Coprostanol in a Sediment Core from the Anoxic Tan-Shui Estuary, Taiwan

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A short sediment core with a length of approximately 40 cm taken from the anoxic Tan-Shui estuary, Taiwan, was analysed for extractable and bound coprostanol (5 β -cholestan-3 β -ol), cholesterol (5 α -cholestan-3 β -ol) and cholesterol (cholest-5-en-3 β -ol). Extractable Σ coprostanol and cholesterol concentrations exhibit an abrupt change at a depth of about 20 cm, which supposedly marks the time when a sewage treatment plant became operational in the estuary in 1980. The Σ coprostanol/cholesterol and cholesterol/cholesterol ratios are comparatively higher in sediment than in the sewage effluent, implying some degree of diagenesis in the extractable phase. Anoxicity must have played a crucial role in the preservation and diagenesis of the sterols. In the upper layer (*c.* top 20 cm), the concentrations of extractable Σ coprostanol, normalized to total organic carbon (TOC), increase down the core. Extractable cholesterol exhibits the same trend, but extractable cholesterol shows the opposite trend. This indicates cholesterol reduction to these two stanols. In addition, both bound Σ coprostanol/TOC and cholesterol/TOC display a decreasing trend with core depth and no pronounced concentration change at 20 cm depth. The averages of percent bound sterols are in the following order: cholesterol > Σ coprostanol > cholesterol. © 1996 Academic Press Limited

Introduction

Coprostanol (5 β -cholestan-3 β -ol) has been used to trace sewage pollution in various sedimentary environments (Goodfellow *et al.*, 1977; Hatcher & McGillivray, 1979; Yde *et al.*, 1982; Brown & Wade, 1984; Readman *et al.*, 1986; Venkatesan & Kaplan, 1990; LeBlanc *et al.*, 1992). A chronological study of coprostanol concentrations in a dated sediment core has been shown to reflect the true sewage inputs over 160 years (Muller *et al.*, 1979). Coprostanol will degrade during aerobic wastewater treatment processes (McCalley *et al.*, 1981). However, coprostanol, along with cholesterol (cholest-5-en-3 β -ol) and cholesterol (5 α -cholestan-3 β -ol), is quite persistent in anoxic sedimentary environments (Nishimura & Koyama, 1977; Hatcher & McGillivray, 1979; McCalley *et al.*, 1980; Readman *et al.*, 1986; Bartlett, 1987). The hydrogenation of naturally occurring unsaturated sterols may be effected extremely rapidly in contemporary aquatic

Figure 1. Map showing the coring sites in the Tan-Shui estuary.

sediments (Gaskell & Eglinton, 1975). Sterol diagenesis has been studied in a bay (Lee *et al.*, 1977), a lake (Nishimura, 1978) and laboratory experiments (Nishimura, 1982).

The Tan-Shui estuary is located on the outskirts of the metropolitan area of Taipei city, with a population of approximately 3 million. The Tan-Shui river carries untreated sewage along its course, and treated sewage effluent has been discharged into the estuary since 1980. High levels of coprostanol have accumulated in the estuary (Jeng & Han, 1991). The estuary is very anoxic; dissolved oxygen is virtually zero. A preliminary survey shows that redox potential (E_h) values of the surface sediments range from 0 to -500 mV approximately. This anoxicity has lasted for a very long period of time which aids in the preservation of coprostanol in sediments. The aim of this study was to investigate the short-term diagenesis of coprostanol, cholestanol and cholesterol in the anoxic environment by analysing their concentrations in the extractable and bound phases.

Experimental

Sediment cores were taken from the Tan-Shui estuary (Figure 1) using a gravity corer with a 6-cm diameter core barrel. Core A had a length of about 40 cm and was sectioned at 5-cm intervals from the top for analysing sterols. Cores 1–6 were measured for the downcore variation of E_{lr} .

For measuring redox potentials, the core liner was drilled with a series of holes (allowing the redox electrode to be inserted into sediment) at 2.5-cm intervals along the liner length. Before sampling, the holes were taped with masking tape. Immediately after

retrieving the core, the masking tape was peeled off the liner, and E_h was measured *in situ* with a combined reference (Ag/AgCl) and platinum electrode from the core top, one hole at a time. The reading was taken after a period of 60 s, to ensure that the electrode had reached a steady potential.

All solvents used were HPLC grade and/or all-glass distilled. All reagents employed were reagent grade.

