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STEROLS IN NATURAL WATER AND SEDIMENT

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Abstract—Sterols were detected in Lake Mendota, Wisconsin and Torch Lake, Michigan, water and sediment and in Lake Wingra, Wisconsin, sediment. These sterols included compounds with gas chromatographic retention times equal to those for coprostanol, cholesterol, stigmasterol and β-sitosterol as well as other, unidentified compounds. In addition, cholestanol and perhaps other saturated sterols were present in Lake Mendota sediment. Sterol concentrations were 0.8 μg cholesterol/l and 3 μg β-sitosterol/l in Lake Mendota water and 0.7 μg cholesterol/l and 2 μg β-sitosterol/l in Torch Lake water. Analysis of sediment sterols was probably not quantitative; however, the minimum concentration in Torch Lake sediment was about 3 ppm sterols on a dry weight basis. Both free sterols and sterol esters were present in Lake Mendota sediment, the sterols present as esters comprising roughly 10% of the total sterol content.

INTRODUCTION

Steroids are a large group of naturally occurring organic compounds, many of which exhibit biological activity in a number of organisms. This study focuses on sterols, the most abundant steroids. Sterols are essential membrane components of all eucaryotic cells and are hormone precursors in a number of animals. Since some aquatic organisms, including insects (Horn, 1971), crustaceans (Teshima & Kanazawa, 1971), a euglenophyte (Droop, 1962) and a coelenterate (Ferezov *et al.*, 1972), may be unable to biosynthesize sterols and thus would have a dietary requirement for them, the presence of sterols in natural water and sediment could be of environmental significance. In addition, certain sterols are somewhat characteristic of algae and of domestic sewage. Thus, the sterol composition of a sediment or body of water could be indicative of major sources of organic material.

Sterols are relatively resistant to bacterial degradation and tend to accumulate under acidic, anaerobic or waterlogged conditions (Turfitt, 1943). Other steroids, especially some synthetic steroid hormones, are even more resistant to degradation than are sterols (Tabak & Bunch, 1970). Because of their resistance to degradation and because of their relatively high concentrations in sediments, sterols have been suggested as possible precursors of some petroleum compounds (Schwendinger & Erdman, 1964). Some fully reduced steroids, the steranes, have in fact been found in the optically active fraction of petroleum (Hills & Whitehead, 1966).

Previous investigators have found sterols present in both natural water and sediment. Matthews & Smith (1968) studied sterols in Gulf of Mexico water using gas chromatography. Analysis of various unfiltered samples revealed compounds with retention times equal to those for cholesterol, stigmasterol and β-sitosterol, plus other unidentified sterols. In one sample they found 10 μg l^{-1} of cholesterol, 17 μg l^{-1} of stigmasterol and 135 μg l^{-1} of β-sitosterol. Since this sample had not been filtered, some of these sterols may have been extracted directly from algal cells.

Murtaugh & Bunch (1967) used gas chromatography to analyze cholesterol and coprostanol in Little Miami River Basin water in Ohio. They found concentrations of coprostanol ranging from <0.02 μg l^{-1}–5 μg l^{-1} and cholesterol ranging from 0.5 μg l^{-1}–2.5 μg l^{-1}. Most of the coprostanol concentrations increased about fifty-fold downstream of sewage outfalls, while cholesterol concentration increases were usually less than two-fold. They suggested that the only source of coprostanol in the environment is mammalian fecal material and that coprostanol could therefore be used as an indicator of fecal pollution.

