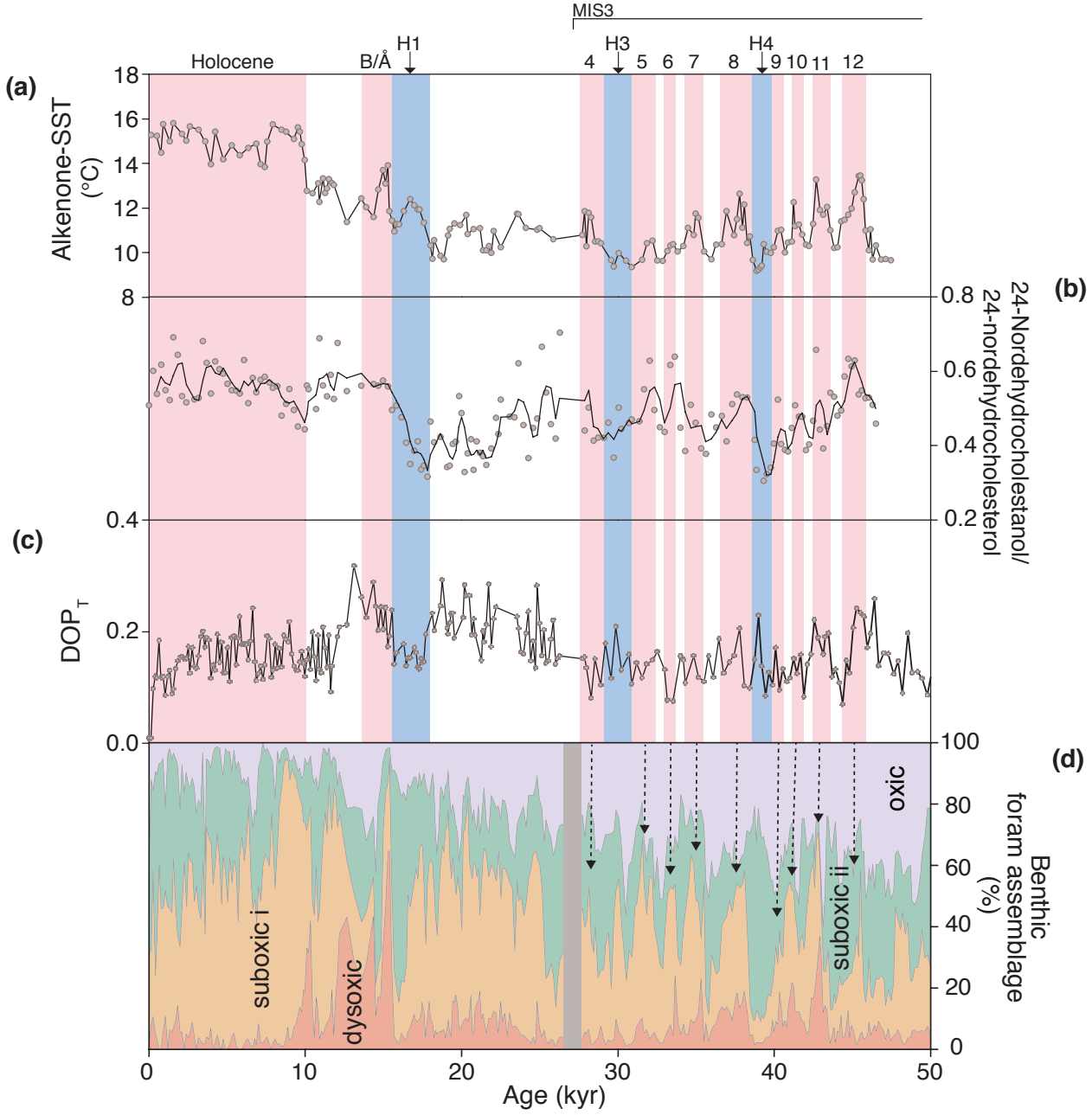

Fig. 3-6. Vertical distribution of alkenone-SST record, 24-norhydrocholestanol / 24-norhydrocholesterol ratio, DOP_T, and Benthic foraminiferal assemblage, classified according to the oxygen level of Hole 1017E. (a) alkenone-SST record (Seki et al., 2002), (b) 24-nordehydrocholestanol / 24-nordehydrocholestanol ratio, (c) DOP_T (Tada et al., 2000), (d) Benthic foraminiferal assemblage (species are grouped into dioxic, suboxic, and oxic indicators) (Cannariato and Kennett, 1999). Black lines in (b) denote three point running average. Interstadials and peaks of benthic foraminifera under low-oxygen conditions are connected with thin dotted lines. MIS3 = marine isotope stage 3. Numbers 4–12 denote interstadial periods. H1, H3 and H4 = Heinrich events 1, 3 and 4. B/Å = Bølling-Ållerød. The timings of those events and the alkenone-SST record are taken from Seki et al., 2002. The chronology is taken from Kennett et al., 2000.

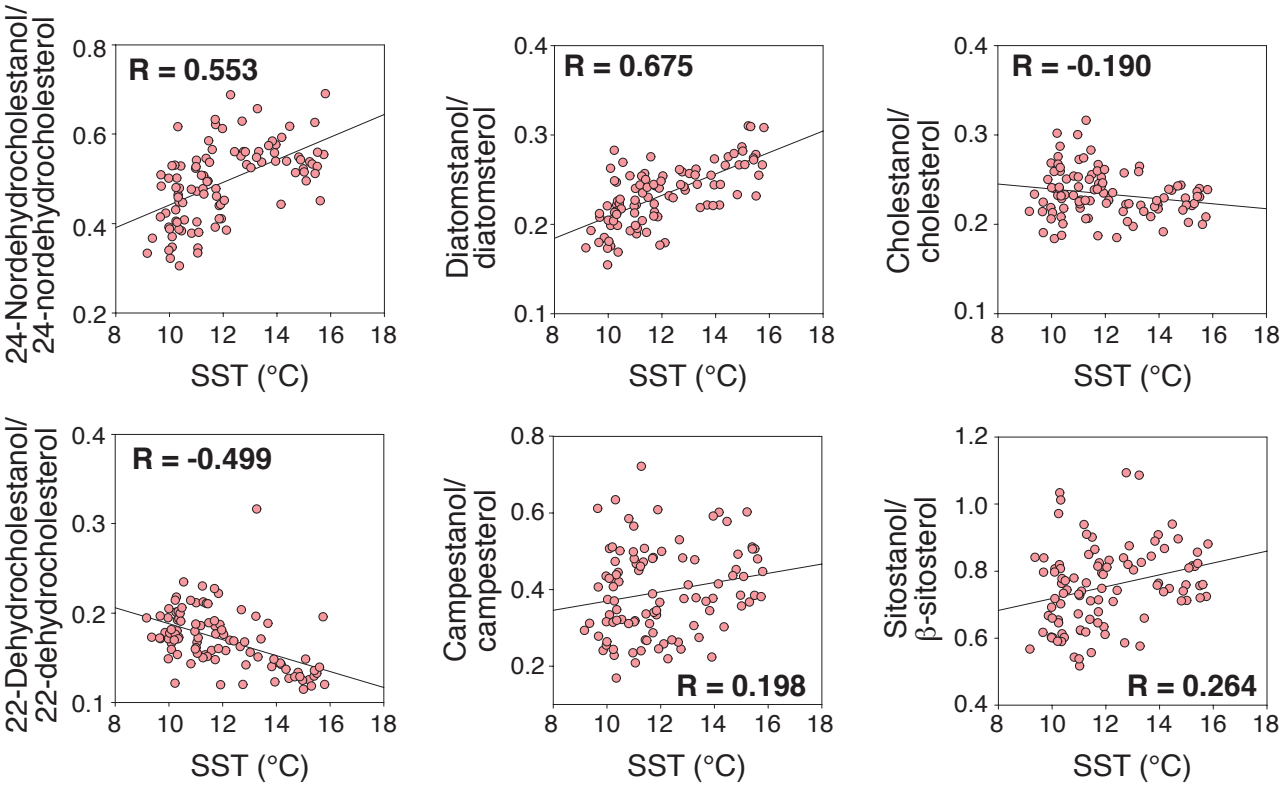

Fig. 3-7. Relationships between 5α(H)-stanol / Δ^5-sterols and alkenone-SST record (n = 100). The alkenone-SST record is taken from Seki et al., 2002.

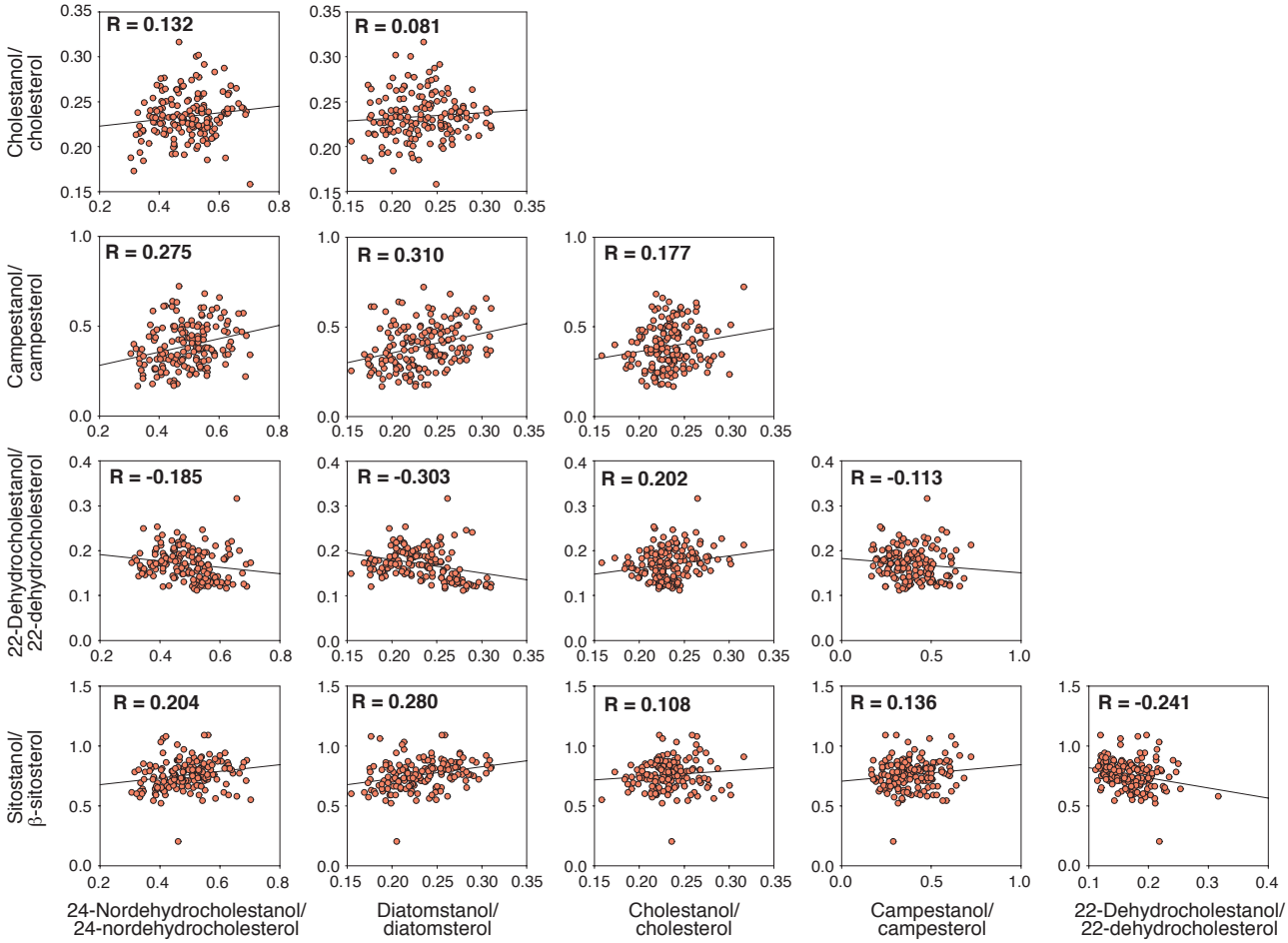

Fig. 3-8. Relationships among 5α(H)-stanol / Δ^5-sterol ratios (n = 163).

Chapter 4
Modern Sediment Records of Sterols and Stanols in Lake Suigetsu, Japan

4.1. Introduction

Redox events occur on various time-scales ranging from the seasonal to millennial scale (e.g., Sholkovitz et al., 1992; Cannariato and Kennett, 1999); these events are important for understanding paleoclimatic changes and for the interpretation of potential factors affecting organic matter preservation (e.g., Didyk et al., 1978; Kennett and Ingram, 1995; Meyers, 2006). For redox event reconstruction, various tracers (e.g., isorenieratene and iron oxide ratios) have been developed (e.g., Sinninghe Damsté et al., 1993; Rouxel et al., 2005). In the field of organic geochemistry, the 5α(H)-stanol / Δ^5-sterol ratio is recognized as a useful redox tracer (e.g., Nishimura and Koyama, 1977; Gaskell and Eglinton, 1975; Wakeham, 1989). A Δ^5 double bond of Δ^5-sterol is reduced by microorganisms under anoxic conditions to form 5α(H)-stanol (Rosenfeld and Hellman, 1971; Eyssen et al., 1973). Thus, 5α(H)-stanol / Δ^5-sterol ratios take high values under anoxic conditions, and have been used as a tracer for environmental changes (e.g., Canuel and Martens, 1993; Bertrand et al., 2012; Zheng et al., 2015).

To analyze Δ^5-sterol and 5α(H)-stanol using gas chromatography (GC), compounds need to be extracted and derivatized; these steps are time consuming and require a large amount of sample. In contrast, for tetramethylammonium hydroxide (TMAH) thermochemolysis with gas chromatography-mass spectrometry (GC–MS) analysis, methylation and hydrolysis are performed simultaneously during the TMAH reaction; therefore, it is possible to analyze in a relatively short time and with a relatively small amount of sample. This makes the method suitable for high time resolution analysis of multiple samples (e.g., sediment cores). The method has been used for the reconstruction of paleo-environments and for the analysis of sediments (e.g., Hatcher and Clifford, 1994; Ishiwatari et al., 2006; Vidal et al., 2016). In this method,

sterols are hydrolyzed to methyl derivatives and can be detected as the sterol methyl ester (Asperger et al., 1999b, 2001); therefore, analysis of 5α(H)-stanol / Δ^5-sterol ratios using this method can be used to reconstruct redox state.

When using the 5α(H)-stanol / Δ^5-sterol ratio, inputs from organisms that directly produce stanols should be considered. Organisms that directly produce stanols include phytoplankton and zooplanktons (e.g., Chardon-Loriaux et al., 1976; Robinson et al., 1984; Volkman et al., 1990; Mansour et al., 1999; Nelson et al., 2000; 2001). Biological production of those stanols can be reflected in the composition of organic matter in sediments (Nishimura and Koyama, 1976, 1977; Nishimura, 1977a; Robinson et al., 1984); therefore, there was a possibility that it would be difficult to capture redox events using 5α(H)-stanol / Δ^5-sterol ratios. However, 5α(H)-stanol / Δ^5-sterol ratios analyzed using the TMAH method as a redox tracer in late-Quarternary sediments from southern California (Ocean Drilling Program; ODP, Leg167, Hole 1017E) in Chapter 3. These sediments had been strongly influenced by variations in the oxygen minimum zone. Of a variety of 5α(H)-stanol / Δ^5-sterol ratios, two specific ratios (24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios) best recorded redox events during the last 45 ka. The study was the first to show the usefulness of 5α(H)-stanol / Δ^5-sterol ratios using the TMAH method as a redox tracer. However, the usefulness of the 5α(H)-stanol / Δ^5-sterol ratio using the TMAH method has so far been limited to southern Californian marine sediments. Improvements to the 5α(H)-stanol / Δ^5-sterol redox tracer method can be expected by applying the approach in other locations (e.g., lake sediments). In this study, I evaluate the usefulness of the 5α(H)-stanol / Δ^5-sterol ratio by the TMAH method using Lake Suigetsu sediment samples, where the redox state has seen dramatic changes.

4.2. Lake Suigetsu study site

Lake Suigetsu is a brackish lake (maximum water depth = 34 m) belonging to the Lake Mikata Five Lakes, Fukui Prefecture, Japan (Fig. 4-1). Lake Suigetsu has become a strongly anoxic environment following a series of events (Table 4-1; Matsuyama, 1973; Masuzawa and Kitano, 1982; Uemura et al., 1992). First, in 1664, in order to prevent flood damage, the Urami Canal was constructed, and Lake Suigetsu changed from being a freshwater lake to a brackish one; it is thought that persistently anoxic conditions formed at this time. In 1800, the Saga Tunnel was opened between Lake Hiruga and Lake Suigetsu, and was then rebuilt in 1848. It is reported that expansion works on the Urami Canal and Saga Tunnel between 1934 and 1935 caused further expansion of the chemocline depth, from 15 to 7 m, in Lake Suigetsu (Matsuyama, 1973). The current Lake Suigetsu is composed of a freshwater upper zone (water depth above 5–7 m) and an anoxic lower zone (water depth below 5–7 m) containing hydrogen sulfide (Kondo et al., 2000, 2009; Mori et al., 2013). At the redox boundary layer, green sulfur bacteria were found to be the major photosynthetic sulfur bacteria (Mori et al., 2013). The 5α(H)-stanol/Δ^5-sterol ratio in Lake Suigetsu is expected to record these redox events.

Lake Suigetsu has an annual varve-thicknesses over the last 70,000 years, which is a rare geologic feature worldwide (Fukusawa et al., 1995; Bronk Ramsey et al., 2012). The varves have been formed by suitable geological surrounding environments of Lake Suigetsu including no large river inputs, blocking strong winds by surrounding mountains, and no benthic organism due to a less oxygen level at the bottom. These varves show different colors according to the year, making it possible to analyze high resolution paleoenvironmental records. Therefore, various studies have been reported using the sediments including varve choronology (e.g., Kitagawa and Van Der Plicht,

1997; Nakagawa et al., 2003, 2006). Likewise, in this study, it is expected that reconstruction of the redox conditions of Lake Suigetsu is possible without influence of disturbances in the sediments.

4.3. Materials and methods

4.3.1 Sediment samples

Lake Suigetsu sediment samples (SUI92-1 and SUI92-2) were collected at a depth of 34 m from the center of the lake in 1992 (Fig. 4-1). The core length of SUI92-1 is 90 cm and that of SUI92-2 is 85 cm. Both cores were cut at intervals of 2 cm between 0 and 50 cm, and cut every 5 cm below 50 cm, and were kept frozen. The frozen samples were freeze-dried and ground into powder at the time of analysis.

4.3.2 Organic carbon analysis and the TMAH method

Sterols were analyzed using the offline TMAH GC–MS method. A dried and finely ground sediment sample (~25 mg) was put into a 10 ml glass ampoule with 150 μl of TMAH reagent (97%, Sigma-Aldrich Co., 25 wt.% in methanol) and 50 μl of nonadecanoic-d_{37} acid (99.1%, CDN isotopes Co.) as an internal standard (100 mg/ml in methanol). After drying the solvent, the sample was sealed under vacuum conditions, and placed in an oven at 300°C for 30 min. The heated sample was then cooled to room temperature and extracted four times with 300 μl of ethyl acetate. The combined extracts were dried in a vacuum desiccator and then dissolved in 50 μl of ethyl acetate.

The dissolved extract was injected and analyzed by capillary GC– MS (6890N GC-5973 MS: Agilent Technologies Co.). The conditions of the GC–MS were as follows: GC column: DB-5MS capillary column, 30 m length, 0.25 mm i.d., and 0.25 μm film thickness; injection type: splitless; injector temperature: 300°C; carrier gas: helium (1.0

ml/min); oven temperature: 60°C for 20 min then increased to 310°C at 6°C/min and held for 20 min; MS ionization: EI mode; MS ion source temperature: 230°C; electron impact spectra: 70 eV; MS quadrupole mass temperature: 150°C; mass spectrometer: full scan ion monitoring mode (50–650 Dalton); MS scanning interval: 0.5 s.

Concentrations of Δ^5-sterols and 5α(H)-stanols were calculated by comparing compound peak areas against internal standard peak areas, from the peak areas obtained by the characteristic mass fragments using a correction factor for each of the compounds. For characteristic mass fragments, *m*/*z* 215 was used for cholestanol, campestanol, and sitostanol; *m*/*z* 255 was used for 24-nordehydrocholesterol, 22-dehydrocholesterol, cholesterol, diatomsterol, campesterol, and β-sitosterol; and *m*/*z* 257 was used for 24-nordehydrocholestanol, 22-dehydrocholestanol, and diatomstanol.

