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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ISOLATING COPROSTANOL FROM SEDIMENT EXTRACTS

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SUMMARY

We report a rapid, largely automated high-performance liquid chromatographic (HPLC) method, which uses an HPLC column packed with alkylnitrile-substituted secondary alkylamine (aminocyano) bonded phase, to isolate coprostanol from interfering compounds in sediment extracts. Coprostanol is then quantitated, as the trimethylsilyl ether, by gas chromatography with flame ionization detection. Results from using the HPLC method to analyze a sediment reference material for coprostanol were statistically comparable to a previously used gravity-flow column method. We also report the coprostanol concentrations in several sediment samples from the California coast which reflect a range of sewage contamination (62–15 000 ng/g).

INTRODUCTION

Continuous discharge of municipal sewage effluents and sludges into the oceans has resulted in the need to monitor the dispersion of sewage-related contaminants throughout the marine environment. Over the past decade, coprostanol (5β-cholestan-3β-ol) has been recognized as a good biochemical indicator of sewage pollution[1]. Coprostanol is formed by the enzymatic hydrogenation of cholesterol in the intestine of higher animals[2,3]. Coprostanol and cholesterol are the major sterols in human feces[3]; however, cholesterol occurs naturally in aquatic environments, limiting its usefulness as a sewage tracer[4].

Many analytical methods for determining coprostanol in sediment have been published over the past decade; all are time-consuming and laborious[1]. For example, typical methods[5–10] involve several steps: (a) freeze- or oven-drying of the sediment; (b) Soxhlet extraction followed by concentration of the extract; (c) saponification; (d) thin-layer or gravity-flow chromatographic cleanup on silica or alumina; (e) derivatization; and (f) quantitation by gas chromatography (GC) with flame ionization (FID) or mass spectrometric (MS) detection.

As the demand for coprostanol analyses grows with increased awareness of the potential environmental impact of ocean dumping, methods must be simplified and improved to lower the costs and to increase the efficiency of these analyses. For

example, McCalley *et al.*[11] report that saponification of the sediment releases only small additional amounts (12–18%) of coprostanol, so they eliminate this step to save time. Furthermore, many laboratories routinely perform determinations of aromatic hydrocarbons (AHs), chlorinated hydrocarbons (CHs) and coprostanol on the same sediment extracts, so a single extraction procedure, such as that reported by Readman *et al.*[10], would prove cost-effective. Our laboratory also uses a sediment extraction procedure which combines the extraction of AHs, CHs and coprostanol; in addition, drying and extraction are combined into a single step[12].

In this manuscript, we report further improvements in the determination of coprostanol in sediment. A rapid high-performance liquid chromatographic (HPLC) separation and fractionation, using two normal-phase columns, isolates coprostanol from interfering compounds. Then, a simple derivatization procedure prepares the sample for GC quantitation. We report the results of analyses for coprostanol in a sediment reference material and in selected sediments acquired as part of the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends (NS&T) Program, a multiyear national program to measure the quality of our nation's coastal environment[13].

EXPERIMENTAL[a]

Preparation of standards

The following standard solutions were prepared for coprostanol analyses: 5α-androstan-17-β-ol (androstanol; 50.0 ng/μl; Sigma, St. Louis, MO, U.S.A.), the internal standard for quantitating coprostanol; [$^2H_{12}$]benzo[*e*]pyrene (56.5 ng/μl; MSD Isotopes, St. Louis, MO, U.S.A.), the HPLC internal standard for calculating the fraction of the extract used for HPLC; hexamethylbenzene (HMB, 76.1 ng/μl; Aldrich, Milwaukee, WI, U.S.A.), the GC internal standard for calculating the recovery of androstanol and [$^2H_{12}$]benzo[*e*]pyrene; an HPLC calibration standard containing coprostanol (180 ng/μl; Supelco, Bellefonte, PA, U.S.A.) and [$^2H_{12}$]benzo[*e*]pyrene (10 ng/μl) for calibrating fraction collection; and a GC calibration standard containing HMB (3.8 ng/μl), coprostanol (4.7 ng/μl), androstanol (2.5 ng/μl) and [$^2H_{12}$]benzo[*e*]pyrene (4.8 ng/μl). Epicoprostanol (5β-cholestan-3α-ol; 5 ng/μl; ICN Biochemicals, Plainview, NY, U.S.A.) was chromatographed to obtain its GC retention time and mass spectrum.