Each section of Core A was freeze-dried and extracted with a mixture of benzene and methanol (1:1, v/v) in a Soxhlet apparatus for 24 h. 1-Nonadecanol was added to the extract as an internal standard. The spiked extract was concentrated and saponified with 0.5 N methanolic KOH. The neutral lipid was extracted with n-hexane four times. The extracted lipid was fractionated by silica gel (deactivated with 5% water) column chromatography. The less polar lipids were removed by elution with 40% hexane in chloroform, and the sterol-containing fraction was isolated using 10% methanol in chloroform.

The extracted sediment residue was subjected to alkaline hydrolysis (16 h) for releasing bound lipids, followed by filtration, internal standard addition, n-hexane extraction and sterol isolation.

The isolated sterols (both extractable and bound) were derivatized with NO-Bis (trimethylsilyl) acetamide prior to gas chromatography (GC) analysis.

Sterols (as TMS ethers) were analysed by capillary GC and gas chromatography-mass spectrometry (GC-MS). Separations were performed with an HP 5890A gas chromatograph equipped with a flame ionization detector (FID) and a split/splitless capillary column inlet system. An OCI-5 cool on-column injector (SGE, Australia) was also fitted in the chromatograph for quantitation. A 30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness, SE-30 bounded-phase fused silica capillary column was used. Hydrogen was used as the carrier gas. Nitrogen served as the make-up gas. The detector was set at 300 °C. Oven temperature was programmed as follows: (1) 45–90 °C at 15 °C min⁻¹; (2) 90–270 °C at 3 °C min⁻¹; (3) 20 min at 270 °C; (4) 270–280 °C at 10 °C min⁻¹; and (5) 20 min at 280 °C. Identification was made by co-injection with authentic standards. Positive confirmation was done with a Finnigan MAT TSQ-46C GC/MS. All GC traces were recorded by a Shimadzu data processor, Chromatopac C-R6A. Quantitation was achieved by comparison of sample peak areas with the areas of the internal standard. The precision of the method was determined by five replicate analyses of the same sediment sample, and the relative standard deviation was 2.1%.

Total organic carbon was determined by the dichromate acid oxidation method (Gaudette *et al.*, 1974) modified by addition of Ag₂SO₄ to H₂SO₄ at the rate of 15 g l⁻¹, with ortho-phenanthroline-ferrous complex used as the indicator. The relative standard deviation of TOC determinations was generally <1%.

Results and discussion

Prior to discussing sterols, it is important to know the redox state of the sediment in the estuary. As shown in Table 1, E_h values are virtually negative and generally in the range between -100 and -200 mV. The general trend of depth profiles is that the E_h values decrease with depth in the upper core and fluctuate with depth in the lower core.

For the extractable phase, Σ coprostanol and cholesterol concentrations *vs.* core depth exhibit a large break between Sections 4 and 5 (i.e. at a depth of about 20 cm), which indicates an extra large input of coprostanol from sometime in the past to the sampling time (Table 2). This depth supposedly marks the time when a sewage treatment plant

TABLE 1. Redox potentials (mV) in sediment cores from the Tan-Shui estuary

Depth (cm)	Core					
	1	2	3	4	5	6
0.0-2.5	-16	4	11	5	-30	-70
2.5-5.0	-112	-96	-7	-49	-59	-95
5.0-7.5	-142	-160	-6	-77	-140	-170
7.5-10.0	-114	-180	-21	-129	-152	-218
10.0-12.5	-179	-176	-41	-162	-147	-194
12.5-15.0	-209	-119	-83	-169	-140	-215
15.0-17.5	-217	-172	-122	-149	-222	-113
17.5-20.0	-216	-150	-139	-160	-119	-152
20.0-22.5	-148	-192	-157	-133	-90	-208
22.5-25.0	-230	-161	-181	-129	-171	-252
25.0-27.5	-214	-152	-120	-168	-131	—
27.5-30.0	-96	-123	-134	-172	-111	—
30.0-32.5	-213	-60	-163	-182	-186	—
32.5-35.0	-134	-155	-81	-42	—	—
35.0-37.5	-200	-171	-39	-195	—	—
37.5-40.0	-125	-177	-60	-58	—	—
40.0-42.5	-206	-110	-108	-55	—	—
42.5-45.0	-172	—	-72	-96	—	—
45.0-47.5	-217	—	-100	-66	—	—

became operational in 1980, and considerably higher levels of coprostanol began to be deposited in the estuary. This record has been well kept due to anoxicity, which aids in the preservation of coprostanol.