Sterols have been identified in a variety of sediments. Schwendinger & Erdman (1964) found evidence of the presence of sterols in every sediment that they tested, ranging from a northern freshwater swamp to those of the Pacific continental shelf. Attaway & Parker (1970) analyzed sediment sterols from Baffin Bay, Texas, and from the San Pedro Basin offshore of southern California. Using gas chromatography, they found evidence of the presence of cholesterol, campesterol, stigmasterol and β-sitosterol. Mass spectrometry indicated that saturated C_{27}, C_{28} and C_{29} stanols were also present. Henderson *et al.* (1972) studied sterols in Recent and Pleistocene sediments of Mono Lake, California. By gas chromatography and mass spectrometry they found that sterols in the bottom ooze were dominated by compounds identified as cholesterol, brassicasterol and campesterol, with a smaller amount of stigmasterol. The only saturated sterol identified was a trace of ergostanol. Older Pleistocene sediments generally

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Thin layer chromatography of fractions three and four as free alcohols on Silica Gel-G (mobile phase, 20% ether in chloroform) revealed that fraction three contained material with a R_f value equal to that for coprostanol plus a good deal of contaminating material, and that fraction four contained only material with a sterol R_f value.

Lake Wingra sediment

A sample of Lake Wingra upper sediment from the middle of this lake was collected through the ice in January, 1973. This sample was extracted, cleaned up, and fractions three and four from the alumina column were analyzed for sterols as various derivatives by gas chromatography on SE-30.

Analysis of fraction three as free alcohol, TMS ester and chloroacetate ester derivatives indicated the presence of a compound with the same retention time as coprostanol for each of these derivatives. Analysis of fraction four for free alcohols, TMS esters chloroacetates and acetates revealed a distribution of sterols very similar to that for Torch Lake sediment. For all derivatives, peaks 2, 5, and 6 had retention times equal to those for cholesterol, stigmasterol and β-sitosterol, respectively. As a free alcohol, peak 1 had a retention time equal to that for coprostanol, but not as any of the other derivatives.

Lake Mendota

A water sample from Lake Mendota was collected in January, 1973, and a sediment sample was collected in May, 1973.

Analysis of the aqueous sterols as free alcohols on SE-30 produced a chromatogram similar to that for Torch Lake, with the exceptions that peak 3 was absent and that the area of the β-sitosterol peak (peak 6) was much greater than that of the cholesterol peak (peak 2). Quantitative analysis indicated that peak 2 represented 0.8 $\mu g\,l^{-1}$ as cholesterol, and peak 6 represented 3.0 $\mu g\,l^{-1}$ as β-sitosterol.

The Lake Mendota sediment extract was separated into four fractions that might contain sterols. These were designated fractions 1–3, 1–4, 2–3, and 2–4, representing coprostanol present as a free alcohol, 5α-stanols and Δ^5-sterols present as free alcohols, coprostanol originally present as an ester, and 5α-stanols and Δ^5-sterols originally present as esters, respectively.

Coprostanol was identified in fraction 1–3 when analyzed as a free alcohol and a TMS ester by GLC on SE-30, and as a TMS ester on QF-1. Coprostanol was not detected in fraction 2–3, thus indicating that all coprostanol in the sediment was present as a free alcohol.

When the samples were analyzed as free alcohols and TMS esters on SE-30, the relative distribution of sterols in fractions 1–4 and 2–4 were similar to each other and to that of sterols in Torch Lake sediment (Figs. 2 and 3). Peaks were present with RRT values equivalent to those for cholesterol, stigmasterol

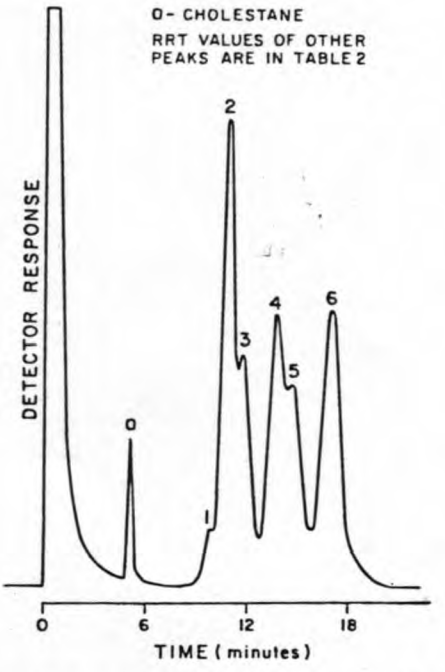

Fig. 2. Gas chromatogram of fraction 1–4 of Lake Mendota sediment extract as sterol TMS ethers on SE-30.

and β-sitosterol (Table 2) as well as peaks which did not correspond to any of these standards. The sterols in fraction 2–4, representing sterols present in the sediment as esters, comprised about 10% of the total sterols present.