Collection of sediments

Sediments were collected with a modified Van Veen grab (see ref. 14). Subsamples were taken from the top 2 cm of three replicate grabs at each of three stations per site, and each replicate was put into a clean glass jar. The samples were returned to the laboratory on ice, frozen, and then thawed before the replicates were composited in the laboratory. Each site (*e.g.* South San Diego Bay) had three stations (A, B and C) located within a 2-km radius. The California sites are a subset of those sam-

[a] Mention of trade names is for information only and does not constitute endorsement by the U.S. Department of Commerce.

pled for NOAA's NS&T Program[13] and represent a range of coprostanol concentrations. The Kellogg Island (Seattle, WA, U.S.A.) sediment is a highly contaminated sediment which had been found by a previous analytical procedure[15] to contain compounds that interfered with analyses for coprostanol. We analyzed this sediment by the HPLC method to demonstrate the efficiency of this procedure in isolating coprostanol from interfering compounds.

Selection of the reference material

A sediment reference material from the Duwamish River (Seattle, WA, U.S.A) was collected and stored as described previously[16]. This Duwamish III reference material has been used in intercalibration exercises (AHs and CHs)[17] for the NS&T Program and currently serves as one of the quality assurance control materials for AHs, CHs and coprostanol in our laboratory. Thus, this material has been analyzed repeatedly.

Extraction of sediment

Sediment samples were extracted to obtain AHs, CHs and coprostanol by a tumbling method as described previously[12]. The sediment extract in methylene chloride was reduced to about 20 ml by evaporation. Then, to eliminate particulate matter which could plug the HPLC system, the extract was filtered through a glass-wool plug packed into a 25-ml disposable pipette. The volume of the filtered extract was reduced by evaporation to 1 ml, and 100 μl of the HPLC recovery standard [$^2H_{12}$]benzo[*e*]pyrene was added to determine the percentage of extract used in the HPLC step.

Instrument conditions for HPLC cleanup

A Spectra-Physics (San Jose, CA, U.S.A.) Model 8800 HPLC equipped with a recorder-integrator (Spectra-Physics, Model 4270), an ultraviolet (UV) detector (Spectra-Physics, Model 8450, set at 254 nm) and a refractive index (RI) detector (Model LC-25, Perkin-Elmer, Norwalk, CT, U.S.A.) were interfaced to a Gilson (Middleton, WI, U.S.A.) Model 231/401 autosampler. Two HPLC columns, 250 × 4.6 mm stainless steel, each packed with Partisil 10-μm PAC (alkylnitrile-substituted secondary alkylamine bonded phase, "amino-cyano"; Phenomenex, Torrance, CA, U.S.A.) were connected in series after a 2-μm in-line filter (Model 7302, Rheodyne, Cotati, CA, U.S.A.). The HPLC columns were connected to a six-port valve (Rheodyne, Model 7000) to allow for a reversal of solvent flow to the columns (backflush). The HPLC solvent, methyl *tert.*-butyl ether (MTBE, "high purity solvent", American Burdick & Jackson, Muskegon, MI, U.S.A.), was reported by Miller[18] to be compatible with chromatographing polar compounds on the amino-cyano columns. However, we found that MTBE caused leaks in some PTFE seals, apparently because this solvent shrinks PTFE[19], so we used Kel-F seals when possible. The MTBE was degassed with helium delivered by a regulator equipped with a stainless-steel diaphragm. The helium was passed through an in-line charcoal filter (200-ml "hydrocarbon trap", Alltech, Deerfield, IL, U.S.A.) to eliminate contaminants which may be transferred by the helium to the HPLC solvent.