4.3.3. Effect of the TMAH reaction on 5α(H)-stanol / Δ⁵-sterol ratio

In Chapter 3, I tested for the effect (e.g., isomerization) of the TMAH process on Δ^5-sterol and 5α(H)-stanol structures using a mixture of cholesterol and cholestanol reagents, and confirmed that no reactions triggered structural changes in the compounds. In this study, to confirm the effect on further ratios, different rates of cholestanol (> 95%, Sigma) and cholesterol (analytical grade reagent, Wako) reagents (cholestanol: cholesterol at 60:40, 40:60, and 20:80) were analyzed. Then, methylated cholesterol and cholestanol were detected; no other compounds were produced under the TMAH reaction conditions, consistent with previous results (Fig. 4-2a). Errors in ratios before and after the TMAH reaction were within $< \pm 3\%$. Furthermore, those relationships show an almost one-to-one linear correlation ($Y = 1.029X$, $R^2 = 0.99$; Fig. 4-2b). These results show that the TMAH process does not alter stanol-to-sterol ratios even in various ratio patterns.

4.3.4. Analytical reproducibility of the 5α(H)-stanol / Δ⁵-sterol ratio results in Lake Suigetsu sediments

Analytical reproducibility of the 5α(H)-stanol / Δ⁵-sterol ratio of sediment samples was estimated using Core SUI92-1 at 17 cm and repeating the analysis four times. Estimated values (the mean coefficient of variation of the four tests) were as follows: 24-nordehydrocholestanol / 24-nordehydrocholesterol, ± 1.7%; 22-dehydrocholestanol / 22-dehydrocholesterol, ± 8.7%; diatomstanol / diatomsterol, ± 8.3%; cholestanol / cholesterol, ± 6.7%; and campestanol / campesterol, ± 7.1%; sitostanol / β-sitosterol, ± 9.0%. For all, errors were within 9.0%. This reproducibility was similar to that in the southern California sediments (Chapter 3).

4.3.5. Age model and presupposition on records

An age model was taken from Fukusawa et al., 1995 (Fig. 4-3). This age model was estimated using the detected ages of historical earthquakes, floods, and human activity. The error of in the age model is calculated to be ±10 years. From the age model, it is estimated that the events in Lake Suigetsu occurred in 1664, 1800, 1848, and 1935–1936, corresponding to depths of 40.3 cm, 20.8 cm, 16.4 cm, and 6.5 cm, respectively (Fig. 4-3; Table 4-1).

In this study, although the age model (Fukusawa et al., 1995), diatom assemblage analysis (Masuzawa and Kitano, 1982), degree of pyritization (Matsuyama, 1974), farnesol concentration (Uemura et al., 1992), and 5α(H)-stanol / Δ⁵-sterol ratio were compared in units of centimeters, the different records are based on data from different cores. However, comparisons by depth units are assumed to be sufficient

because these cores were taken from almost the same place at the center of the Lake and in the same water depth (34 m).

4.4. Results

4.4.1. Detected Δ⁵-sterols and 5α(H)-stanols

I successfully detected five 5α(H)-stanol / Δ⁵-sterol pairs (24-norcholesta-5,22E-dien-3β-ol [24-nordehydrocholesterol] and 24-nor-5α(H)-cholest-22E-en-3β-ol [24-nordehydrocholestanol], cholesta-5,22E-dien-3β-ol [22-dehydrocholesterol] and 5α(H)-cholest-22E-en-3β-ol [22-dehydrocholestanol], cholest-5-en-3β-ol [cholesterol] and 5α(H)-cholestan-3β-ol [cholestanol], 24-methylcholesta-5,22E-dien-3β-ol [diatom-sterol] and 24-methyl-5α(H)-cholest-22E-en-3β-ol [diatomstanol], 24-methylcholest-5-en-3β-ol [campesterol] and 24-methyl-5α(H)-cholestan-3β-ol [campestanol], and 24-ethylcholest-5-en-3β-ol [β-sitosterol] and 24-ethyl-5α(H)-cholestan-3β-ol [sitostanol]; Fig. 4-4, Table 4-2). Hydroxyl groups in the sterols were derivatized to methyl ether during the thermochemolysis reaction with the TMAH reagent. Thus, sterols found through the TMAH GC–MS method were detected as methyl ether compounds (Asperger et al., 1999b, 2001).

4.4.2. Δ⁵-Sterol and 5α(H)-stanol concentrations in Lake Suigetsu sediments

Δ⁵-Sterol and 5α(H)-stanol distributions show similar trends between Core SUI92-01 and Core SUI92-2 (Fig. 4-5). All Δ⁵-sterols were detected in both freshwater and brackish conditions, except for 24-nordehydrocholesterol, which was detected primarily above ~40 cm (SUI92-1; < 13.9 μg/g, SUI92-2; < 11.9 μg/g), and at very low levels below ~40 cm (SUI92-1 and SUI92-2; ≃ 0). Among the six Δ⁵-sterols, cholesterol

is the highest in concentration (SUI92-1; 16.2-112 μg/g, SUI92-2; 10.3-71.5 μg/g), whereas 24-nordehydrocholesterol is detected in the lowest concentration (SUI92-1; 0.00-13.9 μg/g, SUI92-2; 0.00-11.9 μg/g). All Δ^5-sterols show higher concentrations than the opposing stanol concentrations. Among the 5α(H)-stanols, cholestanol showed the highest range (SUI92-1; 3.61-34.8 μg/g, SUI92-2; 2.80-16.5 μg/g) and 24-nordehydocholestanol the lowest (SUI92-1; 0.00-3.99 μg/g, SUI92-2; 0.00-2.14 μg/g). 24-Nordehydrocholestanol was present at low levels below ~40 cm (SUI92-1 and SUI92-2; ≈ 0), similar to 24-nordehydrocholesterol.

4.4.3. Variabilities of 5α(H)-stanol / Δ^5-sterol ratios

The 5α(H)-stanol / Δ^5-sterol ratio distributions show similar trends between Core SUI92-01 and Core SUI92-2 (Fig. 4-6). The values of all ratios are under 1.0. 24-nordehydrocholestanol / 24-nordehydrocholesterol, diatomstanol / diatomsterol, and 22-dehydrocholestanol / 22-dehydrocholesterol ratios show similar trends and the highest values around the surface. In contrast, cholestanol / cholesterol, campestanol / campesterol, and sitostanol / β-sitosterol ratios show decreasing trends from ~40 cm. The cholestanol / cholesterol ratio slightly increases in the surface sediments.

Comparisons of 5α(H)-stanol / Δ^5-sterol ratios in the sediments of fresh water (below 40 cm) and those from brackish water (above 40 cm) conditions are shown in Figure 4-7. The 24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios imply significantly higher values during the brackish water age than those during the fresh water age ($P < 0.01$). The 22-dehydrocholestanol / 22-dehydrocholesterol ratio shows no significant difference between the ages ($p = 0.84$). Finally, the cholestanol / cholesterol, campestanol / campesterol, and sitostanol / β-

sitosterol ratios presented significantly higher values in the freshwater age than those in the brackish water age ($p < 0.01$).

Relationships between 5α(H)-stanol / Δ^5-sterol ratios in the sediments are strong ($R^2 > 0.5$) with 24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol, 24-nordehydrocholestanol / 24-nordehydrocholesterol and 22-dehydrocholestanol / 22-dehydrocholesterol (excepting the relationship in SUI92-01 Core [$R^2 = 0.47$]), diatomstanol / diatomsterol and 22-dehydrocholestanol / 22-dehydrocholesterol, and cholestanol / cholesterol and campestanol / campesterol ratios (Fig. 4-8). In contrast, relationships among other 5α(H)-stanol / Δ^5-sterol ratios show no significant correlation ($R^2 < 0.5$; Fig. 4-9).

4.5. Discussion

4.5.1. Changing redox states in Lake Suigetsu

As described above, environmental change in Lake Suigetsu can be roughly divided into a freshwater age (below ~40 cm, –1664) and a brackish water age (above ~40 cm, 1664–). The brackish water age is characterized by four intervals that reflect the canal and tunnel constructions: **Brackish I**) an interval from immediately after opening the Urami Canal to the construction of the Saga Tunnel (40.3–20.8 cm, 1664–1800); **II**) an interval from the opening of the Saga Tunnel to its re-building (20.8–16.4 cm, 1800–1848); **III**) an interval from the re-building of the Saga Tunnel to 1934 (16.4–6.5 cm, 1848–1934); and **IV**) an interval from 1934 to the present (above 6.5 cm; Table 4-1, Fig. 4-6).

Masuzawa and Kitano (1982) analyzed the diatom assemblage in sediments from Lake Suigetsu (Fig. 4-6a). They reported that the freshwater species of diatoms (*Stephanodiscus carconensis*) abruptly decreased in abundance from ~40 cm, when the

record of brackish conditions begin; diatoms of marine origin (*Coscinodiscus lacustris*) appear from ~35 cm. The variability of diatom assemblage records a dramatic environmental change towards a brackish water environment in the lake.

Matsuyama (1974) analyzed various sulfur compounds (total-, elemental, sulfate, sulfide, and residual sulfur) in sediments from Lake Suigetsu. The most dominant form of sulfur in the sediment was residual sulfur (<40%), which is calculated from the difference between the total sulfur and the sum of elemental, sulfide, and sulfate sulfur. Most residual sulfur should form as pyrite (Kaplan et al., 1963; Berner, 1984), where sulfate ions are reduced to hydrogen sulfide by bacterial action, and then pyrite is produced by the reaction of iron with hydrogen sulfide in surface sediments (Berner, 1984). Therefore, the degree of pyritization (DOP) recorded in sediments has been used as a redox tracer for bottom-water conditions (Calvert and Karlin, 1991; Tada et al., 1992; Tada et al., 1999; Tada et al, 2000). Although DOP is generally estimated from the ratio of pyrite iron to total iron, assuming the residual sulfur content is all pyrite, DOP in this study was calculated using data from Matsuyama (1974; Fig. 4-6b). The DOP in Brackish I shows a relatively higher value than that from the freshwater sediments, but is not high compared with values from the other brackish water intervals, suggesting the redox state in bottom-water was not strong during the onset of brackish conditions. The DOP increases to ~0.27 during Brackish II, and then abruptly increases in Brackish III and IV (Brackish III, < 0.67; Brackish IV, < 0.72). Therefore, it can be concluded that strong redox states occurred in Lake Suigetsu bottom-water after the re-building of the Saga tunnel in 1848. Since there is not a large difference in DOP values between Brackish III and IV, the redox states in both intervals were similar.

Uemura et al. (1992) detected farnesol above 30 cm in Lake Suigetsu sediment (0.02–12.9 mg/g-OC; Fig. 4-6c). Farnesol is derived from green sulfur bacteria (*chlorobiaceae*), which are photoautotrophic bacteria inhabiting an anaerobic environment (Rapoport and Hamlow, 1961; Caple et al., 1978; Otte et al., 1993; Airs and Keely, 2003). Therefore, the presence of farnesol in sediments indicates anoxic conditions. Nevertheless, the Brackish I conditions probably started at an interval corresponding to a depth of ~40 cm, while the first appearance of farnesol is at ~30 cm (Fig. 4-6a–c). This result suggests that there was a delay in the anoxic zone reaching photosynthetic depths, in spite of the anoxic starting conditions.

Interestingly, despite the lack of differences in DOP values, farnesol concentrations have smaller values in Brackish III than in Brackish IV (Brackish III; < 7.11, Brackish IV; < 12.9, Uemura et al., 1992). These differences can be attributed to the characteristics of the two tracers (DOP and farnesol). According to Matsuyama (1973), since further expansion of the anoxic zone occurred in Brackish IV, redox tracers are likely to record these trends. Since farnesol is derived from photosynthetic bacteria, this compound could record the habitat depth of photosynthetic bacteria and of fluctuations controlled by changing the anoxic photosynthetic zone (Fig. 4-10). In fact, this tracer clearly recorded the further expansion of the anoxic zone in Brackish IV (Fig. 4-6c). On the other hand, DOP is a tracer for bottom-water and surface sediment redox state; therefore, under stable anoxic conditions in bottom-water, even though the anoxic zone depth in the water column expanded towards the subsurface, differences in DOP values between Brackish III and IV do not occur (Fig. 4-10). In fact, DOP values during the transition are similar (Fig. 4-6b). Thus, comparison of both tracers (farnesol and DOP) reveals redox states in the water column and bottom conditions of the Lake,

respectively. Therefore, 5α(H)-stanol/Δ^5-sterol ratios can be also expected to record those redox states.

4.5.2. Comparison between 5α(H)-stanol / Δ^5-sterol ratios and other redox tracers

Among trends of 5α(H)-stanol / Δ^5-sterol ratios, 24-nordehydrocholestanol / 24-nordehydrocholesterol, diatomstanol / diatomsterol, and 22-dehydrocholestanol / 22-dehydrocholestanol ratios are most similar to the redox tracers (DOP and farnesol); therefore, these ratios record redox states in the Lake (Fig. 4-6d–f). The significant relationships between the ratios ($R^2 > 0.5$; Fig. 4-8a–c) imply that they record the same events. In particular, the trends of the ratios closely accord with farnesol concentration. Considering that DOP is a tracer for bottom-water redox state and farnesol fluctuates with changing redox state in the water column, these results indicate that 5α(H)-stanol / Δ^5-sterol ratios are more influenced by anoxic conditions in the water column than by the bottom water conditions. Wakeham (1989) studied *in situ* microbial reduction of Δ^5-sterol to 5α(H)-stanol in water columns of the Cariaco Trench and Black Sea, and reported that substantial reduction of Δ^5-sterol to 5α(H)-stanol occurs near oxic–anoxic interfaces. Furthermore, they pointed out that the conversion rate of Δ^5-sterol to 5α(H)-stanol might be reflected in the residence time of Δ^5-sterol at a microbially active redox interface. Therefore, it is suggested that the expansion of the anoxic zone in Lake Suigetsu during Brackish IV made the residence time longer for Δ^5-sterols in the anoxic zone, and promoted the conversion of Δ^5-sterols to 5α(H)-stanols (Fig. 4-10). Although it is reported that stanol conversion also occurs in surface sediments (e.g., Nishimura and Koyama, 1977), our results suggest that this effect is less important than conversion in the water column. A similar result, in which 5α(H)-stanol / Δ^5-sterol ratios are influenced by anoxic conditions in the water column, was recognized in the southern

California sediment by comparison of 5α(H)-stanol / Δ^5-sterol ratios, DOP, and foraminiferal species (Chapter 3). This characteristic might be important for the reconstruction of paleo-environmental changes when 5α(H)-stanol / Δ^5-sterol ratios are used as a redox tracer.

4.5.3. Ratios of 5α(H)-stanol / Δ^5-sterol under fresh and brackish water conditions

Although 24-nordehydrocholestanol / 24-nordehydrocholesterol, diatomstanol / diatomsterol, and 22-dehydrocholestanol / 22-dehydrocholestanol ratios show a significant relationship ($R^2 > 0.5$, Fig. 8a–c), detailed trends in these ratios were different. Although diatomstanol / diatomsterol and 22-dehydrocholestanol / 22-dehydrocholestanol ratios from the freshwater sediments (below 40 cm) showed < 0.10 and < 0.15, respectively, the 24-nordehydrocholestanol / 24-nordehydrocholesterol ratio in freshwater conditions was ≈0 (Fig. 6d–f). This difference might be attributed to variation in sterol sources. According to previous studies on the origins of 24-nordehydrocholesterol, the sterol is found in marine organisms such as phytoplankton (Smith et al., 1982; Nelson et al., 2000; 2001), but there are no reports from freshwater organisms. Therefore, detection, or not, of sterol is important for inferring source organisms and the environmental conditions of the Lake. As shown in Figure 4-6a, freshwater diatoms were replaced by marine diatoms during Brackish I (~20–40 cm). Since neither 24-nordehydrocholestanol nor 24-nordehydrocholesterol were detected in the freshwater sediments, the sources of both are likely marine. In contrast, the significant fluctuation of the 24-nordehydrocholestanol / 24-nordehydrocholesterol ratio in the same period (Fig. 4-6d) suggests that both the source of the sterol and the condition of the lake was unstable.

On the other hand, diatomsterol and 22-dehydrocholesterol were detected in both the freshwater and brackish sediments (Fig. 4-5b–c), suggesting potential for the use of the ratios as a redox tracer in freshwater environments. When comparing the fresh-water conditions and Brackish I, diatomstanol / diatomsterol (freshwater; 0.06 ± 0.02 [mean in SUI92-1 and -2 ± standard deviation], Brackish I; 0.07 ± 0.02 in SUI92-2) and 22-dehydrocholestanol / 22-dehydrocholesterol (freshwater; 0.09 ± 0.04 in SUI92-2, Brackish I; 0.07 ± 0.02 in SUI92-2) ratios are similar (Fig. 4-6e–f). These trends could reflect seasonally anoxic conditions in Lake Suigetsu before the onset of brackish conditions. In Lake Suigetsu, annually laminated sediments suggest that anoxic conditions have occurred throughout the late Quaternary. The sediments have been used for detailed paleo-environmental analysis by recent studies (e.g., Marshall et al., 2012; Schlolaut et al., 2012, 2014; Nakagawa et al., 2013; Smith et al., 2013). Therefore, the trends in the 5α(H)-stanol / Δ^5-sterol ratios during the freshwater period (below ~40 cm) are thought to reflect anoxic conditions in a freshwater environment caused by the reduction of Δ^5-sterol by bacteria, rather than other factors. However, given the small DOP values and non-detection of farnesol in the freshwater sediments, anoxic conditions during the freshwater period were less extreme than those during the brackish water conditions.

4.5.4. Sources of 24-nordehydrocholestanol, diatomstanol, and 22-dehydrocholestanol

To use 5α(H)-stanol / Δ^5-sterol ratios as a redox tracer, it is necessary to select 5α(H)-stanol / Δ^5-sterol ratios that are influenced little by factors other than the reduction of Δ^5-sterol by bacteria. Some organisms contain 24-nordehydrocholestanol, for example, jellyfish, amphipods, and red algae (Chardon-Loriaux et al., 1976; Nelson et al., 2000; 2001). However, since the sources of typical sedimentary sterols are

recognized to be phytoplankton and terrigenous plants (e.g., Huang and Meinschein, 1978, 1979; Gagosian et al., 1983; Volkman, 1986), there is a low possibility that there could be other contributions to sediments. On the other hand, Mansour et al. (1999) compared sterol compositions of nine marine dinoflagellates, and reported that *Scrippsiella* sp. CS-295/c contains 24-nordehydrocholestanol. However, the concentration of 24-nordehydrocholestanol in the dinoflagellate is only 0.2% of the total sterols, and stanol is not detected in other dinoflagellates. Therefore, since the source of 24-nordehydrocholetanol is likely restricted to just a few species, it was speculated that the 24-nordehydrocholestanol / 24-nordehydrocholesterol ratio provides the best tracer for anoxic conditions in sediments (Chapter 3). This speculation is supported by the results from southern California and by those from this study. However, since 24-nordehydrocholesterol have not been detected in the freshwater sediments in Lake Suigetsu, this ratio could be unsuitable for comparison of redox conditions between fresh and brackish conditions.

It is known that diatomstanol is present in red algae and zooplankton (jellyfish; Chardon-Loriaux et al., 1976; Nelson et al., 2000; 2001). However, since these sources are not phytoplankton and terrigenous sources, they are not considered a main source of organic compounds in sediments. Furthermore, since diatomsterol is detected in both marine and freshwater conditions, the ratio has the potential to be a good redox tracer in both of marine and lacustrine environments. On the other hand, diatomstanol is present in some dinoflagellate species (Mansour et al., 1999). Among them, *Gymnodinium sanguineum* has high diatomstanol content (~31.7% of total sterols; Mansour et al., 1999). Since *Gymnodinium sanguineum* is a dinoflagellate species that can occur in red tides, the diatomstanol / diatomsterol ratio might be not useful as a redox tracer in areas where red tides occur.

Only a small number of organisms contain the 22-dehydrochorestanol (e.g., red algae; Chardon-Loriaux et al., 1976). Therefore, it was thought that 22-dehydrochorestanol / 22-dehydrochoresterol ratio would be a useful tracer for redox states. However, in southern California sediments, the 22-dehydrocholestanol / 22-dehydrocholesterol ratio did not record the redox events (Chapter 3). Therefore these results show that the usefulness of the 22-dehydrocholestanol / 22-dehydrhocholesterol ratio is dependent on location. Furthermore, since sources of 22-dehydrocholestanol are also unclear, it is difficult to separate the source of 22-dehydrocholestanol from stanol-creating organisms. Therefore, when applied to other sediment samples, it is important to compare with other 5α(H)-stanol / Δ^5-sterol ratios (e.g., 24-nordehydrocholestanol / 24-nordehydrocholesterol and diatomstanol / diatomsterol ratios).