Core A was located not far from the sewage outfall (Figure 1) which was presumably the predominant coprostanol source for this core. The Σ coprostanol/cholesterol and cholestanol/cholesterol ratios of the effluent from the sewage outfall were 0.69 and 0.043, respectively, and those of Core A sediments had averages of 2.19 and 1.43, respectively (Table 2). The increases in the two ratios are of particular interest. It has been well documented in the literature that there is little decay of coprostanol, cholesterol and cholestanol in anaerobic sediments (Nishimura & Koyama, 1977; Hatcher & McGillivray, 1979; McCalley *et al.*, 1980; Readman *et al.*, 1986; Bartlett, 1987). Furthermore, cholesterol can be transformed into coprostanol and cholestanol in a reducing environment (Gaskell & Eglinton, 1975). The present result with comparatively higher stanol/stenol ratios implies rapid hydrogenation of cholesterol, which could lead to the conversion of cholesterol into coprostanol and cholestanol in the reducing environment.

Since grain size has a significant effect on the distribution of organic compounds in sediment, concentrations of molecular data are usually normalized to TOC (Jeng & Han, 1991; LeBlanc *et al.*, 1992; Jasper & Gagosian, 1993); variation trends and/or meaningful results can readily be obtained. Σ Coprostanol, cholestanol and cholesterol concentrations are normalized to TOC, and their variations, both extractable and bound, *vs.* depth are given in Figures 2-4. It can be seen that the concentrations of extractable Σ coprostanol and cholestanol increase down the core in the upper layer (Sections 1-4). However, extractable cholesterol (top three sections) shows the opposite trend relative to the two stanols. The increase of stanol and decrease of stenol with depth indicates that hydrogenation of stenol (Gaskell & Eglinton, 1975) was occurring in the upper layer.

TABLE 2. Concentrations ($\mu\text{g g}^{-1}$) of extractable coprostanol, cholesterol and cholestanol in Core A from the Tan-Shui estuary

Core section ^a	Coprostanol ($\mu\text{g g}^{-1}$)	Epicoprostanol ($\mu\text{g g}^{-1}$)	Σ Coprostanol ^b ($\mu\text{g g}^{-1}$)	Cholestanol ($\mu\text{g g}^{-1}$)	Cholesterol ($\mu\text{g g}^{-1}$)	Σ Coprostanol		Cholesterol		TOC (%)
						Cholesterol		Cholesterol		
1	3.80	1.33	5.13	2.94	2.40	2.14		1.23		1.63
2	4.46	1.53	5.99	3.63	2.48	2.42		1.46		1.81
3	6.08	1.11	7.19	4.62	2.58	2.79		1.79		2.08
4	4.46	1.42	5.88	4.16	2.32	2.53		1.79		1.61
5	1.68	1.06	2.74	1.92	1.37	2.00		1.40		1.75
6	1.34	0.64	1.98	1.48	1.17	1.69		1.26		1.75
7	1.63	1.18	2.81	1.79	1.30	2.16		1.38		2.08
8	1.96	0.92	2.88	1.88	1.65	1.75		1.14		1.72
Average						2.19		1.43		

^aNumbered from the core top and sectioned at 5-cm intervals.
^b Σ Coprostanol = coprostanol + epicoprostanol (5 β -cholestan-3 α -ol).
TOC, total organic carbon.

Figure 2. Depth profiles of Σ coprostanol (normalized to total organic carbon) for Core A from the Tan-Shui estuary. ●, Bound; ■, extractable.

Figure 3. Depth profiles of cholestanol (normalized to total organic carbon) for Core A from the Tan-Shui estuary. ●, Bound; ■, extractable.

This is thought to be attained by: (1) constant input sources; (2) constant degradation rates (or possibly no degradation); and (3) greater production than degradation of stanols. In the lower layer (Sections 5–8), however, extractable Σ coprostanol, cholestanol and cholesterol all exhibit a similar variation with depth but no depth trend. The reason for this remains unclear. However, one possible factor that might be associated with it is that the redox gradients (or redoxclines) in the sediments around Core A are generally in the top 15–25 cm (Table 1). Beyond the redox gradients, redox potentials stop dropping and fluctuate with depth. In other words, the strength of anoxicity in the lower layer stops increasing and is weakened, consequently affecting sterol hydrogenation.