Cleanup of sediment extracts

MTBE (2 ml) was added to the sediment extract, and the solvent (methylene

TABLE I

MEAN CONCENTRATIONS OF COPROSTANOL [$\bar{x}$, ng/g, DRY WEIGHT (RELATIVE STANDARD DEVIATION, R.S.D., %)] AND MEAN PERCENT RECOVERIES OF THE INTERNAL STANDARD, ANDROSTANOL, BOTH UNDERIVATIZED AND AS TRIMETHYLSILYL ETHERS, IN SEDIMENT SAMPLES AFTER HPLC CLEANUP OF SEDIMENT EXTRACTS

Sample and quantitation method	*Underivatized*		*Trimethylsilyl ethers*		*n*
	Coprostanol, $\bar{x}$ (R.S.D.)	*Androstanol, % recovery (R.S.D.)*	*Coprostanol, $\bar{x}$ (R.S.D.)*	*Androstanol, % recovery (R.S.D.)*	
Duwamish III reference material					
GC–FID	990[a] (15)	84 (14)	920 (17)	84 (7)	5
GC–MS			910[d]	74	1
Kellogg Island sediment					
GC–FID	44 400[b] (2)	87 (1)	33 800 (7)	87 (11)	5
GC–MS	43 400[c]	120	35 000	97	1

[a] Not different by paired Student's *t*-test from concentrations of coprostanol in derivatized samples.
[b] Different by paired Student's *t*-test from concentrations of coprostanol as the trimethylsilyl ether. Sum of coprostanol and epicoprostanol; see Discussion.
[c] Sum of coprostanol and epicoprostanol; see Discussion.
[d] Only a small amount of epicoprostanol was detected.

chloride) was displaced by the MTBE as the volume was reduced to 1 ml. A portion of this extract (100 μl) was injected onto the columns using the autosampler. The elution was isocratic, 100% MTBE at a flow of 2 ml/min for 18 min. The Gilson automatic fraction collector (Model 201C; equipped with a collection valve containing Kel-F seals, Neptune Research, Northbrook, MA, U.S.A.) was calibrated to collect a fraction from the beginning of the [$^2H_{12}$]benzo[*e*]pyrene elution to the end of the coprostanol elution (from 4.2 to 5.8 min). The coprostanol fraction was collected into a conical centrifuge tube (part No. 73785, Kimble Glass, Toledo, OH, U.S.A.). The HPLC columns were backflushed at the completion of fraction collection (from 6 to 16 min) to rapidly remove the remaining polar components from the column.

Hexane (2 ml) was added to the coprostanol fraction and the solvent (MTBE) was displaced by the hexane as the volume was reduced by evaporation to 1 ml. Then, 10 μl of the GC internal standard (HMB) was added, and the volume was further reduced, under nitrogen, to about 100μl.

Preparation of trimethylsilyl ether derivatives

Bis(trimethylsilyl)-trifluoroacetamide (BSTFA with 1% TMCS, Regisil RC-Z; 100 μl; Regis, Morton Grove, IL, U.S.A.) was added to 50 μl of the coprostanol fraction (in hexane), and the mixture was heated on a vial heating module (Pierce, Rockford, IL, U.S.A.) at 60°C for 1 h[20,21]. This mixture was stored in the freezer until GC analyses could be conducted.

Analysis by GC–FID or GC–MS of the sediment fractions

The coprostanol fraction from the sediment extracts and the derivatized fractions were analyzed by capillary GC–FID or GC–MS. A 30 m × 0.25 mm DB-5 capillary column (J & W Scientific, Folsom, CA, U.S.A.) was used in a Hewlett-Packard Model 5880A GC system. The sample (3 μl) was injected splitless, and the split valve was opened after 30 s. An oven temperature of 50°C was held for 1 min and then programmed at 4°C/min to 210°C, at 2°C/min to 280°C and at 8°C/min to 300°C. Helium was the carrier gas, and the flow-rate was set to 30 cm/s at 300°C. Quantitation was made using androstanol as the internal standard.