4.5.5. Cholestanol, campestanol, and sitostanol in freshwater sediments

While 24-nordehydrocholestanol / 24-nordehydrocholesterol, diatomstanol / diatomsterol, and 22-dehydrocholestanol / 22-dehydrocholesterol ratios recorded the redox states in Lake Suigetsu, cholestanol / cholesterol, campestanol / campesterol, and β-sitosterol / sitostanol ratios did not, reflecting the fact that these ratios are significantly higher under freshwater conditions than under brackish conditions (Figs. 6g–i and 7). This likely reflects other contributions besides the reduction of sterol by bacteria. The decreasing trends in cholestanol / cholesterol, campestanol / campesterol, and sitostanol / β-sitosterol ratios from 40 cm show that there was a large input of stanol under freshwater conditions (Fig. 4-6g–i). These sources are presumed to be from two main origins: freshwater phytoplankton that are rich in stanol and/or re-sedimentation from terrestrial sterol.

Robinson et al (1987) identified the sterol composition in four freshwater dinoflagellates (*Woloszynskia coronate*, *Ceratium furcoides*, *Peridinium lomnickii*, and *Peridinium cinctum*), and found that freshwater dinoflagellates have high contents of cholestanol and a high cholestanol / cholesterol ratio (< 20% of total sterols; cholestanol / cholesterol ratio = ~100). Therefore, sterol composition in the sediments, where these phytoplankton are abundant, might be influenced by those cholestanol inputs. Campestanol and sitostanol, although minor relative to cholestanol, are found in freshwater dinoflagellates (campestanol; < 1% of total sterols, sitostanol; < 2% of total sterols; Robinson et al., 1987). The rapid decrease in freshwater diatoms at the boundary between freshwater and brackish conditions seems to support the input of stanol from freshwater dinoflagellates (Fig. 4-6a). However, since these species are not present in the current Lake Suigetsu in any large numbers (Moriyama, 2015), other contributions to the stanols should be considered.

Other possibilities include inputs of stanol concentrated by degraded processes of higher plant-derived sterols. Although campesterol and β-sitosterol are presented in phytoplankton (Paoletti et al., 1976; Matsumoto et al., 1982; Volkman, 1986; Volkamn et al., 1990; Mansour et al., 1999), these sterols are recognized as sterols from terrestrial plants (Yunker et al., 1995; Belicka et al., 2004; Killops and Killops, 2013). Likewise, although present in small amounts, plants contain campestanol and sitostanol (e.g., Nishimura and Koyama, 1977). Nishimura (1977a) pointed out that stanols from living organisms are concentrated by selective degradation processes of Δ^5-sterols under oxidative conditions. Since Δ^5-sterols are easily degraded in oxidative conditions compared to 5α(H)-stanols, high 5α(H)-stanol / Δ^5-sterol ratios in sediments are expected even if the amount of 5α(H)-stanol in living organisms is low. Furthermore, it

is suggested that 5α(H)-stanols, especially of terrestrial origins (terrestrial and soils), could contribute to sediments through this degradation process (Nishimura, 1997a).

In the southern California sediments, high sitostanol contents were considered to potentially reflect terrestrially derived degraded products (sitostanol / β-sitosterol ratio; < 1.09, Chapter 3). Rontani et al (2014) suggested that high contents of sitostanol and campestanol in the Mackenzie Shelf (sitostanol / β-sitosterol ratio; < 7.21, campestanol / campesterol ratio; < 6.00) were influenced by inputs from degraded sterols. As these previous studies suggested, the influence of terrestrial stanol by degraded products cannot be ignored. Therefore, the significantly high values of these ratios in the freshwater conditions (under 40 cm) of Lake Suigetsu could be influenced by inputs from terrestrial stanol through degraded products.

The good correlation between campestanol / campesterol and cholestanol / cholesterol ratios (Fig. 4-8d) indicates the same sources; thus, I speculate that cholestanol is also affected by the contribution of terrestrial degraded products. Indeed, Arzayus and Canuel (2005) interpreted high 5α(H)-stanol / Δ^5-sterol ratios (including cholestanol / cholesterol ratio) in the York River as being possibly derived from a large contribution of older organic material. There is a high possibility that the cholestanol / cholesterol ratio in Lake Suigetsu also was influenced by terrestrial degraded products. On the other hand, at Brackish I, the cholestanol / cholesterol ratios show similar trends with the redox tracers of the 5α(H)-stanol/Δ^5-sterol ratios (i.e., high values of surface layers). Additionally, this trend appeared when sitostanol / β-sitosterol ratio was the lowest. These results indicate that if the stanol contributions of degraded products of terrestrial origin are less, the ratio could possibly be used as a redox tracer.

4.6. Conclusions

In this study, 5α(H)-stanol / Δ^5-sterol ratios using the TMAH method were applied to lacustrine depositional sediments for reconstruction of redox states, and compared with other redox tracers. Characteristics of the obtained 5α(H)-stanol/Δ^5-sterol ratios from Lake Suigetsu sediments are as follows:

- The 24-nordehydrocholestanol / 24-nordehydrocholesterol, diatomstanol / diatomsterol, and 22-dehydrocholestanol / 22-dehydrocholesterol ratios in Lake Suigetsu sediments record redox events that occurred from the 17th century onward. Therefore, the results confirm the usefulness of 5α(H)-stanol/Δ^5-sterol ratios as redox tracers in lake sediments.
- Diatomsterol and 22-dehydocholesterol were detected in both freshwater and seawater environments and showed potential as redox tracers in both.
- On the other hand, other 5α(H)-stanol/Δ^5-sterol ratios (cholestanol / cholesterol, campestanol / campesterol, and sitostanol / β-sitosterol ratios) appeared not to record the redox events in Lake Suigetsu, likely because they are influenced by inputs of terrestrial stanol by degraded products.

Table. 4-1 Lake Suigetsu events recorded in changing redox conditions

Events*	Year*	Depth estimated from dating**	Intervals
			Fresh-water (-1664)
Urami Canal opened	1664	40.3 cm	
			Brackish I (1664-1800)
Saga Tunnel construction	1800	20.8 cm	
			Brackish II (1800-1848)
Saga Tunnel reconstruction	1848	16.4 cm	
			Brackish III (1848-1935)
Chemocline increase	1934-1935	6.5 cm	
			Brackish IV (1935-)

* Events and years from Matsuyama, 1973; Masuzawa and Kitano, 1982; Uemura et al., 1992.
** Chronology taken from Fukusawa et al., 1995.

Table. 4-2 Identified sterol and stanol compositions from Lake Suigetsu sediments (SUI92-1 and SUI92-2).

Symbol*	Systematic Name	Trivial name
	Δ^5-Sterols	
i	24-Norcholesta-5,22E-dien-3β-ol	24-Nordehydrocholesterol
ii	Cholesta-5,22E-dien-3β-ol	22-Dehydrocholesterol
iii	Cholest-5-en-3β-ol	Cholesterol
iv	24-Methylcholesta-5,22E-dien-3β-ol	Diatomsterol
v	24-Methylcholest-5-en-3β-ol	Campesterol
vi	24-Ethylcholest-5-en-3β-ol	β−Sitosterol
	5α(H)-Stanols	
vii	5α(H)-Cholestan-3β-ol	Cholestanol
viii	24-Methyl-5α(H)-cholestan-3β-ol	Campestanol
ix	24-Ethyl-5α(H)-cholestan-3β-ol	Sitostanol
x	24-Nor-5α(H)-cholest-22E-en-3β-ol	24-Nordehydrocholestanol
xi	5α(H)-Cholest-22E-en-3β-ol	22-Dehydrocholestanol
xii	24-Methyl-5α(H)-cholest-22E-en-3β-ol	Diatomstanol

* Symbols refer to Fig. 4-4

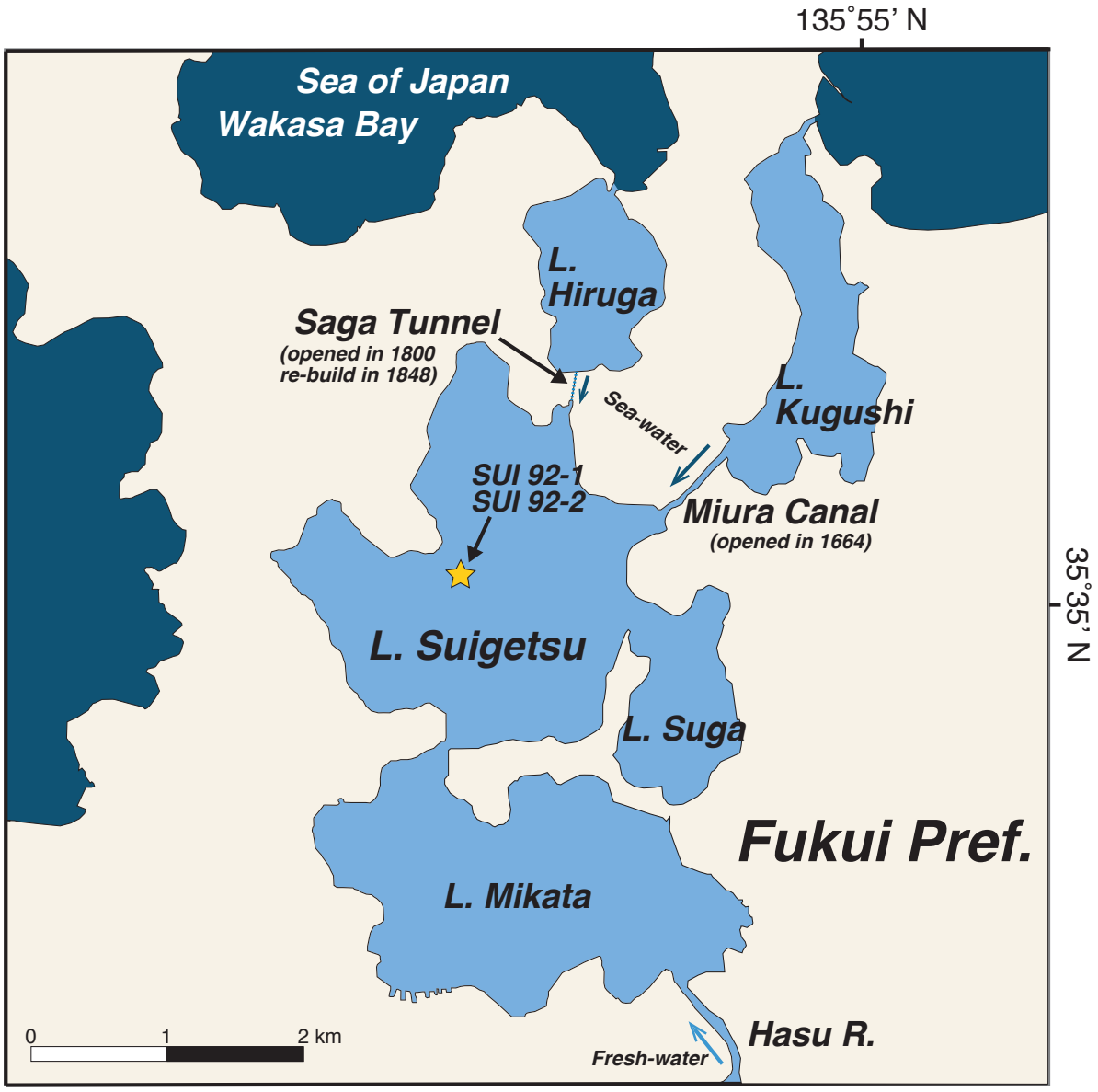

Fig. 4-1 Location map of Lake Suigetsu, showing surrounding lakes, connected rivers, and a river tunnel. The star (★) denotes the sampling point. River water from the Hasu River flows through Mikata Lake, and seawater flows through Lake Hiruga and Lake Kugashi before flowing into Lake Suigetsu.

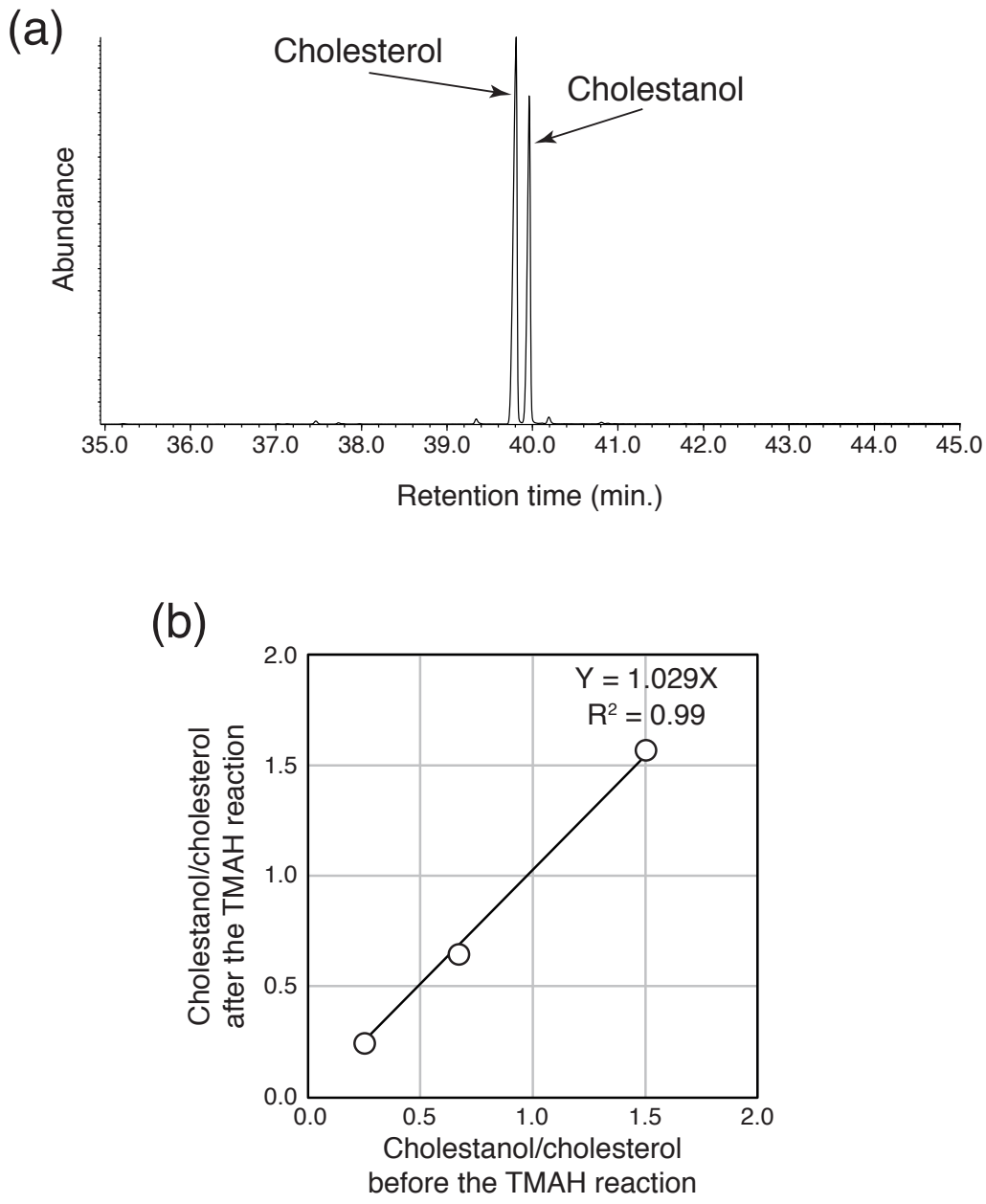

Fig. 4-2 (a) Representative total ion chromatogram of the sterols obtained by the TMAH method. (b) Relationship between cholestanol / cholesterol ratio before and after the TMAH reactions.

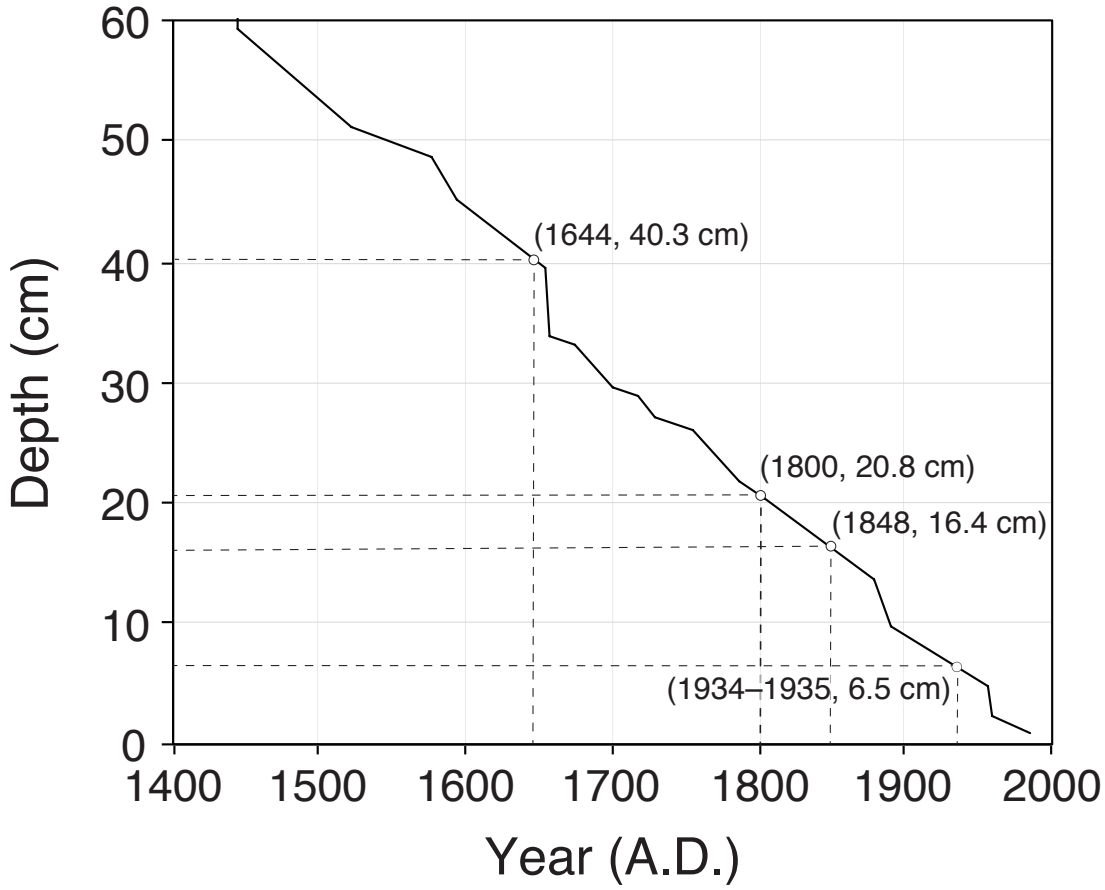

Fig. 4-3 Age model for Lake Suigetsu sediment. The age model is taken from Fukusawa et al., 1995.

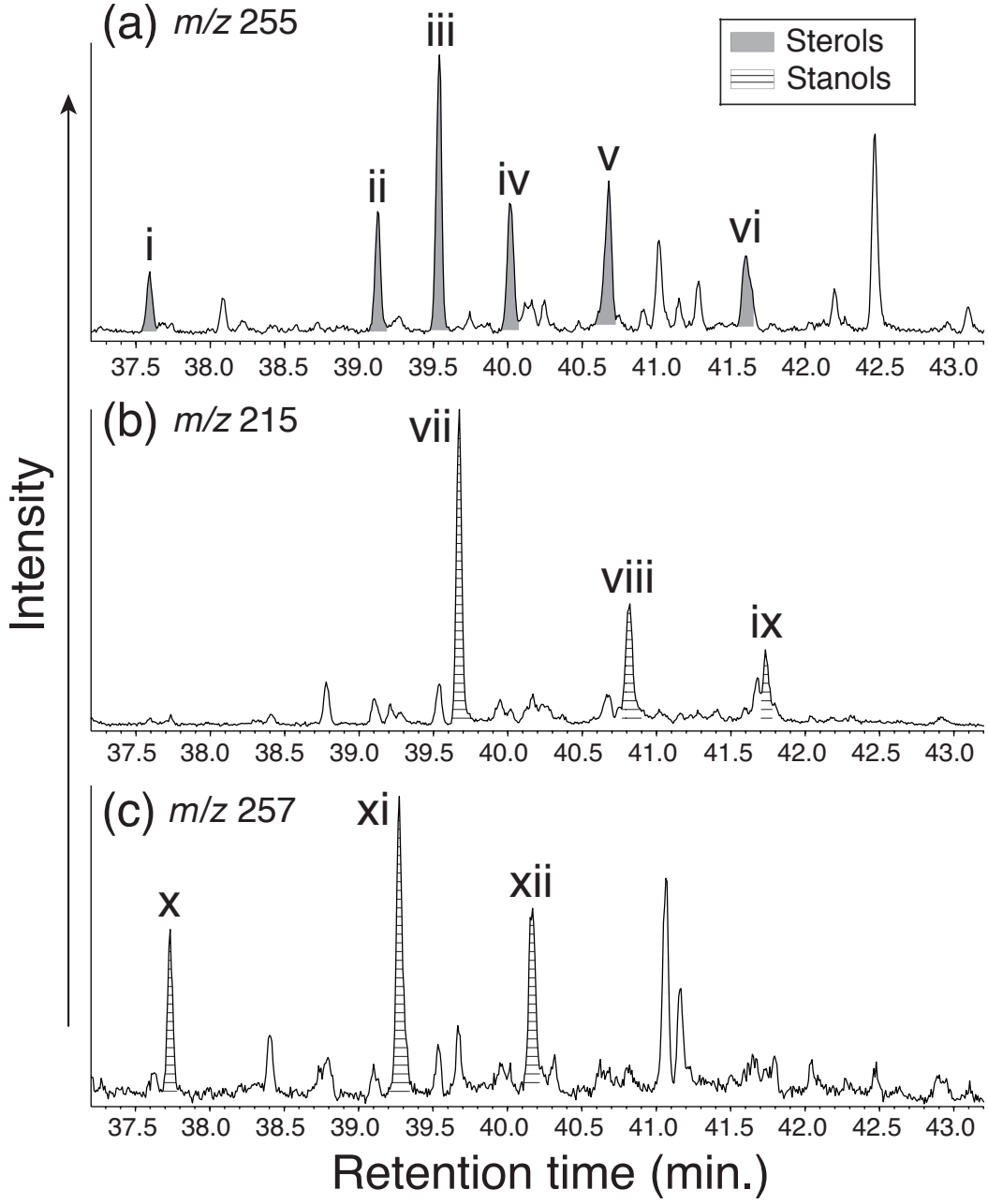

Fig. 4-4 Mass chromatograms from gas chromatography-mass spectrometry (GC–MS) using the tetramethylammonium hydroxide thermochemolysis (TMAH) method. Data were collected for sterols and stanols (as methyl ethers) of Lake Suigetsu sediments (SUI-92-2) [(a) *m*/*z* 255, (b) *m*/*z* 215, (c) *m*/*z* 257]. Identified sterols and stanols are listed in Table 4-2.