Although chromatograms of fractions 1–4 and 2–4 were similar when analyzed on SE-30, they were quite different when analyzed as TMS esters on QF-1 (Figs. 4 and 5). For both of these chromatograms, peaks

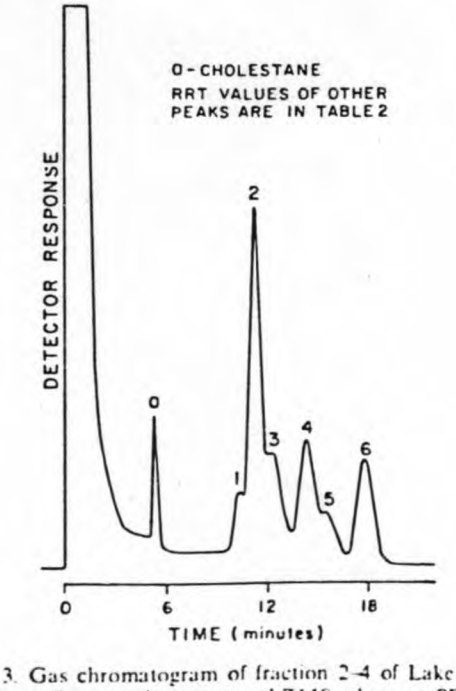

Fig. 3. Gas chromatogram of fraction 2–4 of Lake Mendota sediment extract as sterol TMS ethers on SE-30.

Table 2. RRT* values of standard sterols and sterols from Lake Mendota sediment as TMS esters on SE-30

Standard	RRT
coprostanol	1.52
cholesterol	2.06
stigmasterol	2.60
β-sitosterol	3.24

	Peak Number					
Fraction	1	2	3	4	5	6
1–4	1.66	2.06	2.20	2.47	2.60	3.20
2–4	1.70	2.08	2.24	2.52	2.66	3.25

* Relative retention times.

2a, 3a, and 7a corresponded to cholesterol, cholestanol and β-sitosterol, respectively (Table 3). The RRT values of other peaks did not correlate well to RRT values of any of the standard sterols.

DISCUSSION

Column, thin layer and gas chromatographic evidence indicates that sterols are present in all of the water and sediment samples analyzed. Although this is fairly good evidence for the existence of sterols in these samples, it would be desirable to have further confirmatory data, such as infra-red and mass spectra, not only to prove the occurrence of sterols but also to aid in identifying individual compounds. Unfortunately, such data were not collected as part of this study.

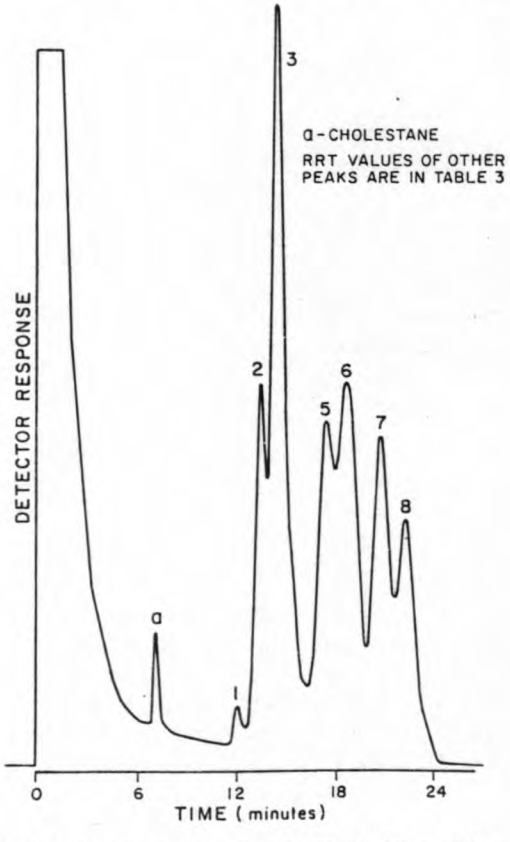

Fig. 4. Gas chromatogram of fraction 1–4 of Lake Mendota sediment extract as sterol TMS ethers on QF–1.