RESULTS

Analyses by GC–FID and GC–MS for coprostanol in sediment extracts

Extracts from Duwamish III and Kellogg Island sediments (n = 5 for each sediment) were cleaned up by HPLC, and the resulting fractions were analyzed for coprostanol by GC–FID. A portion of each fraction was derivatized with BSTFA and was analyzed for the trimethylsilyl ether of coprostanol. The results are shown in Table I.

One underivatized and one derivatized fraction from the Kellogg Island sediment and one derivatized fraction from the Duwamish III reference material were also analyzed by GC–MS to search for any compounds which could interfere with the quantitation of coprostanol and also to compare the quantitations by GC–FID and GC–MS (Table I). The GC–MS determinations of coprostanol agreed well with the GC–FID results for all the samples (Table I).

In a previous analysis of the Kellogg Island sediment by the column chroma-

tographic procedure of MacLeod *et al.*[15], a "cholestadienol" interferent was found which coeluted with coprostanol on the DB-5 GC column. This cholestadienol interferent had the same molecular weight (384) and fragment ions (*m/z* 367, 351, 255 and 213) as cholesta-5,22-dien-3β-ol, which was tentatively identified (from the mass spectrum) by Matusik *et al.*[22]. No cholestadienol interferent was found in any of the sediment fractions cleaned up by HPLC, indicating a satisfactory separation of this compound from coprostanol.

Epicoprostanol, a product of bacterial action on sewage sludge during digestion[11], coeluted with coprostanol on the DB-5 capillary column (see Discussion). This was unexpected because in previous analyses (*ca.* 1–2 years earlier), the DB-5 columns in use separated the epimers by about 0.8 min. Because coprostanol and epicoprostanol have similar mass spectra, neither selected ion monitoring nor the full-scan mode could effectively distinguish the coeluting epimers. FID also would be unable to differentiate between these compounds. GC–MS chromatograms of one Kellogg Island fraction, both before (Fig. 1A) and after derivatization (Fig. 1B), are shown in order to demonstrate the resolution of the epimers after derivatization.

Statistical comparisons of amounts of coprostanol in derivatized vs. underivatized sediment extracts

When concentrations of coprostanol in underivatized fractions from Kellogg Island sediment were compared by Student's *t*-test (paired) to those in portions of the same fractions derivatized with BSTFA, a significant difference was found; the underivatized fractions had larger concentrations of coprostanol (Table I). In contrast, the concentrations of coprostanol in underivatized and derivatized extracts of Duwamish III reference sediments (Table I) were not significantly different by Student's *t*-test (paired).

Statistical comparisons of amounts of coprostanol in Duwamish III sediment reference material cleaned up by two different methods

Concentrations of coprostanol in samples of Duwamish III sediment reference material analyzed by the HPLC cleanup ($n = 15$) were compared statistically to those in Duwamish III samples cleaned up by the column chromatography method of MacLeod *et al.*[15] (quality assurance for NS&T, 1984–1985; $n = 29$); no differences were found (Table II).

Analyses for coprostanol in sediment samples from California

Several sediments samples, collected in California for the NS&T Program, were extracted, cleaned up by the HPLC method, derivatized, and analyzed for coprostanol (Table III). These samples were chosen, based on analyses for coprostanol in sediments collected at these sites in earlier years, to include a range of coprostanol contamination levels. The lowest coprostanol concentrations were found at the nonurban site, Dana Point, located between San Diego and Los Angeles, while the highest levels were in Santa Monica Bay, adjacent to the Hyperion sewer outfall (Table III). San Pedro Outer Harbor and South San Diego Bay showed intermediate levels of contamination. The variability among the three stations from each site is due to the individual samples taken at each station rather than to analytical method variability; note that the duplicate samples (San Pedro Outer Harbor-C) were in good agreement.