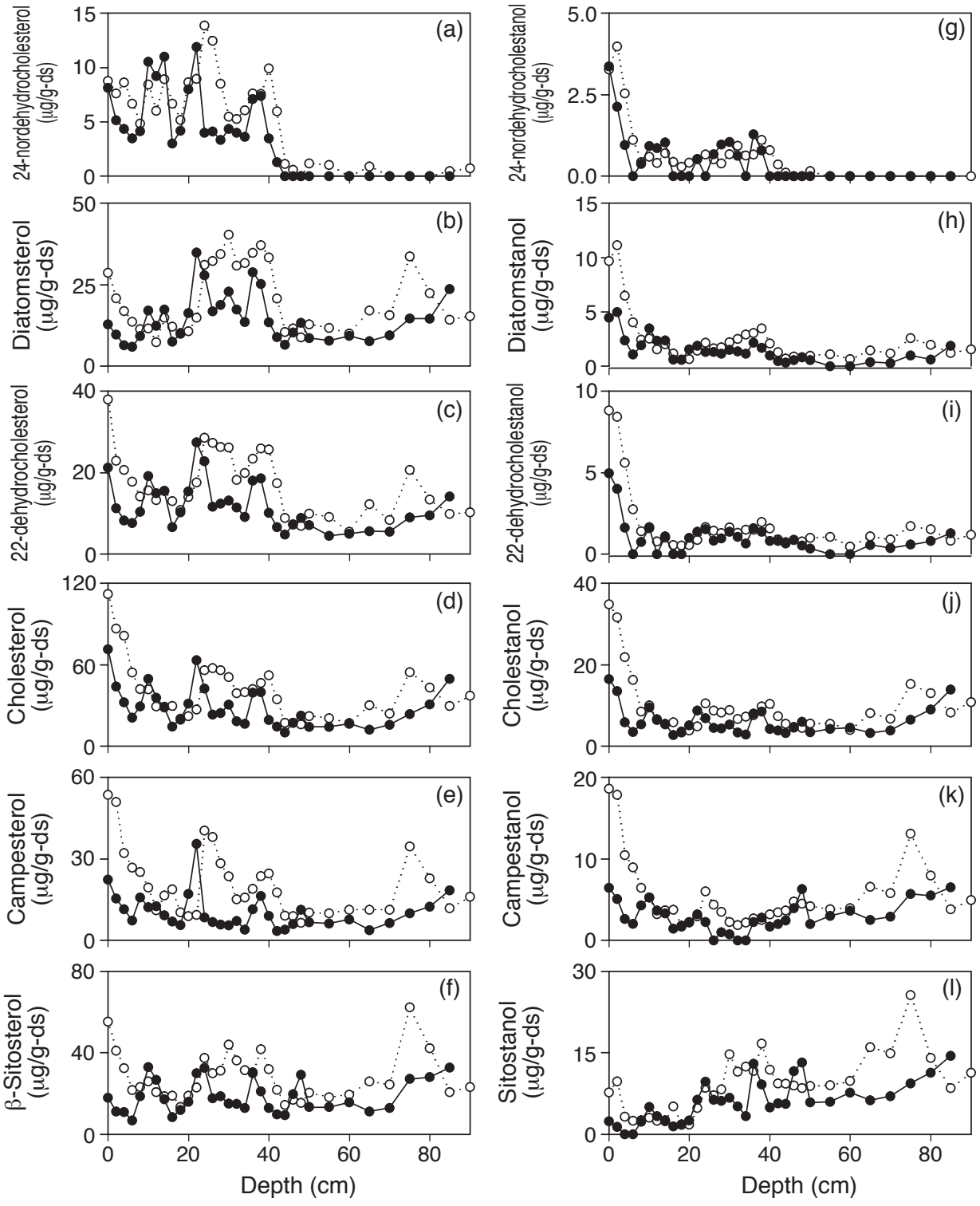

Fig. 4-5 Vertical distributions of sterol and stanol concentrations (μg/g) in Lake Suigetsu sediments. SUI92-1 and SUI92-2 are represented by open circles (○) and black circles (●), respectively.

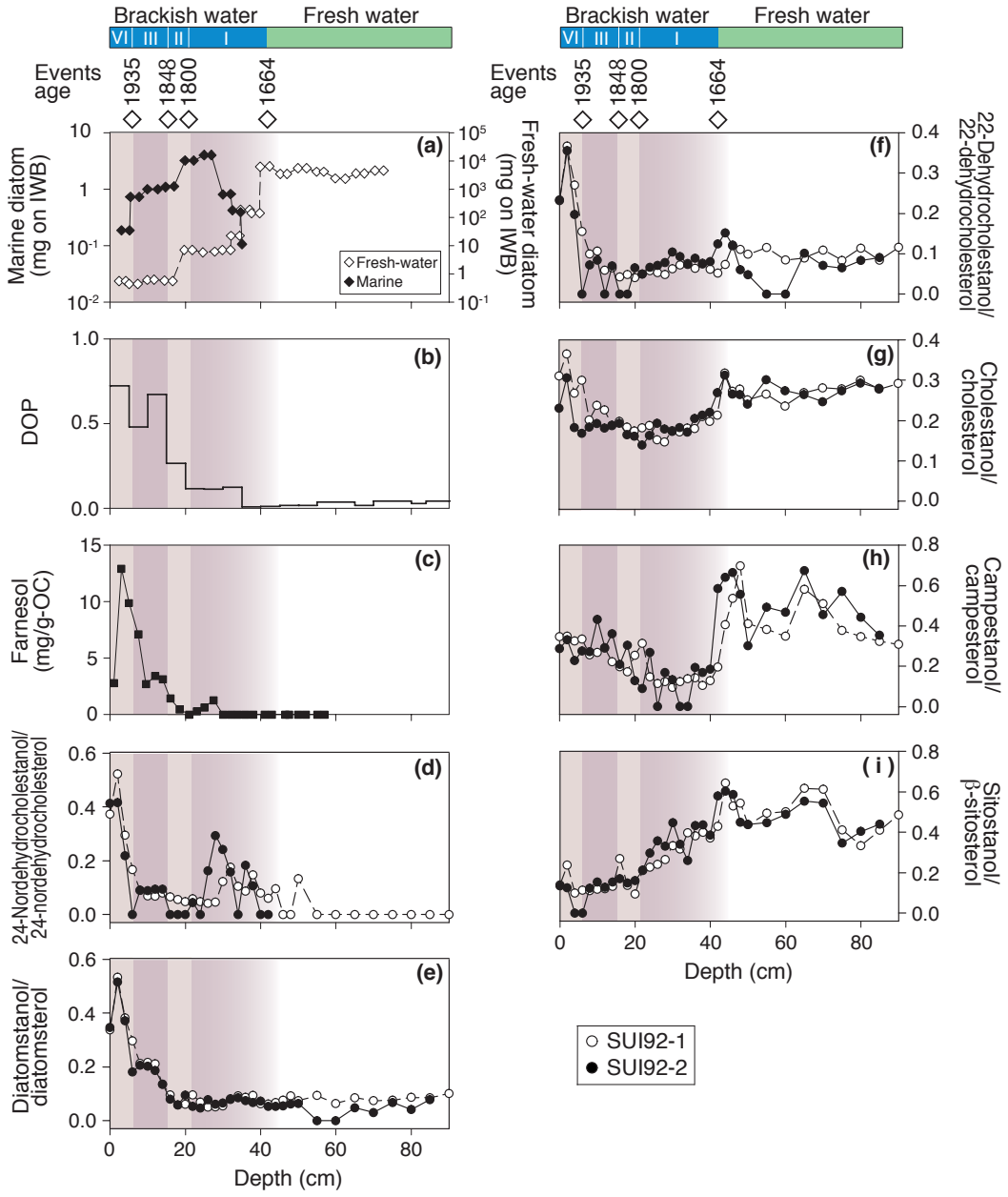

Fig. 4-6 Vertical distribution of diatom species, degree of pyritization (DOP), farnesol, and 5α(H)-stanol / Δ^5-sterol ratios recorded in Suigetsu Lake sediments. (a) Number of diatom valves (mg on an ignited weight basis [mg on IWB]) of marine diatom (*Coscinodiscus lacustris* [open diamonds, ◇]) and freshwater diatom (*Stephanodiscus carconensis* [black diamonds, ◆]) (Masuzawa and Kitano, 1982); (b) DOP, calculated using data from Matsuyama (1974); (c) farnesol concentration (mg/g-TOC [total organic carbon]) by Uemura et al. (1992); (d) 24-Nordehydrocholestanol / 24-nordehydrocholesterol ratio; (e) diatomstanol / diatomsterol ratio; (f) 22-dehydrocholestanol / 22-dehydrocholesterol ratio; (g) cholestanol / cholesterol ratio; (h) campestanol / campesterol ratio; and (i) sitostanol / β-sitosterol ratio. Core SUI92-1 and SUI92-2 are represented by open circles (○) and black circles (●), respectively. The chronology is taken from Fukusawa et al., 1995.

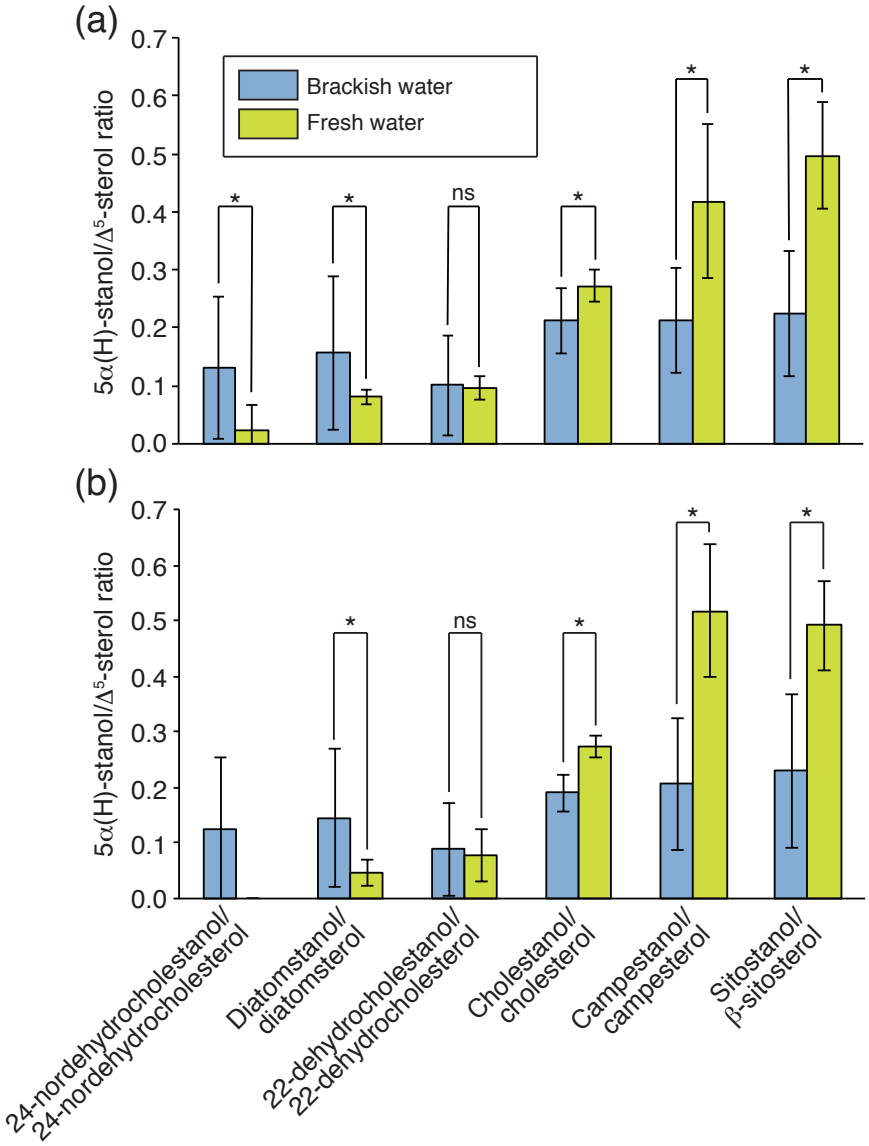

Fig. 4-7 Comparisons of 5α(H)-stanol / Δ^5-sterol ratios between the freshwater age (below 40cm; green color) and the brackish water age (above 40 cm; blue color) in Lake Suigetsu [(a) SUI92-1, and (b) SUI92-2]. Values are shown as means ± standard deviation. For freshwater, n = 12; for brackish water, n = 21. * = significance ($p < 0.01$). ns = no significance ($p > 0.01$).

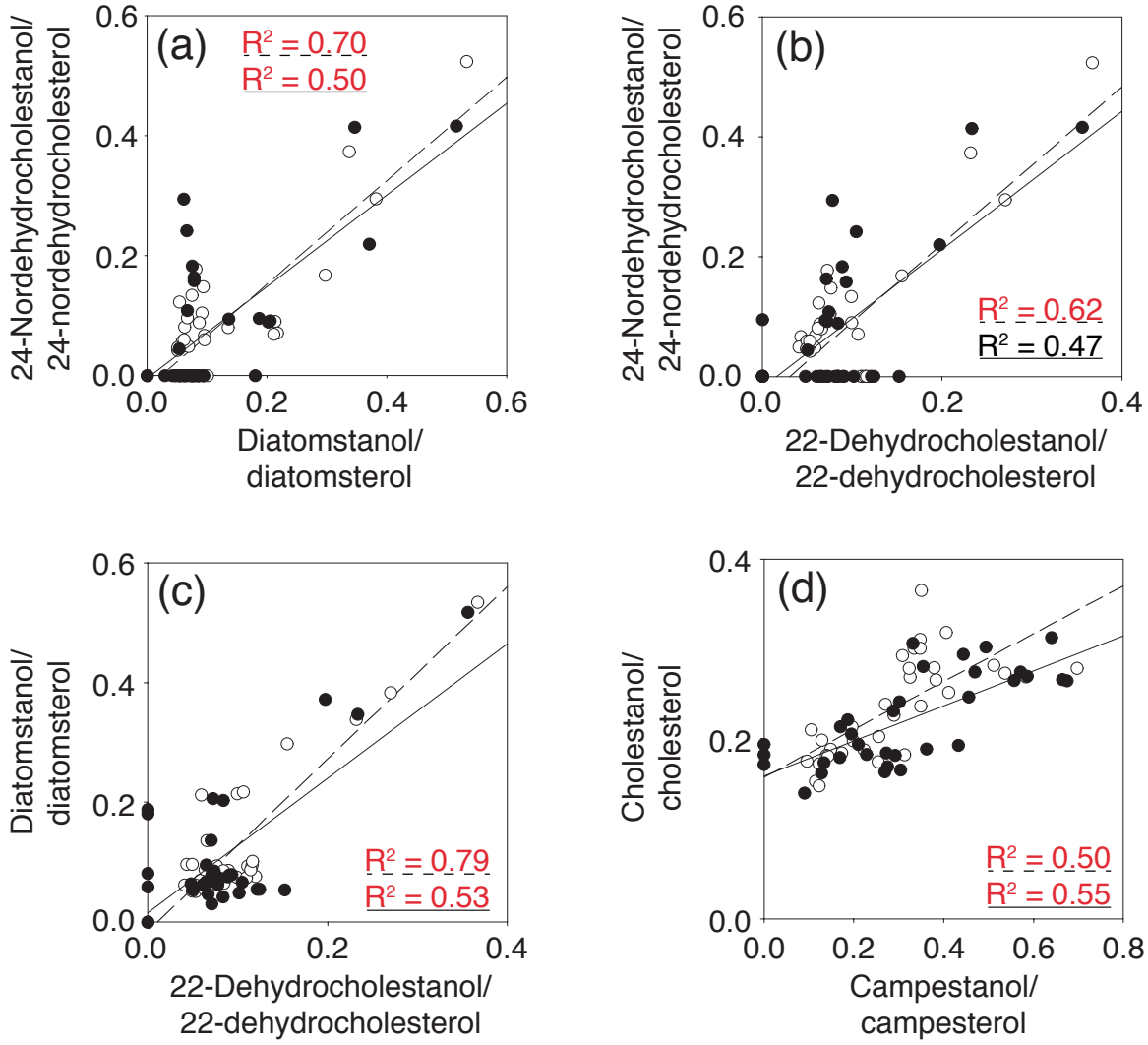

Fig. 4-8 Relationships between 5α(H)-stanol / Δ^5-sterol ratios (n = 34). Red colored numbers are significantly correlated at $R^2 > 0.05$. Relationships between other 5α(H)-stanol/Δ^5-sterol ratios show no significant correlation (See Fig. 4-9).

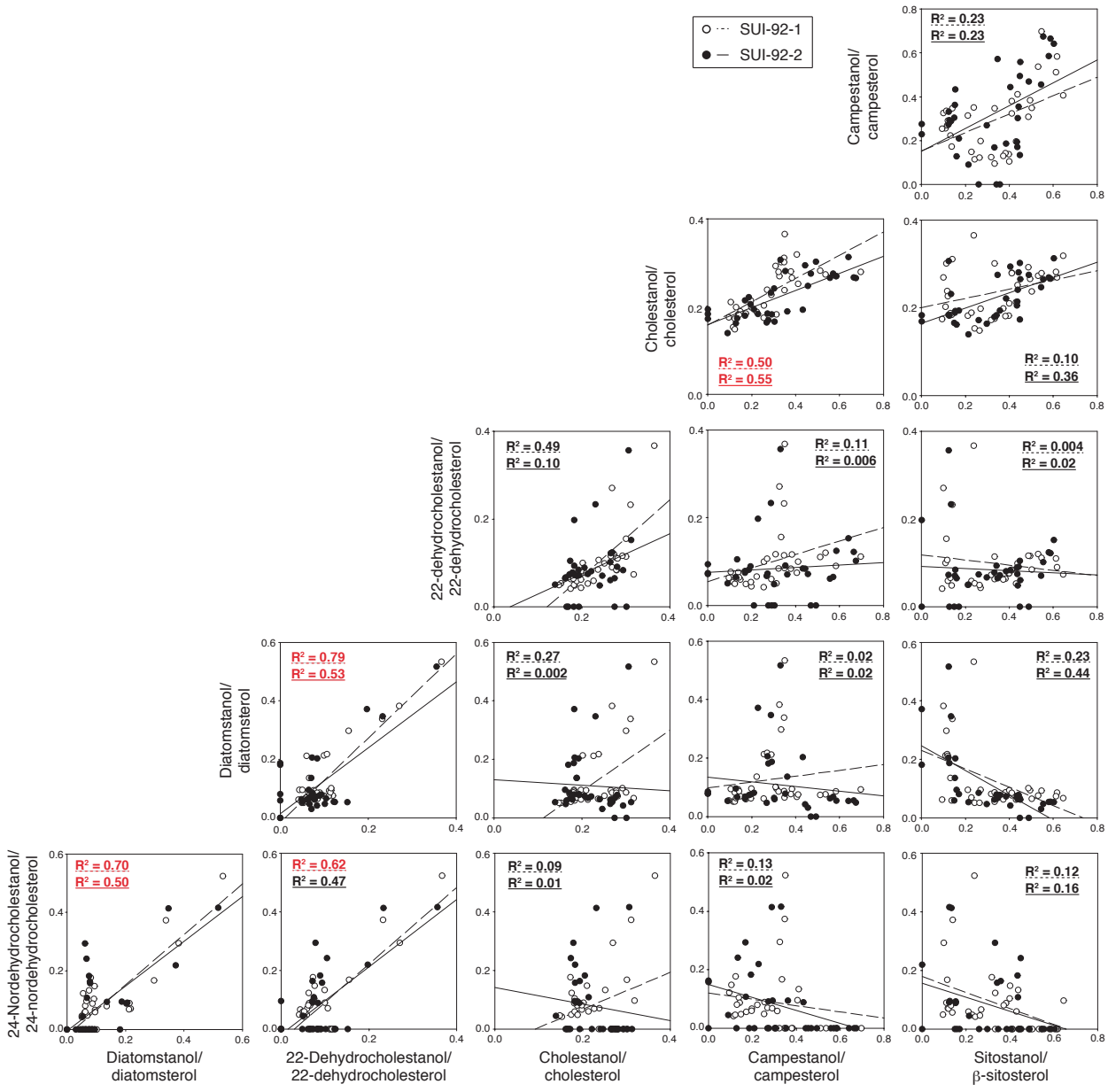

Fig. 4-9 Relationships between 5α(H)-stanol / Δ^5-sterol ratios.