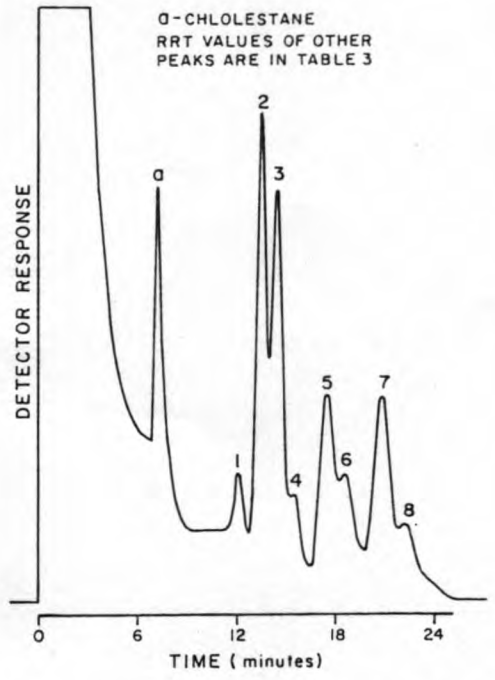

Fig. 5. Gas chromatogram of fraction 2–4 of Lake Mendota sediment extract as sterol TMS ethers on QF-1.

The similarity among the gas chromatograms of sterol fractions of the various sediment extracts is striking. These chromatograms are also similar to those obtained by Attaway & Parker (1970) for marine sediment sterols. In all cases, the major components have retention times equal to those for cholesterol (peak 2) and β-sitosterol (peak 6). Minor components include one with a retention time equal to that of stigmasterol (peak 5) and one which Attaway & Parker (1970) identify as having a retention time equal to that of campesterol (peak 4). Since several sterols may elute under the same gas chromatographic peak, the exact identity of these compounds is presently unknown. For example, cholestanol,

Table 3. RRT* values of standard sterols and sterols from Lake Mendota sediment as TMS ethers on QF-1

coprostanol	1.51
cholesterol	1.89
cholestanol	2.02
stigmasterol	2.50
β-sitosterol	2.92

	Peak Number							
Fraction	1a	2a	3a	4a	5a	6a	7a	8a
1–4	1.69	1.88	2.01	—	2.43	2.60	2.89	3.11
2–4	1.67	1.87	2.00	2.12	2.41	2.55	2.88	3.06

* Relative retention times.

Δ^{14}-cholestanol and $\Delta^{7,22}$-cholestadienol have the same retention time on SE-30 as cholesterol, while stigmasterol, fucosterol, chondrillasterol and α-spinasterol have the same retention time as β-sitosterol (Ikekawa *et al.*, 1968). The reason for the similarity among these chromatograms is not known. Since all samples are from nearshore areas, the sterols may be of terrestrial origin rather than having been produced by organisms in the water. Further characterization of the sterols may reveal differences that were not detected by gas chromatography on SE-30.

Analysis of Lake Mendota sediment sterols by gas chromatography on QF-1 indicated some differences in the sterol composition of the free sterol and sterol ester fractions. The most noticeable difference is that the ratio of cholestanol (peak 3a) to cholesterol (peak 2a) is much higher in the free sterol fraction (Fig. 4) than in the sterol ester fraction (Fig. 5). The ratios of peaks 6a to 5a and of peaks 8a to 7a are also greater in the free sterol fraction. Peak 7a has a RRT value equal to that for β-sitosterol, and retention time of peak 8a relative to peak 7a is the same as the retention time of cholestanol relative to cholesterol. Thus it is possible that peak 7a represents a C_{29} Δ^5-sterol and peak 8a represents a saturated C_{29} sterol. Peaks 5a and 6a may represent a pair of C_{28} sterols; however, no standards were available to confirm this.