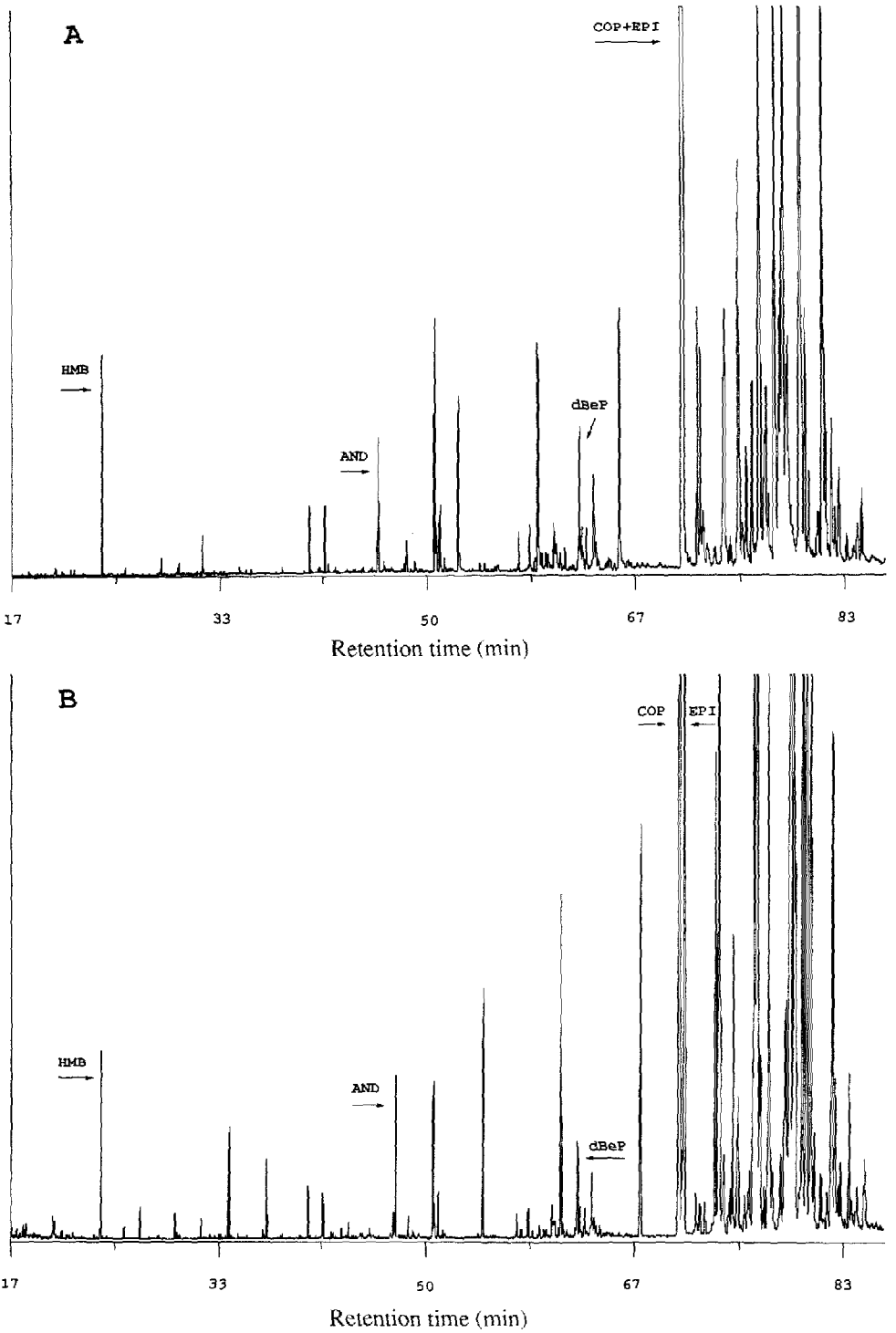

Fig. 1. GC–MS chromatogram of the sediment extract from Kellogg Island after HPLC cleanup and fractionation (see Experimental for details). (A) Prior to derivatization, coprostanol and epicoprostanol coelute. (B) The fraction resulting from HPLC cleanup was derivatized with BSTFA and the epimers were resolved. Abbreviations: HMB = hexamethylbenzene; AND = androstanol; dBeP = [$^2H_{12}$]benzo[*e*]pyrene; COP = coprostanol; EPI = epicoprostanol.