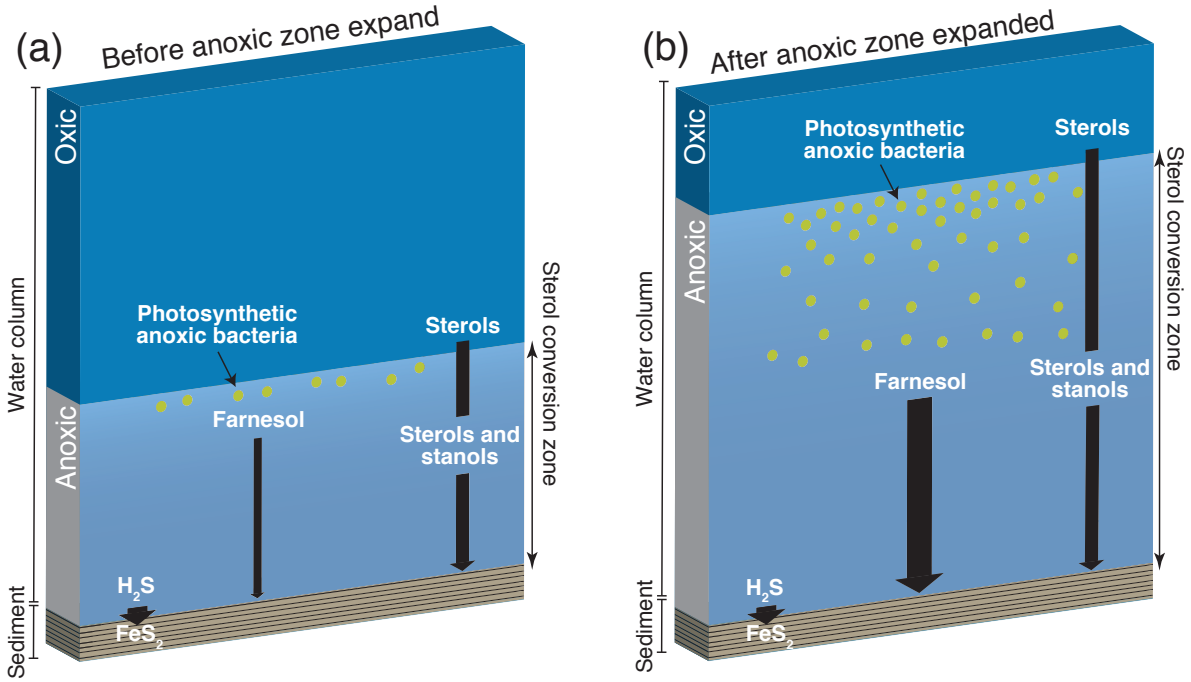

Fig. 4-10 Characteristics of each tracer (degree of pyrization, farnesol, and stanols and sterols) between different anoxic zone environments. Pyritization occurs in surface sediments (Berner, 1984), therefore the degree of pyritization records bottom-water conditions. Farnesol is derived from green sulfur bacteria (Rapoport and Hamlow, 1961; Caple et al., 1978; Otte et al., 1993; Airs and Keely, 2003). An expansion of the anoxic zone can be speculated to increase the habitat of green sulfur bacteria and its related farnesol concentration in sediment. Reduction of sterol to stanol occurs in the water column and surface sediments (e.g., Nishimura and Koyama, 1977; Wakeham, 1989), therefore the expansion of an anoxic zone in the water column promotes the reduction of Δ^5-sterol to 5α(H)-stanol, and increases the 5α(H)-stanol / Δ^5-sterol ratio. However, when there are other contributions besides the reduction of Δ^5-sterol by bacteria, those inputs are added into this model figure. (a) Stable anoxic condition during Brackish III; (b) expanded stable anoxic condition during Brackish IV. The chemocline in the present Lake Suigetsu is at around 6–7 m depth and phototrophic sulfur bacteria (mostly *Chlorobiaceae*) has a habitat depth of 5–10 m (the maximum densities are at 6–7 m; Mori et al., 2013), as shown in (b). Fig. 9a sets the oxic-anoxic boundary to 15 m depth. Photosynthetic active radiation of the present Lake Suigetsu has reached > 10 m (Mori et al., 2013), therefore it is expected that a phototrophic sulfur bacteria habitat at around 15 m could be possible.

Chapter 5

Sterol and Stanol Compositions in Sediments from Pond of Literature

5.1. Introduction

Sterols have been widely distributed from marine and lacustrine sediments (e.g., Ishiwatari et al., 2009a; Bertrand et al., 2012; Yamamoto et al., 2015; Huang et al., 2016). Sources of sterols in sediments are phytoplankton and terrestrial plants (Volkman, 1986). 24-Ethylcholest-5-en-3β-ol (β-sitosterol) and 24-methylcholest-5-en-3β-ol (campesterol) are major sterol components in higher plants (Yunker et al., 1995; Belicka et al., 2004; Killops and Killops, 2013). 24-Methylcholesta-5, 22E-dien-3β-ol (diatomsterol or brassicasterol) is mainly derived from phytoplankton such as diatom and haptophyte algae (e.g., Volkman et al., 1981b, 1998; Rampen et al., 2010). Utilization of their structural features, such as the number and position of the double bonds, types of functional groups, and the carbon content, sterols can be used as tracers for photo- and auto-oxidation (Christodoulou et al., 2009; Rontani et al., 2012, 2014). On the contrary, the 5α(H)-stanol / Δ^5-sterol ratio can be used as a tracer for redox conditions because the Δ^5-sterol is reduced to 5α(H)-stanol by bacterial reactions under anoxic conditions (Rosenfeld and Hellman, 1971; Eyssen et al., 1973; Fig. 1C). Therefore, in anoxic conditions, high value of the 5α(H)-stanol/Δ^5-sterol ratio are expected (Nishimura and Koyama, 1977; Wakeham, 1989). In Chapter 3, it was shown that this tracer was useful for reconstructing the redox events recorded in continuous sediment sequences (marine sediments off southern California, Ocean Drilling Program, Leg 167, Hole 1017E) over the last 45 kyr.

In contrast, inputs of 5α(H)-stanol were obtained by not only the bacterial reduction of Δ^5-sterol but also the other sources (e.g., Nishimura, 1977a; Gagosian et al., 1980; Volkman et al., 1990), which complicate the interpretation of 5α(H)-stanol/Δ^5-sterol ratio as redox tracer. In fact, high 5α(H)-stanol inputs was reported at various sites other than anoxic environments (Kondo et al., 1994; Rontani et al., 2014).

Furthermore, it is suggested that living organisms such as higher plants and phytoplankton containing high 5α(H)-stanols might be important sources of 5α(H)-stanols in sediments (Nishimura and Koyama, 1977; Robinson et al., 1984; Volkman et al., 1990; Fig. 1A). Also, the sedimentary 5α(H)-stanol/Δ^5-sterol ratio can significantly change by selective degradation during sedimentation processes of Δ^5-sterols (Nishimura, 1977a; Nishimura and Koyama, 1977; Fig. 1B). Regarding the selective degradation of Δ^5-sterols, there have been reports in the sediments of Lake Shirakoma-ike (Nagano, Japan), suggesting 5α(H)-stanol compositions in the sediments were significantly affected by plant-derived degradation products because plant-derived 5α(H)-stanols (such as 24-ethyl-5α(H)-cholestan-3β-ol; sitostanol) could be abundantly found (Nishimura, 1977a). From these insights, it is suggested that such selective degradation of Δ^5-sterol can occur under oxidative environments, although our understanding of the origins and fate of 5α(H)-stanols still require further examination. The investigation of production and diagenetic processes for the 5α(H)-stanol in oxic environments should provide better understanding of its source and behavior.

In addition, tetramethylammonium hydroxide (TMAH) thermochemolysis was employed for the 5α(H)-stanol / Δ^5-sterol analysis of the sediments in our study. Organic compound analysis using the TMAH method was utilized since hydrolysis and methylation are simultaneously performed, and in a relatively short time. Using the TMAH method, various sterols including Δ^5-sterols, 5α(H)-stanols, and 4α-methyl-sterols were identified from marine sediment, and thus, this method makes it possible to analyze variety of sterols, as analyzed by trimethylsilyl derivatization (Asperger et al., 1999b, 2001).

In the present study, I observed Pond of Literature (Bungaku-no-ike pond) within Soka University as a new site where the 5α(H)-stanol / Δ^5-sterol ratios were high

in the sediments of oxidative conditions, and report the sterol compositions to examine the applicability of redox indicator using the 5α(H)-stanol / Δ^5-sterol ratios.

5.2. Material and methods

5.2.1. Sampling location and pond samples

Pond of Literature is a small pond (ca. 3000 m^2) located in Soka University, Hachioji, Tokyo, Japan (35°41'24"N, 139°19'41"E) (Fig. 5-2). The water depth is < 2 m, and the pond is not connected to a river system.

Sediment samples (St. 1 and St.2) were obtained on August 20, 2017 using an Ekman–Birge grab sampler. The sediment samples were frozen, lyophilized and finely powdered for tetramethylammonium hydroxide (TMAH) thermochemolysis. The vegetation around the pond includes: konara oak (*Quercus serrate*), several cherry trees (*Cerasus yedoensis*), and azalea (*Rhododendron*). The distribution of vegetation surrounding the pond is summarized in Fig. 5-3.

At the sampling location, dissolved oxygen (DO) was measured in triplicate using a DO water test kit (Kyoritsu Chemical-Check Lab. Corp.). The DO at the water surface (ca. 10 cm in water depth) and bottom (ca. 1.8 m in water depth) were 7.3 ± 0.6 mg/L and 5.5 ± 0.5 mg/L, respectively, indicating that the pond is under oxidative conditions. The DO values were measured on the same day of sampling of the sediments (August 20, 2017). Similar values of DO were also confirmed on July 25, 2017 during a preliminary experiment (the surface; ca. 6 mg/L, the bottom; ca. 5 mg/L). Although DO data are limited from July to August, a fountain water circulation system is operational throughout the year preventing stagnation of the aquatic conditions in the pond.

5.2.2. Plant samples around the pond

Leaf samples of *Quercus serrate, Cerasus yedoensis*, *Rhododendron* satsuki, and *Rhododendron* tsutsuji were taken on November 5 and 6, 2017. The samples were lyophilized and powdered for sterol composition analysis.

5.3.2. Methods

5.3.2.1 Carbon content and stable isotope analysis

Total organic carbon (TOC), total nitrogen (TN), and the carbon isotope ratio in the sediment samples were analyzed using an elemental analyzer (EA1110, Thermo Fisher Co.) and an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Co.). Powdered sediment samples (ca. 10 mg) were wrapped in tin foil, and then analyzed with the instruments. The carbon isotope ratio was expressed in δ notation referenced to Vienna Pee Dee Belemnite limestone. The analysis error was < ± 0.24‰.

5.3.2.2 Analysis of organic matter using the TMAH method

Sterol compositions in the sediments were analyzed using TMAH thermochemolysis gas chromatography-mass spectrometry (TMAH GC-MS). The samples (ca. 100 mg for the sediment and 5 mg for the leaf samples) were placed in a 10 mL glass ampoule and the TMAH reagent (25 wt.% in methanol; 150 μL) was added. Nonadecanoic-d_{37} acid (100 ng/μL in methanol; 50 μL) was added as an internal standard. After the methanol (MeOH) evaporated, the ampoule was sealed under vacuum conditions and placed in a 300°C oven for 30 min. The combined extracts (with ethyl acetate) were dried in a vacuum desiccator and were re-dissolved in 100 μL of ethyl acetate. Lastly, 2 μL of the dissolved sample was injected (splitless injection at 300 °C) and analyzed with a GC-MS (6890N GC5973 MS; Agilent Technologies Co.) on

a DB-5MS capillary column (0.25 mm internal diameter (i.d.), 0.25 μm film thickness (Agilent Technologies Co.), 30 m in length) using helium as the carrier gas at 1.0 mL/min. The oven temperature was set at 60°C for 2 min, changed to 310°C (6°C/min) and was then maintained at 310°C for 20 min. The mass spectrometer was set to a full scan ion monitoring mode (50–650 Dalton) with an MS scanning interval of 0.5 s. Sterol concentrations were calculated by comparing with internal standards.

5.3. Results

5.3.1. Bulk carbon, nitrogen content, and sterol compositions in the sediment samples

TOC and TN in the sediment samples are similar (Table 5-1); high TOC (12.8%–13.7%), low TN (1.2%–1.3%) with C/N ratio of 10.2–10.6. The carbon isotope ratios ($\delta^{13}C$) are -30.5‰ in both of the sediment samples.

The major sterols were identified in the sediment samples included cholest-5-en-3β-ol (cholesterol), diatomsterol, campesterol, β-sitosterol, 5α(H)-cholestan-3β-ol (cholestanol), 24-methyl-5α(H)-cholestan-3β-ol (campestanol), 24-methyl-5α(H)-cholest-22E-en-3β-ol (diatomstanol or brassicastanol), and sitostanol (Fig. 5-4). The sterol compositions are listed in Table 5-2. Among the Δ^5-sterols, concentration of β-sitosterol is the highest (9.43–13.52 μg/g), whereas that of diatomsterol is much lower (1.48–1.77 μg/g). Sitostanol is the major 5α(H)-stanol component in the sediments (2.78–3.88 μg/g). Although the diatomstanol was detected in the sediment, only trace amounts were found (< 0.28 μg/g).

The ratios of cholestanol / cholesterol and campestanol / campesterol in the sediments show high values of 0.76–0.85 and 0.60–0.80, respectively. The sitostanol / β-sitosterol ratios are smaller (0.29) than the cholestanol / cholesterol and campestanol / campesterol ratios despite the high concentration of sitostanol. The diatomstanol /

diatomsterol ratio has a significantly lower value (0.11–0.16) than the other ratios.

5.3.2. Sterols of higher plants from the area surrounding a pond.

Sterol compositions of the leaves obtained from the area surrounding the pond are listed in Table 5-2, and the representative mass chromatograms are shown in Fig. 5-5. The β-sitosterol was identified as the major sterol component from all leaf samples (373.9–426.4 μg/g). The concentrations of campesterol in leaf samples of *Cerasus yedoensis* are lower than that of the β-sitosterol (9.29 μg/g). As for the 5α(H)-stanols, only trace amounts of sitostanol were detected in *Quercus serrate*.

5.4. Discussion

5.4.1. Major sterol sources in the sediment

β-Sitosterol is documented in higher plants as the major sterol components (Yunker et al., 1995; Belicka et al., 2004; Killops and Killops, 2013). The β-sitosterol has the highest concentration in the Δ^5-sterols in the sediment samples, and is the main sterol found in the leaf samples from the area around the pond (Table. 5-2). Diatomsterol is found in phytoplankton including diatoms and haptophyte algae (Kanazawa et al., 1971; Orcutt and Patterson, 1975; Teshima et al., 1980; Volkman et al., 1981b, 1998; Lin et al., 1982; Marlowe et al., 1984; Rampen et al., 2010). The concentration of diatomsterol is much lower in the sediments (Table 5-2). Huang and Meinschein (1979) indicated that the ternary plots of C_{27}, C_{28}, and C_{29} sterol compositions can be used as an ecological indicator. Phytoplankton contain high amounts of C_{27} sterols, while terrestrial plants contain high amounts of C_{29} sterols. Plots of C_{27}, C_{28}, and C_{29} sterol compositions in the sediments show closer distribution to terrestrial plants (Fig. 5-6), suggesting that the sterols in the sediments are mainly derived from higher terrestrial

plants.

5.4.2. Environmental condition of the pond evaluated by 5α(H)-stanol compositions in the sediments

In general, high contribution of 5α(H)-stanol in natural environment is thought to be resulted from three routes; (1) bacterial reduction of Δ^5-sterol under anoxic condition (e.g., Gagosian et al., 1979; Wakeham, 1989, Fig. 5-1C), (2) direct inputs from unique living organisms containing high 5α(H)-stanols (Robinson et al., 1984; Volkman et al., 1990, Fig. 5-1A), and (3) selective degradation of Δ^5-sterol during sedimentation processes (e.g., Nishimura, 1977a, Fig. 1B). For the first route of bacterial reduction of Δ^5-sterols, the 5α(H)-stanol generates by the bacterial reduction of Δ^5-sterol under anoxic conditions (Rosenfeld and Hellman, 1971; Eyssen et al., 1973). Therefore, the 5α(H)-stanol / Δ^5-sterol ratio can be used as a tracer of bacterial anoxic activity, and also can apply to evaluation of depositional environments including paleo-studies (Canuel and Martens, 1993; Bertrand et al., 2012; Zheng et al., 2015). Under anoxic condition, the microbial conversion of Δ^5-sterol to 5α(H)-stanol occurs in surface sediments and water columns (Nishimura and Koyama, 1977; Wakeham, 1989; Fig. 5-1C). Also, the 5α(H)-stanol / Δ^5-sterol records were reported in marsh environment and peat sequences in the Miocene, and increase of their ratios may be attributed to degradation and microbial hydrogenation of biosterols during the early diagenetic stage (Sawada et al., 2013; Stefanova et al., 2016). However, it should be noted that variations of the 5α(H)-stanol / Δ^5-sterol ratios can also be related to environmental conditions, and the 5α(H)-stanols are derived directly from organisms or that the Δ^5-sterols were converted to 5α(H)-stanols in the early diagenesis (Stefanova et al., 2016).

The higher 5α(H)-stanol / Δ^5-sterol ratios was reported in severe anoxic water

and sediment of the Black Sea (0 μmol/kg in DO below ca. 100m); remarkable high values at redox boundaries of the water column (>1; Wakeham, 1989) and the surface sediments (>1; Gagosian et al., 1979). In sediments from offshore of the southern California, 24-nordehydrocholestanol / 24-nordehydrocholestanol and diatomstanol / diatomsterol ratios indicated high values (~ 0.7) during suboxic (warming) intervals of the Marine Isotope Stage (MIS) 3 (Chapter 3).

Among the 5α(H)-stanol / Δ^5-sterol ratios, diatomstanol / diatomsterol ratio is suggested to be a good redox tracer, because inputs other than reduction of sterols in anoxic conditions is considered to be small in both marine and lacustrine sediments (See Chapters 3 and 4). However, the diatomstanol / diatomsterol ratios are low (< 0.16) in the Pond of Literature of the present study, which is strikingly lower than the 5α(H)-stanol / Δ^5-sterol records in the anoxic conditions reported previously. The result suggests that the pond in our work is an oxidizing environment based on the organic chemical trace, as well as the DO contents in the bottom water (5.5 ± 0.5 mg/L).

On the other hand, the ratios of cholestenol / cholesterol, campestanol / campesterol, and sitostanol / β-sitosterol shows markedly higher values compared to diatomstanol / diatomsterol ratio, similar to typical anoxic condition levels found in the Black Sea and southern California. The results indicate use of these ratios as a redox tracer is not suitable, and the higher contents of 5α(H)-stanol (cholestanol, campestanol, sitostanol) in the sediments are possibly caused by biological/geochemical effect(s) other than the bacterial reduction of the Δ^5-sterols.