The presence of saturated sterols in Lake Mendota sediment indicates formation by some diagenic process since they are not commonly found to any significant extent in organisms. In Lake Mendota sediment, the most likely source of stanols is saturation of sterol double bonds by microorganisms in the sediment. Saturated sterols have been detected by mass spectrometry in recent marine sediments by Attaway & Parker (1970); however, Henderson *et al.* (1972) did not detect saturated sterols in recent sediments of a saline lake by either gas chromatography or mass spectrometry. The higher ratio of saturated to unsaturated sterols in the free sterol fraction than in the sterol ester fraction may indicate that hydrolysis of the ester occurs more rapidly than saturation of double bonds.

The presence of detectable amounts of coprostanol in the sediment samples in this study may be environmentally significant. The most likely source of coprostanol in these lakes is sewage. Thus if coprostanol is present, other organic compounds from sewage, including other steroids, may also be present. Since many of the steroids, including steroid hormones, that may be present in sewage are more resistant to bacterial decomposition than are the sterols (Tabak & Bunch, 1970), it is possible that other steroids are accumulating in the sediments. Although it is not known what other steroids are present in sediments or what their environmental significance might be, it has been found that some steroids have toxic effects on fish at $mg\,l^{-1}$ concentrations in water (Miller & Mumma, 1973).

Although Lake Mendota and Torch Lake represent two different environments, Lake Mendota being more productive than Torch Lake, the concentrations of cholesterol and β-sitosterol in water, based on a few samples, are surprisingly similar. The reason for this similarity may be that samples from both lakes were taken during mid-winter when productivity was low and the conditions in the two lakes were more similar. It would be interesting to see how the concentrations compare during the more productive summer months when the differences between the two lakes are more pronounced.

The cholesterol concentrations are also similar to those found by Murtaugh & Bunch (1967) in the Little Miami River system. Matthews & Smith (1968), however, found much higher sterol concentrations in marine waters than those found in this study. The high concentrations found by Matthews & Smith may have been due to algal cells present in the water sample.

An interesting sidelight of this investigation is the observation that less than 10% of the sterols in a spiked ($100\,\mu g\,l^{-1}$) Lake Mendota sample were recovered by extraction of the nonacidified water, but that 100% recovery was possible from nonacidified distilled water. This indicates that something is present in Lake Mendota water that is capable of holding sterols in aqueous solution at concentrations higher than their solubility in distilled water. Since acidification greatly enhanced recovery of sterols, the substance responsible for their increased solubility may be an ionized material such as humic matter (dissolved organic carbon). Material which increases the solubility of sterols may affect not only the environmental chemistry of sterols but also that of other organic compounds. For example, Wershaw *et al.* (1969) found that sodium humates greatly increased the solubility of DDT. Water soluble sterol associations have been found in a number of organisms, including the alga *Euglena gracilis* (Brandt *et al.*, 1970). Some of these sterols were not extractable even after acidification.

CONCLUSIONS

C_{27}, C_{28} and C_{29} sterols are present in the three lake sediments studied. In Lake Mendota sediments, both saturated and unsaturated sterols were present, but this distinction was not made for Lake Wingra and Torch Lake sediments. The fecal sterol coprostanol was detected in all three samples, thus indicating that organic compounds from sewage are accumulating in these sediments. The presence of saturated sterols in Lake Mendota sediment indicates that sterols are being reduced in the sediment. Since the degree of saturation is greater in free sterols than in sterol esters, hydrolysis of the esters may be more rapid than reduction of double bonds.

Sterol concentrations in Lake Mendota and Torch Lake water are at least 1000 times less than the dry

weight concentration in Torch Lake sediment. Thus, sterols appear to be accumulated in sediments.

The exact identity and origin of sterols in these lakes is unknown. Algae and terrestrial runoff probably contribute most sterols; however, it is not known which is the more important source. The presence of coprostanol indicates that sewage is also a sterol source.

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