Linear regression of extractable Σ coprostanol and cholestanol vs. depth (using the mean depth of each core section) for the upper layer gives the following equations:

Figure 4. Depth profiles of cholesterol (normalized to total organic carbon) for Core A from the Tan-Shui estuary. ●, Bound; ■, extractable.

$$\Sigma\text{coprostanol/TOC} = 3.3 \text{ depth} + 306 \quad (r=0.999)$$

$$\text{cholestanol/TOC} = 5.1 \text{ depth} + 164 \quad (r=0.990)$$

where $\Sigma\text{coprostanol/TOC}$ and cholestanol/TOC are in $\mu\text{g gC}^{-1}$ and depth is in cm. It is interesting to note that the slope for cholestanol (5.1) is greater than that for $\Sigma\text{coprostanol}$ (3.3). This is attributed to the fact that the 5α stanol is thermodynamically more stable than its 5β epimer (Van Graas *et al.*, 1982). Therefore, the sedimentary reduction process of cholesterol gives rise to stanol mixtures in which 5α stanol predominates (Gaskell & Eglinton, 1975; Nishimura & Koyama, 1977; Boudou *et al.*, 1986; Robinson *et al.*, 1986).

Coprostanol is only found in the faeces of higher mammals and is nearly human-specific (Walker *et al.*, 1982), especially in urbanized estuaries where human waste predominates. Cholestanol is not a common constituent of most organisms but has been found in some living organisms (Nishimura & Koyama, 1977). Compared with these two stanols having relatively limited sources, cholesterol is quantitatively the most important sterol of animals (Tissot & Welte, 1978) and widespread in plant life (Volkman, 1986). The concentration of cholesterol in sediment depends on the amount of input sources during deposition; therefore, irregular variation with depth may result. The concentration of extractable cholesterol in Section 4 may result from a large input (Figure 4).

Table 3 presents the concentrations of bound sterols. In Figures 2 and 3, excluding the Section 8 data point, both bound $\Sigma\text{coprostanol/TOC}$ and cholestanol/TOC exhibit a decrease with core depth and no pronounced concentration change between Sections 4 and 5. It appears that bound stanols are not influenced markedly by the enhanced sewage input change. The decreasing trend with depth might be ascribed to assimilation by bacteria and/or chemical decomposition. Lee *et al.* (1977) studying sterol diagenesis in recent sediments from Buzzards Bay found that extractable sterols decreased in concentration while bound sterols increased in concentration with depth. From this result, they inferred that a transformation between extractable and bound sterols

TABLE 3. Concentrations ($\mu\text{g g}^{-1}$) of bound coprostanol, cholestanol and cholesterol in Core A from the Tan-Shui estuary

Core section	Coprostanol ($\mu\text{g g}^{-1}$)	Epicoprostanol ($\mu\text{g g}^{-1}$)	Σ Coprostanol ($\mu\text{g g}^{-1}$)	Cholestanol ($\mu\text{g g}^{-1}$)	Cholesterol ($\mu\text{g g}^{-1}$)
1	1.24	0.22	1.46	0.19	0.61
2	1.20	0.22	1.42	0.19	0.81
3	1.11	0.21	1.32	0.18	0.87
4	0.66	0.15	0.81	0.12	0.54
5	0.35	0.10	0.45	0.065	0.36
6	0.29	0.10	0.39	0.059	0.33
7	0.28	0.13	0.41	0.066	0.44
8	0.52	0.15	0.67	0.099	0.49

TABLE 4. Percent bound Σ coprostanol, cholestanol and cholesterol in Core A from the Tan-Shui estuary

Core section	Σ Coprostanol	Cholestanol	Cholesterol
1	22	6.1	20
2	19	5.0	25
3	16	3.8	25
4	12	2.8	19
5	14	3.3	21
6	16	3.8	22
7	13	3.6	25
8	19	5.0	23
Average	16	4.2	23

appeared to be occurring over the entire core. However, it is worthy of note that their depth trends are the reverse of those found in the present study. The main cause of this difference is that the core was in a reducing environment in the present study. It is quite probable that extractable stanol formation rates greatly exceed sterol degradation rates.

Percent bound sterols are calculated as follows:

$$\% \text{ Bound} = [\text{Bound}/(\text{Extractable} + \text{Bound})] \times 100$$

The results are presented in Table 4. It is noted that the averages of percent bound sterols are in the order, cholesterol > Σ coprostanol > cholestanol. Before diagenesis, it is assumed that the three sterols have about the same percentage of bound fractions. Part of the extractable cholesterol is transformed into stanols during diagenesis resulting in an increase in percent bound cholesterol. At the same time, extractable coprostanol and cholestanol increased due to diagenesis, thus decreasing the percent bound stanol. As mentioned above, since there is less diagenetic coprostanol than diagenetic cholestanol, there is more percent bound Σ coprostanol than percent bound cholestanol.

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