5.4.3. Possibility of source of 5α(H)-stanol in the sediment

In previous studies, high 5α(H)-stanol contents (cholestanol, campestanol, and sitostanol) have been documented even in oxidative conditions. Kondo et al (1994) found

high choletanol (cholestenol / cholesterol ratio; 0.66–0.85), campestanol (campestanol / campesterol ratio: 0.63–0.67) and sitostanol (sitostanol / β-sitosterol ratio: 0.59–0.63) contents (Table. 5-4) in the sediments from acidic lake such as Lake Fudo-ike (Kirishima, Japan). Nishimura (1977a) also reported high values of 5α(H)-stanol / Δ^5-sterol ratios (cholestenol / cholesterol; 0.56–0.82, campestanol / campesterol; 0.37–1.00, sitostanol / β-sitosterol; 0.67–1.22) from the sediments of Lake Shirakoma-ike (Nagano, Japan). Previous studies suggested that the contribution of 5α(H)-stanols except the bacterial reduction of Δ^5-sterols was significantly large to the sterol composition in freshwater sedimentary environments, which agrees with our results. The high 5α(H)-stanol inputs complicate interpretation of the 5α(H)-stanol / Δ^5-sterol ratio as an indicator of redox condition. Thus, it is important to explore the cause of high 5α(H)-stanol contents other than reducing environments, and as the results suggest, it is expected to provide better interpretation of the 5α(H)-stanol / Δ^5-sterol ratio as the indicator.

5.4.3.1. Terrestrial sources

The high 5α(H)-stanol contents in the sediments from the Pond of Literature are likely to be related to terrigenous sources, because the ternary plots of C_{27}, C_{28}, and C_{29} sterols indicate significant contribution from higher plants (Fig. 5-6). In general, the content of 5α(H)-stanol in living higher plants are low (e.g., Nishimura, 1977b; Table 3), which is similar to the results in the present study (Table 5-2). However, according to Nishimura (1977b), only *Quercus serrata* is known to have relatively high sitostanol content (30.3% of all the sterols; sitostanol / β-sitosterol ratio: 0.48; Table 3). Since the Pond of Literature is surrounded by *Quercus serrata*, there is a possibility that the plant-derived 5α(H)-stanols contributed to the sediments. However, content of sitostanol in the leaf samples of *Quercus serrata* around the pond were trace in the

present study (Table 2). The difference of the sterol compositions in *Quercus serrate* between Nishimura (1977b) and our study may be attributed to chemical compositional variability as a result of seasonal and environmental effects.

Another possibility is a mechanism that the 5α(H)-stanols from living organisms are concentrated by selective degradation process of Δ^5-sterols under oxidative environments (Nishimura, 1977a; Nishimura and Koyama, 1977). Since the Δ^5-sterols are easily degraded in oxidative conditions compared to 5α(H)-stanols, high 5α(H)-stanol / Δ^5-sterol ratios in sediments are expected even if the direct contribution of 5α(H)-stanol from living organisms is low (Fig. 5-1B). Furthermore, Nishimura (1977a) suggests that 5α(H)-stanol, especially derived from terrestrial organism, could contribute to sediment through the degradation process. Since sitostanol was detected in the leaf samples, the high sitostanol / β-sitosterol ratio in the sediments can be interpreted as the results that the sitostanol was concentrated by the selective degradation process of β-sitosterol (Table 5-2). Although the sitostanol / β-sitosterol ratio in our work is lower than those in Lake Shirakoma-ike (0.67–1.22; Nishimura, 1977a), fresh plant inputs around the Pond of Literature might be the cause. In fact, the sterol compositions in living plants around the pond showed high β-sitosterol concentrations (Table 5-2). Similarly, high 5α(H)-stanol contents in sediments from oxic conditions have been interpreted by inputs of degradation products from terrestrial sources associating with such the mechanism (Kondo et al., 1994; Arzayus and Canuel, 2005; Rontani et al., 2014). Since cholestanol and campestanol were not detected in the investigated plants (Table 5-2), it is difficult for the sources of campesterol and cholesterol to account for factors of the terrestrial plant sources and the degradation processes.

5.4.3.2. Phytoplanktonic sources

In aquatic organisms, the 5α(H)-stanol contents are conclusively low, although the same 5α(H)-stanols have been identified in zooplankton and phytoplankton (Nishimura and Koyama, 1976, 1977; Chardon-Loriaux et al., 1976). However, high contents of 5α(H)-stanol are known in some organisms as unique cases (Volkman et al., 1990). In freshwater phytoplankton species, to the best of our knowledge, high 5α(H)-stanol contents were reported in some dinoflagellates only (Robinson et al., 1984, 1987). Therefore, the 5α(H)-stanols from dinoflagellates could contribute to organic composition in sediments (Fig. 5-1A). In fact, the dinoflagellates have cholestanol and campestanol, which cannot be explained by the terrestrial sources. On the other hand, freshwater dinoflagellates are known to have high contents of 4α-methyl sterols, such as 4α-methyl-5α-cholestan-3β-ol and 4α,23,24-trimethyl-5α-cholest-22-en-3β-ol (Robinson et al., 1987). Although dinoflagellate was not investigated in the present study, the high contents of 5α(H)-stanols in the pond sediments are not thought to be derived from the dinoflagellates because of no detection of 4α-methyl sterols.

Based on our results, it is difficult to reveal the cause of high 5α(H)-stanol sources in the sediments by only comparing the sterol compositions of plants and phytoplankton. As other possibility, it is pointed out that degradation and microbial hydrogenation of biosterols during the early diagenetic stage cause increasing of 5α(H)-stanol / Δ^5-sterol ratio in the Miocene geological records (Sawada et al., 2013; Stefanova et al., 2016). Although the time scale is more widely than our study site, these results suggest that effects of the early diagenetic stage should be consider for interpretation of 5α(H)-stanol / Δ^5-sterol ratio. Future studies will need to identify the specific sources of high 5α(H)-stanol in the pond sediment, such as multi-source analyses from

phytoplankton, plants and soils during long term studies including various seasons.

5. Conclusions

The present study reports the sterol compositions in an oxidative pond surrounded by higher plants. Compositions of Δ^5-sterol in the sediments are characterized as high contribution of terrestrial sterol (β-sitosterol) and low autogenous sterol (diatomsterol). High contents of cholestanol, campestanol, and sitostanol and high ratios of cholestenol / cholesterol and campestanol / campesterol were found in the sediments. A new case of high amounts of 5α(H)-stanols were found in an oxidative environment. The sources of 5α(H)-stanols and high 5α(H)-stanol / Δ^5-sterol ratio in the oxidative pond were dependent on three factors; 1) phytoplankton, 2) higher plants, and 3) preferential degradation of Δ^5-sterol under oxidative conditions. Although critical sources of cholestanol, campestanol, and sitostanol in the pond were not determined, the sitostanol might be formed as a result of the selective degradation of Δ^5-sterols during the early diagenesis, as suggested previously. These specific sources and production processes of the 5α(H)-stanols are expected to become clearer with more studies being performed on this model site.

Table 5-1 Organic carbon and nitrogen data in the sediment sample from the Pond of Literature.

	TOC (%)	TN	C/N	$\delta^{13}C$ (‰)
St. 1	13.7	1.3	10.5	-30.5
St .2	12.8	1.2	10.7	-30.5

Table 5-2 List of major sterols detected in the sediment samples from the Pond of Literature and sterols of higher plants collected the area around the pond.

	Sediment samples				Higher plants			
	St. 1		St. 2		Q.s.	C.y.	R.s.	R.t.
	(μg/g)	(%)*	(μg/g)	(%)*	(μg/g)			
Δ⁵-Sterols								
Cholesterol	2.00	7.5 (11.2)	1.42	7.7 (11.8)	-	-	-	-
Campesterol	2.28	8.5 (11.6)	1.21	6.5 (9.0)	-	9.29	-	-
Brassicasterol	1.77	6.6 (9.1)	1.48	8.0 (10.9)	-	-	-	-
β-Sitosterol	13.52	50.4 (69.1)	9.43	50.9 (69.6)	399.0	394.6	426.4	373.9
5α(H)-Stanols								
Cholestanol	1.70	6.3 (23.5)	1.08	5.8 (21.6)	-	-	-	-
Campestanol	1.37	5.1 (19.0)	0.97	5.2 (19.5)	-	-	-	-
Brassicastanol	0.28	1.1 (3.9)	0.16	0.9 (3.3)	-	-	-	-
Sitostanol	3.88	14.5 (53.6)	2.78	15.0 (55.6)	trace	-	-	-
Ratios								
Cholestanol/cholesterol	0.85		0.76		-	-	-	-
Campestanol/campesterol	0.60		0.80		-	-	-	-
Brassicastanol/brassicasterol	0.16		0.11		-	-	-	-
Sitostanol/β-sitosterol	0.29		0.29		-	-	-	-

Q.s. = *Quercus serrata*, C.y. = *Cerasus yedoensis*, R.s. = *Rhododendron* satsuki, and R.t. = *Rhododendron* tsutsuji.

* The values are given as % of the eight sterols, and the values in parentheses are given as % of total Δ^5-sterols and total 5α(H)-stanols.

Table 5-3 Sterols of freshwater dinoflagellates and higher plants reported by previous studies (Robinson et al., 1987; Nishimura, 1977b; Nishimura and Koyama, 1977).

	Freshwater dinoflagellates			Higher plants			
	P.l.	P.c.	C.f.	I.p.	P.d.	Q.s.	Z.l.
	(%)			(%)			
Δ⁵-sterols							
Cholesterol	trace	94.6	80.2	trace	trace	2.9	trace
Campesterol	-	1.8	-	3.0	7.8	3.6	5.3
Brassicasterol	-	-	-	-	-	-	-
β-Sitosterol	17.9	-	-	93.0	90.2	62.5	93.8
5α(H)-stanols							
Cholestanol	71.4	3.6	18.7	trace	trace	trace	trace
Campestanol	3.5	-	1.1	0.2	0.1	0.6	0.1
Brassicastanol	-	-	trace	-	-	-	-
Sitostanol	7.2	-	-	3.8	1.8	30.3	0.9
Ratios							
Cholestanol/cholesterol	-	0.04	0.23	-	-	-	-
Campestanol/campesterol	-	-	-	0.05	0.02	0.17	0.02
Brassicastanol/brassicasterol	-	-	-	-	-	-	-
Sitostanol/β-sitosterol	0.40	-	-	0.04	0.02	0.49	0.01

P.l. = *Peridinium lomnickii*, P.c. = *Peridinium cinctum*, C.f. = *Ceratium furcoides*. I.p. = *Ilex pedunculosa*, P.d. = *Pinus densiflora*, Q.s. = *Quercus serrata*, Z.l. = *Zizania latifolia*.

The sterol composition data in freshwater dinoflagellates and higher plants are taken from Robinson et al. (1987), Nishimura (1977b), and Nishimura and Koyama (1977), respectively.

*The values are given as % of the eight sterols.

Table 5-4 Sterols in sediment samples from other sites reported by previous studies (Nishimura, 1977a; Kondo et al., 1994).

	Sediment samples					
	S-1	S-2	S-3	S-4	F-1	F-4
			(μg/g)			
Δ^5-sterols						
Cholesterol	8.4	8	5.3	6.4	1.38	0.81
Campesterol	8.4	10.3	4.1	8.6	1.20	0.78
Diatomsterol	trace	6.6	trace	2.1	1.08	0.54
β-Sitosterol	45.8	39.7	27.6	47.3	2.38	1.76
5α(H)-stanols						
Cholestanol	6.9	4.5	3.2	4.2	1.12	0.69
Campestanol	5.4	3.8	4.1	4.6	0.80	0.49
Brassicastanol	-	-	-	-	0.35	0.15
Sitostanol	43.6	26.6	33.7	37.8	1.40	1.11
Ratios						
Cholestanol/cholesterol	0.82	0.56	0.60	0.66	0.81	0.85
Campestanol/campesterol	0.64	0.37	1.00	0.53	0.67	0.63
Brassicastanol/brassicasterol	-	-	-	-	0.32	0.28
Sitostanol/β-sitosterol	0.95	0.67	1.22	0.80	0.59	0.63

S-1 = Lake Shirakoma-ike (0-3 cm), S-2 = L. Shirakoma-ike (3-6 cm), S-3 = L. Shirakoma-ike (6-12 cm), S-4 = L. Shirakoma-ike (12-20 cm), F-1 = L. Fudo (0-2 cm), F-4 = L. Fudo (6-8 cm).
The sterol composition data in sediment samples of Lake Shirakoma-ike and Lake Fudo are taken from Nishimura (1977a) and Kondo et al. (1994), respectively.

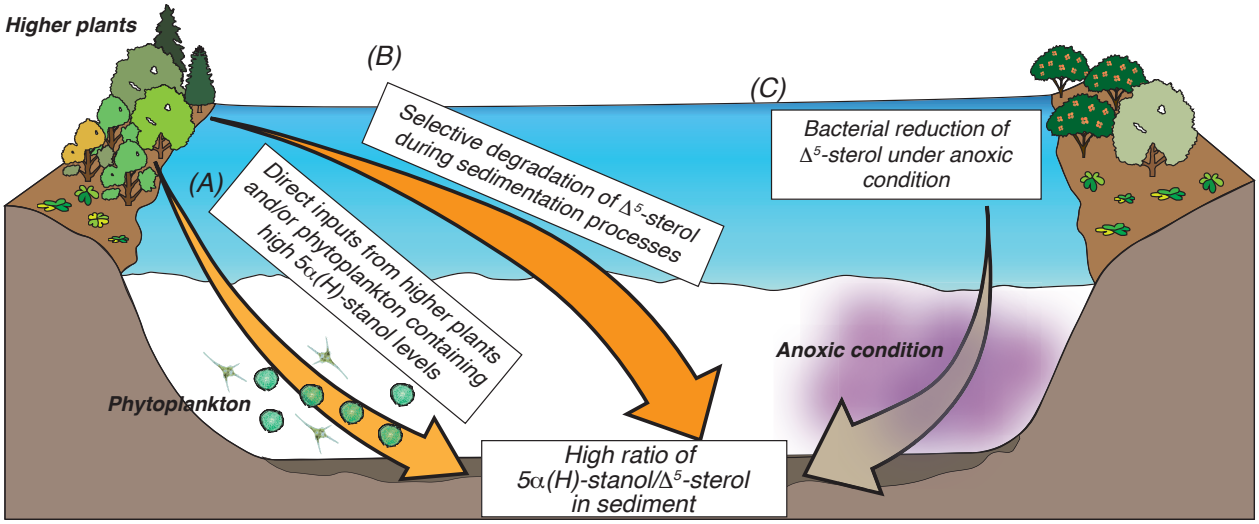

Fig. 5-1 Scheme of the high 5α(H)-stanol / Δ^5-sterol ratio in sediment. (A) Direct inputs from higher plants and/or phytoplankton containing high 5α(H)-stanol levels. Contributions of 5α(H)-stanol derived from living organisms are few, although organisms with high 5α(H)-stanol levels have also been reported (Robinson et al., 1984; Volkman et al., 1990; Nishimura, 1977b). (B) Selective degradation of sterol during sedimentation processes. Since the sterols are more susceptible to decomposition than the 5α(H)-stanols under oxidative conditions, 5α(H)-stanols from living organisms can be concentrated during the sedimentation process (Nishimura, 1977a; Nishimura and Koyama, 1977). (C) Bacterial reduction of Δ^5-sterol under anoxic condition. The reduction occurs in the water column and surface sediments (e.g., Nishimura and Koyama, 1977; Wakeham, 1989). Therefore, the 5α(H)-stanol / Δ^5-sterol ratio can be used as a redox tracer (e.g., Bertrand et al., 2012; Zheng et al., 2015).

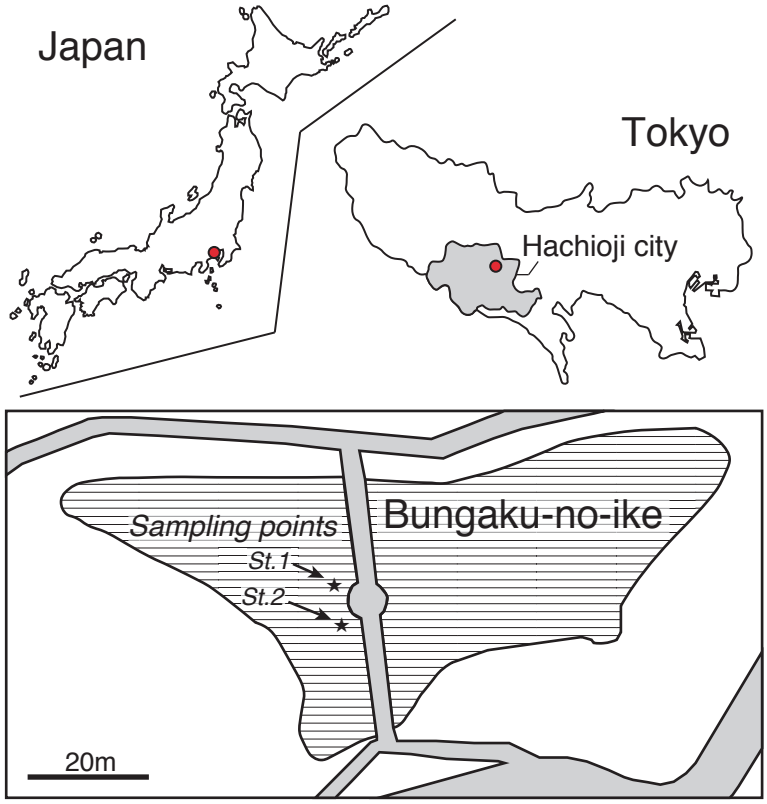

Fig. 5-2 Location of the Pond of Literature. The stars (★) denote sampling sites. The upper and the lower sites are St. 1 and St. 2, respectively.

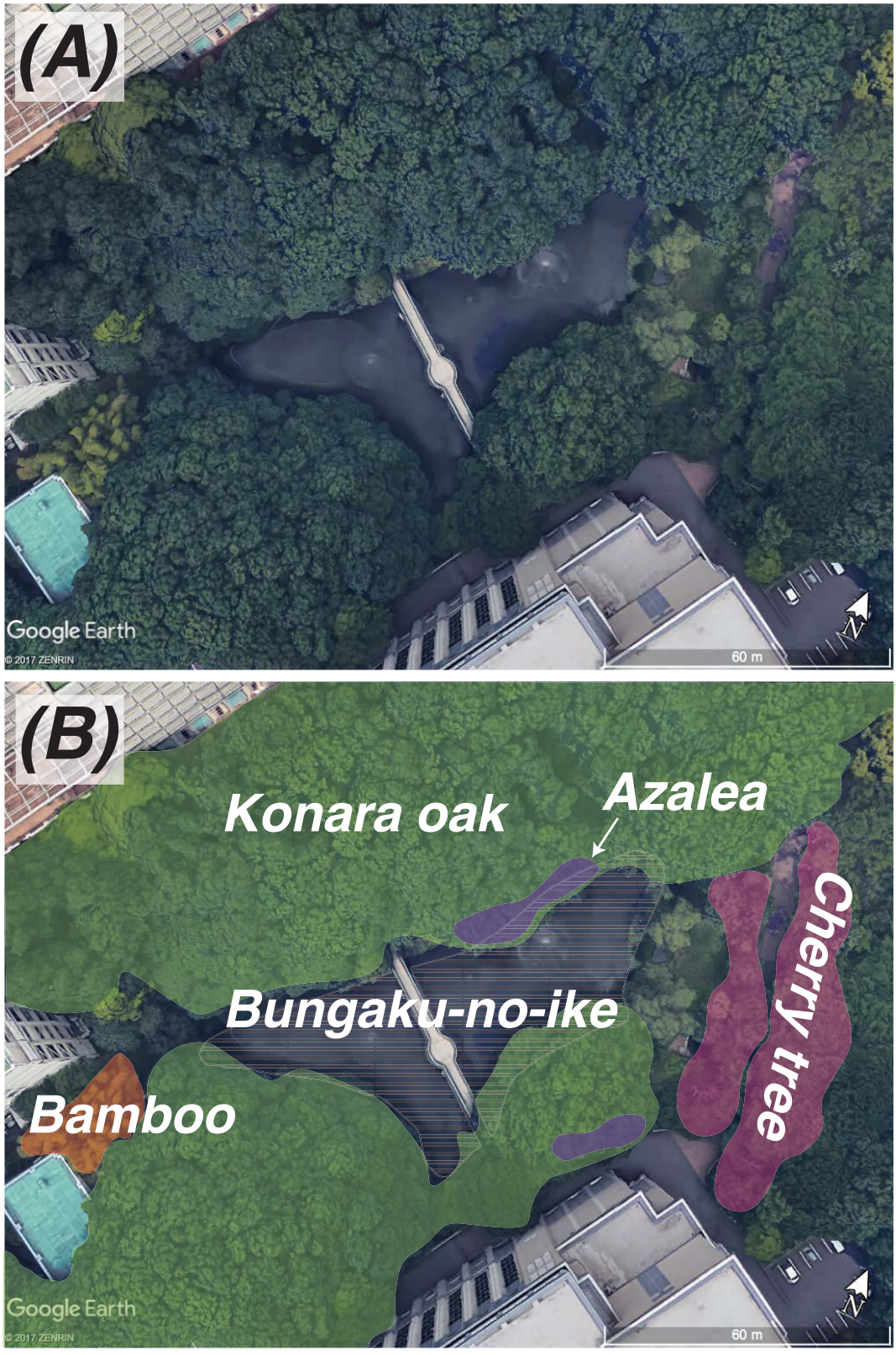

Fig. 5-3 Distribution of vegetation around the Pond of Literature: (A) Original picture. (B) Distribution of vegetation. The original picture was obtained from Google Earth (https://www.google.com/earth/).