TABLE II

COMPARISON OF MEAN CONCENTRATIONS OF COPROSTANOL [$\bar{x}$, ng/g, DRY WEIGHT, (R.S.D., %)] AND MEAN PERCENT RECOVERIES OF THE INTERNAL STANDARD, ANDROSTANOL, IN DUWAMISH III REFERENCE MATERIAL SEDIMENTS CLEANED UP BY HPLC AND BY A COLUMN CHROMATOGRAPHIC METHOD

Cleanup + quantitation method	*Coprostanol*[a] $\bar{x}$ *(R.S.D.)*	*Androstanol % recovery (R.S.D.)*	*n*
HPLC + GC–FID[b]	900 (24)	89 (27)	15
Column chromatography + GC–FID[c]	860 (28)	86 (20)	29

[a] The concentrations of coprostanol found by the two methods were not statistically different by Student's *t*-test.

[b] All samples were analyzed by the HPLC cleanup method described in this manuscript. Ten samples were part of the quality assurance program for NS&T Program (1986–1988)[13].

[c] Analyzed by an earlier column chromatographic cleanup method[15] as a part of the quality assurance for the NS&T Program (1984–1985).

The limit of detection for coprostanol was $< 14 \pm 9$ ng/g, dry weight ($n = 7$, from methods blanks). For the spiked blanks ($n = 3$), mean percent recoveries of coprostanol were 98 ± 12 and the mean percent recoveries of androstanol were 90 ± 23.

DISCUSSION

The largely automated HPLC method for isolating coprostanol from interfering compounds in sediment extracts is a significant advance over previous methods which used either thin-layer or column chromatographic cleanup steps[5–10]. Although our method is more efficient (less time per sample) than our previous procedure[15], accuracy and precision are maintained as demonstrated by comparing results from analyzing the Duwamish III reference material for coprostanol by both procedures (Table II).

Other advantages of the HPLC method are decreased costs of solvents and the ability to monitor instrument conditions. For example, smaller quantities of highly pure solvent are needed for the cleanup step, *i.e.*, from 10–40% of the solvent used for typical column chromatographic cleanups[5,6,15]. Also, the ability to monitor the UV signal and the system pressure during the HPLC cleanup alerts the operator to problems with the system and reduces the need to rechromatograph the extracts.

In the first two years of the Benthic Surveillance Project of the NS&T Program (1984–1985), we conducted analyses for coprostanol in sediment by a laborious column chromatographic procedure[15]. For samples collected in the years since then, we have used our HPLC procedure to analyze for coprostanol. Data are given for four sites sampled in 1986 from among the 50 NS&T sites (Table III). The degree of sewage contamination reflects the location of these sites with respect to sources of sewage. For example, relatively remote sites such as Dana Point show low levels (62 to 96 ng/g) of coprostanol contamination. In contrast, Santa Monica Bay, which is within a short distance of major urban sewage outfalls, has high levels of coprostanol from sewage (4700–15 000 ng/g). Coprostanol levels in sediments from more typical urban sites fall between these concentrations. The urban South San Diego Bay and

TABLE III

CONCENTRATIONS OF COPROSTANOL (AS THE TRIMETHYLSILYL ETHER) AND RECOVERIES OF THE INTERNAL STANDARD, ANDROSTANOL, IN SEDIMENT SAMPLES

Sediments were sampled in 1986 at sites chosen for the NS&T Program[13]. Individual sediment samples were obtained within a site at each of three stations (A–C) located within a 2 km diameter (see Experimental).

Site	*Coprostanol (ng/g, dry weight)*			*Recovery of androstranol (%)*			*Source of contamination*
	Station			*Station*			
	A	*B*	*C*	*A*	*B*	*C*	
Dana Point	82	96	62	110	120	100	Non-urban site
South San Diego Bay	360	310	370	110	100	100	Urban harbor
San Pedro Outer Harbor	880	700	990	120	120	120	5 miles from the Los Angeles County sewer outfalls at Palos Verdes
			1000[a]			120	
Santa Monica Bay	15 000	10 000	4700	100	100	140	1–2 miles from the Los Angeles city sewer outfalls (Hyperion)

[a] Replicate sample.