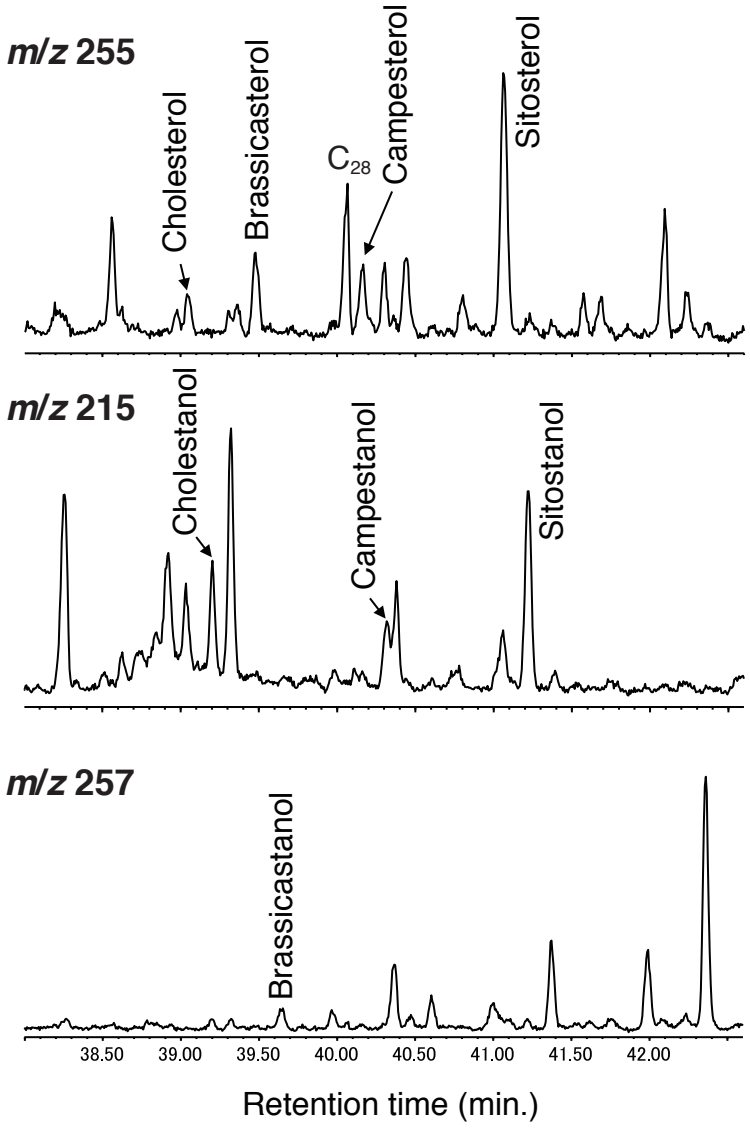

Fig. 5-4 Mass chromatograms (*m*/*z* 255, *m*/*z* 215, and *m*/*z* 257) of sterols from the sediment of St. 1.

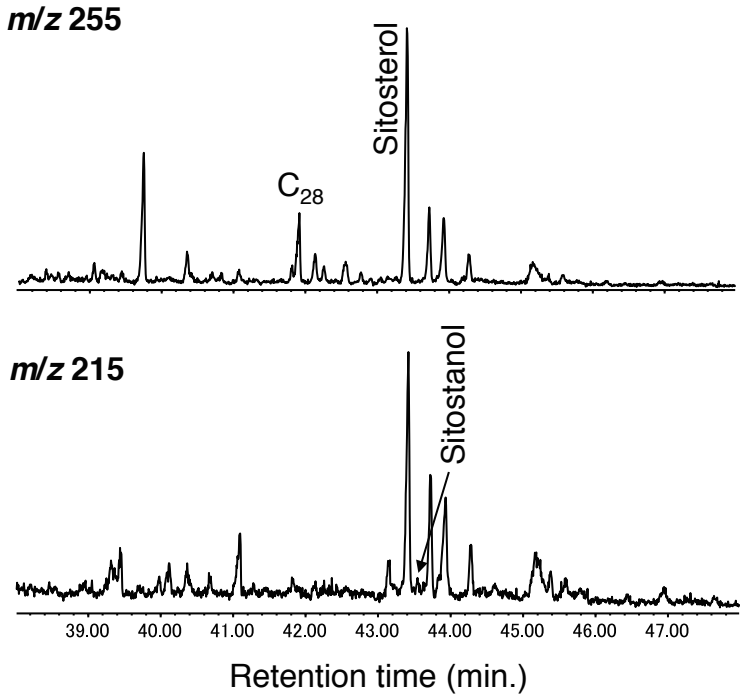

Fig. 5-5 Representative mass chromatograms (*m*/*z* 255 and *m*/*z* 215) of sterols from the plants (*Quercus serrate*).

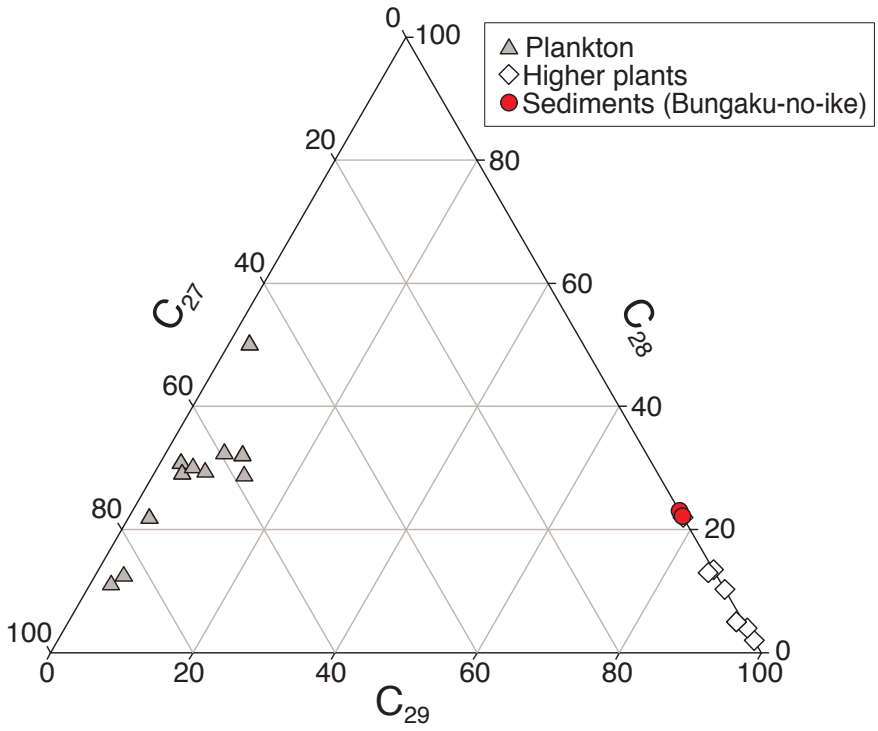

Fig. 5-6 Distribution of C_{27}, C_{28}, and C_{29} sterols in aquatic plankton, higher plants, and sediment samples. Gray triangles (▲) = plankton. White diamonds (◇) = higher plants. Red circles (●) = sediment samples from the Pond of Literature. C_{27} sterols = 22-dehydrocholesterol and cholesterol. C_{28} sterols = diatomsterol, campesterol, and 24-methylenecholesterol. C_{29} = stigmasterol and β-sitosterol. Plankton and higher plant distributions obtained from Huang and Meinschein (1979), including Attaway et al. (1971), Nishimura and Koyama (1976), Pryce (1971), and Meinschein and Kenney (1957).

Chapter 6
General Discussion

6.1. Application and expectation of the TMAH method for paleo-enviromental studies.

In this study, the Offline TMAH–GC–MS method was applied for analysis of organic compounds. In this method, TMAH reagent is added to the sample in an ampoule tube, and then organic matters are hydrolyzed and derivatized under thermochemical reaction. This method can also be performed with smaller amounts of sample (5 mg–100 mg dry weight) than in extraction method. Additionally, since pre-treatment of samples can be performed in shorter time, more number of samples can be treated with shorter time using the method.

As applications for sediment samples in previous studies, the TMAH method has been recognized as analysis method for lignin phenols and fatty acids which are mainly derived from terrestrial plants (e.g., Van Heemst et al., 2000; Ishiwatari et al., 2006; Bertrand et al., 2013; Ohira et al., 2014). On the other hand, in the TMHA method, detection cases and spectral patterns of other compounds such as cutin acids (e.g., del Rio and Hatcher, 1998), sterols (e.g., Asperger et al., 1999b, 2001; Yamamoto et al., 2015a), sugars (e.g., Schwarzinger et al., 2002; Tanczos et al., 2003), and amino acids (e.g., Gallois et al., 2007) have been reported from other than sediment samples. These compounds are organic tracers that are also widely found in sediments. If we can analyze such numerous organic compounds from sediment samples at once by the TMAH method, it could help us obtain a lot of paleoenvironment information stored in sediments. Therefore, this method has good potentials for grasping compositions of organic matter in geo-samples such as sediments and for reconstructing paleoenvironment using these various organic tracers.

In this study, I have selected sterols as a case of application of the TMAH method, although over 100 types of organic compounds have been detected from

California sediments using this method. The lists of organic compounds from the sediment is shown in Table 6-1 and Fig 6-1. From the sediment, not only fatty acids and lignin phenols but also various other organic compounds including cutin acids, di-ols, and hopanols as useful tracers have been detected. Cutin acids are organic compounds derived from cuticular layer of leaves, and these compositions vary depending on types of plants (Goni and Hedges, 1990). Therefore, it can be accepted that compositions of cutin acid in sediments can be used for tracking detailed vegetation changes in paleo-environment. Di-ols are aliphatic hydrocarbons having two hydroxyl groups. Rampen et al (2012) proposed that long chain 1,13- and 1,15-diols can be used as paleo-temperature indicator, and, after this report, these compounds have been attracting attention in the field of organic geochemistry. To the best of my knowledge, there has been no report on an application of di-ols in sediments using the TMAH method, and in future, it can be said that it is important to show usefulness of di-ol analysis by TMAH method as a more convenient method. Hopanols are compounds derived from a bacterial (prokaryotic organisms) lipids. Hopanol is widely detected in sapolopel-like sedimentary rocks (e.g., in Oceanic Anoxic Events) having high organic compound (e.g., Ohkouchi, 1997; Summons et al., 1999). This indicates that the high organic layers were generated by prokaryotes (cyanobacteria) in an anoxic environment. In the case of California, hopanols may be derived from prokaryotic organisms living in the poor oxygen water which is described in Chapter 3.

Advantages of the TMAH method, which can detect many compounds, can be found even in comparison with classical methods. The solvent extraction method is commonly used and well-established for analysis of organic matters including di-ols, hopanols, and sterols, but this pretreatment relatively requires more labor and time. Furthermore, in the solvent extraction method, lignin phenols, strong land biomarker,

cannot be detected because *β–O–4* linkages which are bonds of lignin to lignin cannot be cleaved by the method. On the other hand, the CuO oxidation method can analyze lignin phenols and this method has been mainly used for lignin analysis in the field of organic geochemistry (e.g., Requejo et al., 1991; Goñi et al., 1998; Hu et al., 1999). However, this method is not suitable for analysis of organic compounds such as sterols other than lignin phenols, and it has a complicated pretreatment and extraction method. Therefore, to analyze organic compounds of terrestrial origin such as lignin phenol and aquatic origin such as sterol from the same sediment, it was necessary to analyze separately by the two methods (i.e., the solvent extraction and the CuO oxidation methods, e.g., Bertrand et al., 2012, 2013). On the other hand, the TMAH method can analyze both of terrestrial and marine origin compounds such as lignin phenols and sterols, and makes it possible to interpret terrestrial and aquatic contributions at the same time. This is a great advantage of the TMAH method.

As shown above, organic compounds which have a potential for various indicators are widely found from the TMAH method. This shows a strong possibility that the TMAH method will be one of the general methods for analysis of organic compounds in sediments. Therefore, the proposal of sterol analysis using the TMAH method in this study is a pioneer studies for detection and application of various compounds in sediments using the TMAH method.

6.2. Significance of sterols in sediments as geochemical proxies

Sterols in sediments are mainly derived from terrestrial plants or phytoplankton. They can be used to trace input of terrestrial plants and primary production of phytoplanktons in aquatic environment. Especially, primary production is caused by regional to global climatic changes such as ocean current changes according

with atmospheric circulations. Therefore, it is an important tracer for reconstruction of paleo-environmental changes.

Until now, total organic carbon (TOC) and foraminifera fossils in sediments have been used as powerful indicators for primary production (e.g., Ishiwatari et al., 2000). However, the sterol tracer provides advantages that are not found in TOC amount and foraminifera fossils. Basically, a high organic content in sediments with respect to high primary productivity and high TOC shows that the productivity was high in paleo-environments. However, TOC in sediments is affected by other than autogenous source inputs. In fact, form marine sediments, fragments of plants are observed, and plant-derived organic compounds are widely detected. Ohira et al (2014) interpreted that organic compounds in lacustrine sediments (Lake Biwa) are constituted by terrestrial sources during the last 147,000 years, from strong correlation between TOC content and lignin phenols in the sediments. These previous studies strongly indicate that TOC contents in sediments is not completely controlled by aquatic primary productions. On the other hands, sterols can demark origins into terrestrial and aquatics using their structural features. For example, campesterol and β-sitosterol are major sterol component in terrestrial plants, and phytoplankton has characteristic sterols such as diatomsterol and 4α-methyl sterols. Therefore, it sterols can be used as tracers that are not influenced by something other than autogenous source inputs.

Foraminifera fossils are preserved in sediments by keeping their original forms. Since foraminiferal differ in their skeleton depending on species, foraminiferal fossil in sediments makes it possible to estimate paleo-environment from differences in their habitat such as warm and cold currents (e.g., Barron et al., 2003). Although foraminiferal fossils are affected by storage and dissolution of skeletons, dilution by clastic particles and organic matter, the changes in their amount reflects the primary

production. However, foraminiferal are usually not dominant species in aquatic environments, and thus it is possible that the fossils may not reflect overall primary productivity changes in paleoenvironments. Kouduka et al. (2017) attempted to reconstruct a biota of late Pleistocene using eukaryotic DNA stored in cold deep sediments from the Japan Sea. As the result, the most dominant species was Sargassum vachellianum, which is a seaweed that inhabitates in the shallow waters around the Japanese archipelago from the East China Sea, and the second of that was radiolarians of Chaunacanthida which is not preserved as microfossils. The diatom species were less than 10% of the total. Therefore, reconstruction of primary production using foraminifera fossils is very useful for certain species, but a whole phytoplanktonic variability may not be captured. On the other hand, sterols in sediment reflect a whole phytoplanktonic primary production as sterols are detected from nearly all phytoplankton species, which is strong advantage of sterol analysis in sediments as the tracer.

6.3. Application and proposal of stanol / sterol ratio as a redox tracer

This study has showed the utility of 5α(H)-stanol/Δ^5-steorl ratio as a redox tracer in sediment samples. Furthermore, in this study, 5α(H)-stanol/Δ^5-steorl ratios could be divided into useable ratios for oxic–anoxic tracer and unsuitable ratios for one. In generally, 5α(H)-stanol/Δ^5-steorl ratio has been used as ratio of total 5α(H)-stanols to total Δ^5-sterols in previous studies. For example, Bertrand et al. (2012) calculated the 5α(H)-stanol/Δ^5-steorl ratio from total Δ^5-sterols (cholesterol + campesterol + stigmasterol + b-sitostrol) and total 5α(H)-stanols (cholestenol + campestanol + stigmastanol + sitostanol). Zheng et al (2015) defined the 5α(H)-stanol/Δ^5-steorl ratio as ratio of campestanol and sitostanaol to campesterol and sitoterol. However, these

equations include 5α(H)-stanols (cholestenol, campestanol, and sitostanol) which are considered not suitable as redox tracer in this study. Therefore, the total 5α(H)-stanols/Δ^5-steorls ratio is likely to be composed by high contribution by unsuitable 5α(H)-stanols as redox tracer. Contribution to the total 5α(H)-stanols/Δ^5-steorls ratio of 5α(H)-stanols that are influenced other than reduction of Δ^5-sterol in anoxic environments is examined using the sediments used in present study. The ratio has been defined as:

$$\frac{\text{5α(H)-stanols}}{\Delta^5\text{-sterols}} = \frac{\text{24-nordehydrocholestanol + diatomstanol + cholestanol + campestanol + sitostanol + 22-dehydrocholestanol}}{\text{24-nordehydrocholesterol + diatomsterol + cholesterol + campesterol + sitosterol + 22-dehydrocholesterol}} \quad (1)$$

5α(H)-Stanols which were not useful as redox tracers as 5α(H)-stanol/Δ^5-steorl ratios in California sediments are 22-dehydrocholestanol, cholestanol, campestanol and sitostanol. In a total 5α(H)-stanols/Δ^5-steorls ratio determined by the formula, these 5α(H)-stanols accounted for 54%–67% (ave. 59%) of the total 5α(H)-stanols. Likewise the California sediments, in the Suigetsu sediment, contribution of unusable 5α(H)-stanols (cholestenol, sitostanol, and campestnaol) to total 5α(H)-stanols are calculated, which imply 70%–94% (ave. 85%). These results show that the major constituent is of unusable 5α(H)-stanols as the redox index, and it is clearly indicated that 5α(H)-stanols / Δ^5-sterols ratio determined from the total amount cannot capture past oxic-anoxic conditions well. Therefore, for reconstruction of pleo-redox conditions recorded in sediments, suitable specific 5α(H)-stanols and Δ^5-sterols as the tracer should be selected,

such as diatomstanol and diatomsterol, and 24-nordehydrocholestanol and 24-nordehydrocholesterol.

To using organic matter as biological indicators for reconstruction of paleoenvironments, interpretation of their preservation and degree of decomposition are required. Therefore, some organic indicators have been proposed for interpreting their preservation. For example, lignin phenols, a strong indicator of terrestrial plants, have a degradation tracer called Ad/Al ratio (Hedges, et al., 1982). This tracer, since aldehyde group of lignin phenols is altered to acid group by oxidizing action of fungi, can estimate the degree of decomposition of lignin phenols. In general, Ad / Al ratio in sediments is obtained as ratio of vanillic acid to vanillin or syringic acid to syringaldehyde (e.g., Thevenot et al., 2010). In previous studies using lignin phenol in sediment as an indicator of plants, the lignin phenol distributions have been interpreted together with the Ad/Al ratio to discuss whether the lignin phenols vertical distributions can indicate their preservation or input of terrestrial plants (e.g., Ishiwatari et al., 2006; Bertrand et al., 2013; Ohira et al., 2014). Conversely, the presence of Ad/Al ratio as preservation tracer of lignin is one of reason that lignin phenols have been used as a good terrestrial tracer.

On the other hand, sterols in sediments has not been proposed as a good tracer to interpret their preservation and degradation. Therefore, it was difficult to interpret whether sterol fluctuations in sediments reflect primary production. However, it is expected that the 5α(H)-stanol/Δ^5-steorl ratio can become such a tracer. For example, when both of 5α(H)-stanols/Δ^5-steorls ratio and their respective concentrations are high, it may be interpreted to be obtained by high preservation of the sterols. Likewise, high sterol concentrations with low 5α(H)-stanols/Δ^5-steorls ration may indicate high primary productivity. Furthermore, since conversion from Δ^5-sterol to 5α(H)-stanol

under anoxic condition occurs in water column, it is suitable for discussing preservation of sterols associated with fluctuation of oxic-anoxic conditions during sedimentation process.

Table. 6-1. Identification of organic compounds found by the TMAH method in California marine sediment.

Peak No.	Retention Time	Compound	Formula	MW	Methylated Formula	MW
1	7.476	butanedioic acid, dimethyl ester			$C_6H_{10}O_4$	146
2	8.23	2-methyl-butan-1, 4-dioic acid methyl ester			$C_7H_{12}O_4$	160
3	9.95	dimethyl pentane-1,5-dioate			$C_7H_{12}O_4$	160
4	10.178	1,2-dimethoxy-benzen			$C_8H_{10}O_2$	138
5	10.634	1,4-dimethoxy-benzene			$C_8H_{10}O_2$	138
6	10.964	1,2,6,-trimethoxyhexsan			$C_9H_{20}O_3$	176
7	12.943	4-methoxy- benzaldehyde (*p*-hydroxybenzaldehyde:Ph)	$C_7H_6O_2$	122	$C_8H_8O_2$	136
8	13.218	methyl 3-phenylpropanoate			$C_{10}H_{12}O_2$	164
9	14.703	3-methoxybenoic acid methyl ester (Ma)	$C_8H_8O_3$	152	$C_9H_{10}O_3$	166
10	15.119	3-methoxy acetophenone (*p*-hydroxyacetophenone:Po)	$C_8H_8O_2$	136	$C_9H_{10}O_2$	150
11	15.363	1,2,4-Trimethoxybenzene			$C_9H_{12}O_3$	168
12	15.512	4-methoxy-benzoic acid methyl ester (*p*-hydroxybenzoic acid:Pa)	$C_8H_8O_3$	152	$C_9H_{10}O_3$	166
13	15.704	2-propenoic acid-3-phenyl-methyl ester			$C_{10}H_{10}O_2$	162
14	16.235	1,3,5-trimethoxy benzene			$C_9H_{12}O_3$	168
15	16.619	methyl 3-methoxy-4-methylbenzoate			$C_{10}H_{12}O_3$	180
16	17.79	3,4-dimethoxy benzaldehyde(vanilin:Vh)	$C_8H_8O_3$	152	$C_9H_{10}O_3$	166
17	18.52	*n*-dodecanoic acid methyl ester (*n*-C_{12} FA)	$C_{12}H_{24}O_2$	200	$C_{13}H_{26}O_2$	214
18	19.447	3,4-dimethoxy acetophenone(acetovanillon: Vo)	$C_9H_{10}O_3$	166	$C_{11}H_{16}O_2$	180
19	19.863	3,5-dimethoxy benzoic acid methyl ester (3,5-diOH)	$C_9H_{10}O_4$	182	$C_{10}H_{12}O_4$	196
20	19.934	3,4-dimethoxy benzoic acid methyl ester (vanillic acid:Va)	$C_9H_{10}O_4$	182	$C_{10}H_{12}O_4$	196
21	20.107	3,4,5-trimethoxybenzaldehyde (syringealdehyde:Sh)	$C_9H_{10}O_4$	182	$C_{10}H_{12}O_4$	196
22	21.529	3,4,5-ttimethoxy acetophenone (acetosyringone:So)	$C_{10}H_{12}O_4$	196	$C_{11}H_{14}O_4$	210
23	21.615	3-(4-methoxyphenyl)2-propenoic acid methylester (*p*-coumaric Acid: Pc)	$C_{10}H_{10}O_3$	178	$C_{11}H_{12}O_3$	192
24	22.275	3,4,5-trimethoxy benzoic acid-methyl ester (syringic acid: Sa)	$C_{10}H_{12}O_5$	212	$C_{11}H_{14}O_5$	226
25	22.393	*n*-tetradecanoic acid methyl ester (*n*-C_{14} FA)	$C_{14}H_{28}O_2$	228	$C_{15}H_{30}O_2$	242
26	23.509	9-methyl- (*br*-C_{14} FA)	$C_{14}H_{28}O_2$	228	$C_{15}H_{30}O_2$	242
27	23.509	erythreo- or threo-3,4-dimethoxy-1-(1,2,3-trimethoypropane)-benzen (V_{tmp1})	$C_9H_{10}O_5$	198	$C_{14}H_{20}O_5$	268
28	23.642	methyl 12-methyltetradecanoate (*br*-C_{14} FA)	$C_{14}H_{28}O_2$	228	$C_9H_{10}O_5$	198
29	23.736	erythreo- or threo-3,4-Dimethoxy-1-(1,2,3-trimethoypropane)-benzen (V_{tmp2})	$C_9H_{10}O_5$	198	$C_{14}H_{20}O_5$	268
30	24.161	*n*-pentadecanoic acid methyl ester (*n*-C_{15} FA)	$C_{15}H_{30}O_2$	242	$C_{16}H_{32}O_2$	256
31	24.844	5,9,13-trimethyl-tetradecanoic acid methyl ester (*br*-C_{14} FA)	$C_{17}H_{34}O_2$	270	$C_{18}H_{36}O_2$	284
32	25.237	3-(3,4-dimethoxyphenyl)-2-propenoic acid methylester (ferulic acid: Vc)	$C_{10}H_{10}O_4$	194	$C_{12}H_{14}O_4$	222
33	25.339	erythreo- or threo-3,4,5-trimethoxy-1-(1,2,3-trimethoypropane)-benzen ($S_{tmp\ 1}$)	$C_{10}H_{12}O6$	214	$C_{15}H_{22}O_6$	298
34	25.496	9-hexadecenoic acid, methyl ester ($C_{16:1}$ FA)	$C_{16}H_{30}O_2$	264	$C_{17}H_{32}O_2$	268
35	25.543	erythreo- or threo-3,4,5-trimethoxy-1-(1,2,3-trimethoypropane)-benzen ($S_{tmp\ 2}$)	$C_{10}H_{12}O_6$	214	$C_{15}H_{22}O_6$	298
36	25.881	*n*-hexadecanoic acid methyl ester (*n*-C_{16} FA)	$C_{16}H_{32}O_2$	256	$C_{17}H_{34}O_2$	270
37	25.983	glucuronic acid	$C_6H_{10}O_7$	194	$C_{11}H_{20}O_7$	264
38	28.049	phytol	$C_{20}H_{40}O$	296	$C_{21}H_{42}O$	310

Table. 6-1. Identification of organic compounds found by the TMAH method in California marine sediment (continue).

Peak No.	Retention Time	Compound			Methylated Formula	MW
39	29.054	*n*-octadecanoic acid methyl ester (*n*-C_{18} FA)	$C_{18}H_{36}O_2$	284	$C_{19}H_{38}O_2$	298
40	31.387	8,16-/9,16-/10,16-dimethoxy C_{16} acid methyl ester (Cutin acid A)	$C_{15}H_{30}O_4$	274	$C_{18}H_{36}O_4$	316
41	31.599	11-eicosenoic acid	$C_{20}H_{38}O_2$	310	$C_{21}H_{40}O_2$	324
42	31.695	11-eicosenoic acid	$C_{20}H_{38}O_2$	310	$C_{21}H_{40}O_2$	324
43	31.992	*n*-eicosanoic acid methyl ester (*n*-C_{20} FA)	$C_{20}H_{40}O_2$	312	$C_{21}H_{42}O_2$	326
44	32.731	7-/8-methoxy C_{16} diacid dimethyl ester (Cutin B)	$C_{16}H_{30}O_5$	302	$C_{17}H_{32}O_5$	316
45	33.021	13-docosen-1-ol	$C_{22}H_{44}O$	324	$C_{23}H_{46}O$	338
46	33.108	13-docosen-1-ol	$C_{22}H_{44}O$	324	$C_{23}H_{46}O$	338
47	33.375	docosan-1-ol	$C_{22}H_{46}O$	326	$C_{23}H_{48}O$	340
48	34.011	11,18-dimethoxy $C_{18:1}$ acid methylester (11,18-diOHC$_{18:1}$(cis)FA)	$C_{17}H_{34}O_4$	302	$C_{20}H_{40}O_4$	344
49	34.121	octadecanoic acid-11,18-diol C_{18}FA	$C_{17}H_{36}O_4$	304	$C_{20}H_{42}O_4$	346
50	34.341	13-tetracosanoic acid	$C_{22}H_{42}O_2$	338	$C_{23}H_{44}O_2$	342
51	34.443	13-tetracosanoic acid	$C_{22}H_{42}O_2$	338	$C_{23}H_{44}O_2$	342
52	34.71	*n*-docosanoic acid methyl ester (*n*-C_{22} FA)	$C_{22}H_{44}O_2$	340	$C_{23}H_{46}O_2$	354
53	34.828	11,18-dimethoxy $C_{18:1}$ acid methylester (11,18-diOHC$_{18:1}$(trans)FA)	$C_{17}H_{34}O_4$	302	$C_{20}H_{40}O_4$	344
54	35.229	9,10,18-trimethoxy C18 acid methyl ester (Cutin C)	$C_{17}H_{34}O_5$	318	$C_{21}H_{42}O_5$	374
55	35.653	13-tetracosan-1-ol	$C_{24}H_{46}O$	352	$C_{25}H_{48}O$	366
56	35.739	13-tetracosan-1-ol	$C_{24}H_{46}O$	352	$C_{25}H_{48}O$	366
57	35.959	1-docosanol	$C_{24}H_{48}O$	354	$C_{25}H_{50}O$	368
58	36.446	9,10-dimethoxy C_{18} diacid dimehtyl ester	$C_{17}H_{36}O_4$	304	$C_{20}H_{42}O_4$	346
59	36.878	tetracosenoic acid	$C_{24}H_{46}O_2$	366	$C_{25}H_{48}O_2$	380
60	36.965	tetracosenoic acid	$C_{24}H_{46}O_2$	366	$C_{25}H_{48}O_2$	380
61	37.192	*n*-tetracosanoic acid methyl ester (*n*-C_{24} FA)	$C_{24}H_{48}O_2$	366	$C_{25}H_{50}O_2$	382
62	37.478	13-hydroxy-tetradocosen-1-ol (1,13-diOHC$_{24}$)	$C_{23}H_{48}O_2$	356	$C_{25}H_{52}O_2$	384
63	38.654	14-hydroxy-pentacosan-1-ol (1,14-diOH C_{25})	$C_{24}H_{50}O_2$	370	$C_{26}H_{54}O_2$	398
64	38.866	24-norcholesta-5,22E-dien-3β-ol	$C_{26}H_{42}O$	370	$C_{27}H_{44}O$	384
65	39.038	24-norcholest-22E-en-3β-ol	$C_{26}H_{44}O$	372	$C_{27}H_{46}O$	386
66	39.172	glucuronic acid				482
67	39.533	*n*-Hexacosanoic acid methyl ester (*n*-C_{26} FA)	$C_{26}H_{52}O_2$	396	$C_{27}H_{54}O_2$	410
68	39.769	11-/12-/13-/14-hydroxy-hexacosan-1-ol (1,11-/1, 12-/1,13-/1, 14-di OH C_{26})	$C_{25}H_{52}O_2$	384	$C_{27}H_{56}O_2$	412
69	39.824	γ-tocopherol	$C_{27}H_{46}O_2$	402	$C_{28}H_{48}O_2$	416
70	40.232	27-nor-24-methylcholesta-5,22E-dien-3β-ol	$C_{27}H_{44}O$	384	$C_{28}H_{46}O$	398
71	40.413	5α-cholest-22-en-3b-ol	$C_{27}H_{46}O$	386	$C_{28}H_{48}O$	400
72	40.437	cholesta-5,22E-dien-3β-ol (22-dehydrocholesterol)	$C_{27}H_{44}O$	384	$C_{28}H_{46}O$	398
73	40.61	α-tocopherol	$C_{29}H_{50}O_2$	430	$C_{30}H_{52}O_2$	444

Table. 6-1. Identification of organic compounds found by the TMAH method in California marine sediment (continue).

Peak No.	Retention Time	Compound			Methylated Formula	MW
74	40.877	cholest-5-en-3β-ol (cholesterol)	$C_{27}H_{46}O$	386	$C_{28}H_{48}O$	400
75	40.884	13-/12-hydroxy-heptacosan-1-ol (1, 13-/1, 12-diOHC_{27})	$C_{26}H_{54}O_2$	398	$C_{28}H_{58}O_2$	426
76	40.939	α-tocoenol	$C_{29}H_{48}O_2$	428	$C_{30}H_{50}O_2$	442
77	41.042	5α(H)-cholestan-3β-ol (cholestanol)	$C_{27}H_{48}O$	388	$C_{28}H_{50}O$	402
78	41.324	24-methylcholesta-5,22E-dien-3β-ol (diatomsterol)	$C_{28}H_{46}O$	398	$C_{29}H_{48}O$	412
79	41.552	5α(H)-cholest-7-en-3β-ol (lathosterol)	$C_{27}H_{46}O$	386	$C_{28}H_{48}O$	400
80	41.489	24-methyl-5α(H)-cholest-22E-en-3β-ol	$C_{28}H_{48}O$	400	$C_{29}H_{50}O$	414
81	41.733	*n*-octacosanoic acid methyl ester (*n*-C_{28} FA)	$C_{28}H_{56}O_2$	424	$C_{29}H_{58}O_2$	438
82	41.928	24-methylcholesta-5,24(28)-dien-3β-ol	$C_{28}H_{46}O$	398	$C_{29}H_{48}O$	412
83	41.937	12-/13-/14-hydroxy-octacosan-1-ol (1,12-/1,13-/1, 14-diOH C_{28})	$C_{27}H_{56}O_2$	412	$C_{29}H_{60}O_2$	440
84	42.008	24-methylcholest-5-en-3β-ol (campesterol)	$C_{28}H_{48}O$	400	$C_{29}H_{50}O$	414
85	42.008	24-methyl-5α(H)-cholest-24(28)-en-3β-ol	$C_{28}H_{48}O$	400	$C_{29}H_{50}O$	414
86	42.102	4α,24-dimethyl-5α(H)-cholest-22E-en-3β-ol	$C_{29}H_{50}O$	414	$C_{30}H_{52}O$	428
87	42.157	23,24-dimethylcholesta-5,22E-dien-3β-ol	$C_{29}H_{48}O$	412	$C_{30}H_{50}O$	426
88	42.157	24-methyl-5α(H)-cholestan-3β-ol	$C_{28}H_{50}O$	402	$C_{29}H_{52}O$	416
89	42.283	24-ethylcholest-5,22E-dien-3β-ol (stigmasterol)	$C_{29}H_{48}O$	412	$C_{30}H_{50}O$	426
90	42.33	23,24-dimethyl-5α(H)-cholest-22E-en-3β-ol	$C_{29}H_{50}O$	414	$C_{30}H_{52}O$	428
91	42.448	24-ethyl-5α(H)-cholest-22E-en-3β-ol	$C_{29}H_{50}O$	414	$C_{30}H_{52}O$	428
92	42.817	23,24-dimethylcholest-5-en-3β-ol	$C_{29}H_{50}O$	414	$C_{30}H_{52}O$	428
93	42.895	4α,23,24-trimethyl-5α(H)-cholest-22-en-3β-ol (dinosterol)	$C_{30}H_{52}O$	428	$C_{31}H_{54}O$	442
94	42.927	24-ethylcholest-5-en-3β-ol (β-sitosterol)	$C_{29}H_{50}O$	414	$C_{30}H_{52}O$	428
95	42.974	23,24-dimethyl-5α(H)-cholestan-3β-ol	$C_{29}H_{52}O$	416	$C_{30}H_{54}O$	430
96	42.982	13-hydroxy-nonacosan-1-ol (1,13-diOH C_{29})	$C_{28}H_{58}O_2$	426	$C_{30}H_{62}O_2$	454
97	43.068	24-ethyl-5α(H)-cholestan-3β-ol (sitostanol)	$C_{29}H_{52}O$	416	$C_{30}H_{54}O$	430
98	43.068	24-ethylcholesta-5,24(28)E-dien-3β-ol (fucosterol)	$C_{29}H_{48}O$	412	$C_{30}H_{50}O$	426
99	43.312	24-ethylcholesta-5,24(28)Z-dien-3β-ol (isofucosterol)	$C_{29}H_{48}O$	412	$C_{30}H_{50}O$	426
100	43.563	24-ethyl-5α(H)-cholest-7-en-3β-ol	$C_{29}H_{50}O$	414	$C_{30}H_{52}O$	428
101	43.642	4α, 23, 24-trimethyl-5α-cholestan-3β-ol	$C_{30}H_{54}O$	430	$C_{31}H_{56}O$	444
102	43.799	*n*-triacontanoic acid methyl ester (*n*-C_{30} FA)	$C_{30}H_{60}O_2$	452	$C_{31}H_{62}O_2$	466
103	43.964	15-/14-/13-tricontan-1-ol (1,15-/1,14-/1,13-diOHC_{30})	$C_{29}H_{60}O_2$	440	$C_{31}H_{64}O_2$	468
104	44.867	*n*-hentriacontanoic acid methyl ester (*n*-C_{31} FA)	$C_{31}H_{62}O_2$	466	$C_{32}H_{64}O_2$	480
105	45.095	15-hydroxy-hentriacontan-1-ol (1, 15-diOH C_{31})	$C_{30}H_{62}O_2$	454	$C_{32}H_{66}O_2$	482
106	46.124	*n*-dotriacontanoic acid methyl ester (*n*-C_{32} FA)	$C_{32}H_{64}O_2$	480	$C_{33}H_{66}O_2$	494
107	46.328	15-hydroxy-dotriacontan-1-ol (1,15-diOH C_{32})	$C_{32}H_{66}O_2$	482	$C_{34}H_{70}O_2$	510
108	47.821	17β,21β-homohomohopanol methylation	$C_{31}H_{54}O_2$	442	$C_{32}H_{56}O_2$	456
109	49.777	17β,21β-bishomohopanol methylation	$C_{32}H_{56}O_2$	456	$C_{33}H_{58}O$	470
110	52.377	17β,21β-trishomohopanol methylation	$C_{33}H_{58}O$	470	$C_{34}H_{60}O$	484

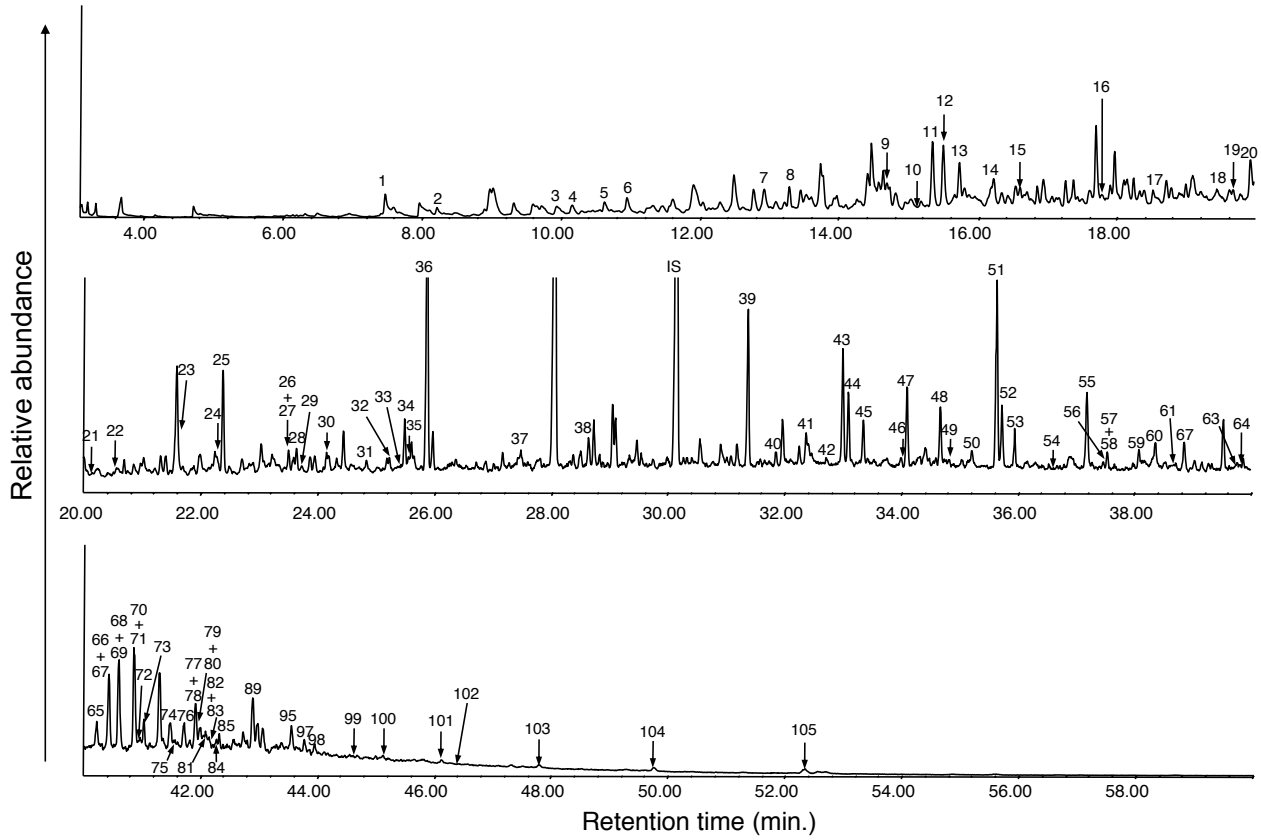

Fig. 6-1. Total ion chromatogram found in offline TMAH–GC–MS method form a California marine sediment at section 6–9cm. Peak assignments are given in Table 6-1. IS = internal standard (nonadecanoic-d_{37} acid).

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