San Pedro Outer Harbor sites, receiving general urban runoff and vessel sewage inputs, have coprostanol concentrations from 310 to 1000 ng/g.

The range of coprostanol concentrations found in sediments at these California sites is typical of those reported by researchers working in other locations. Hatcher and McGillivary[23] report a range of 56 to 5200 ng/g coprostanol in sediment for sites in the New York Bight; Readman *et al.*[10] found a range of 1400 to 9000 for estuaries in the U.K.; Goodfellow *et al.*[5] report levels of 3 to 13 500 ng/g for Clyde Estuary in Glasgow, U.K.; and Itoh and Tatsukawa[24] find coprostanol levels of 300 to 3600 ng/g in sediments from Osaka Bay, Japan.

Some of these earlier coprostanol measurements may not be strictly comparable with our results. For example, coprostanol determinations, especially those obtained from packed GC columns, may be artificially high due to coeluting compounds, such as cholesta-5,22-dien-3β-ol and epicoprostanol. Although we separated cholestadienol from coprostanol in the HPLC cleanup step, epicoprostanol was only partially resolved. Therefore, it was necessary to assure the separation of the epimers in the GC analysis. Before derivatization, the coprostanol concentration in the Kellogg Island extract –a sum of the concentrations of the epimers– was 30% high (Table I). After a simple derivatization with BSTFA, the trimethylsilyl ethers of the epimers were resolved on the DB-5 GC column (Fig. 1B).

In conclusion, the HPLC cleanup method for isolating coprostanol is rapid, precise and efficient. Also, the cleanup can be totally automated. Use of this method will permit environmental managers to assess the extent of sewage pollution more rapidly and less expensively. Derivatization of the coprostanol fraction from the cleanup step is recommended because: (a) epicoprostanol is often present in samples in significant amounts; (b) coprostanol and epicoprostanol are reliably separated on the DB-5 GC column only when derivatized; and (c) the condition of the GC column is not as critical in obtaining sharp peaks and good resolution from derivatized compounds.

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REFERENCES

1 R. W. Walker, C. K. Wun and W. Litsky, *CRC Crit. Rev. Environ. Control*, 12 (1982) 91–112.
2 J. Ferezou, E. Gouffier, T. Coste and F. Chevallier, *Digestion*, 18 (1978) 201–212.
3 P. Eneroth, K. Hellstrom and R. Ryhage, *J. Lipid Res.*, 5 (1964) 245–262.

4 R. B. Schwendinger and J. G. Erdman, *Science, (Washington, D.C.)*, 144 (1964) 1575.
5 R. M. Goodfellow, J. Cardoso, G. Eglinton, J. P. Dawson and G. A. Best, *Mar. Poll. Bull.*, 8 (1977) 272–276.
6 P. G. Hatcher, L. E. Keister and P. A. McGillivary, *Bull. Environ. Contam. Toxicol.*, 17 (1977) 491–498.
7 D. V. McCalley, M. Cooke and G. Nickless, *Bull. Environ. Contam. Toxicol.*, 25 (1980) 374–381.
8 R. C. Brown and T. L. Wade, *Water Res.*, 18 (1984) 621–632.
9 R. H. Pierce and R. C. Brown, *Bull. Environ. Contam. Toxicol.*, 32 (1984) 75–79.
10 J. W. Readman, M. R. Preston and R. F. C. Mantoura, *Mar. Poll. Bull.*, 17 (1986) 298–308.
11 D. V. McCalley, M. Cooke and G. Nickless, *Water Res.*, 15 (1981) 1019–1025.
12 M. M. Krahn, C. A. Wigren, R. W. Pearce, L. K. Moore, R. G. Bogar, W. D. MacLeod, Jr., S.-L. Chan and D. W. Brown, *NOAA Technical Memorandum NMFS F/NWC-153*, National Oceanic and Atmospheric Administration, Seattle, WA, 1988; available from the National Technical Information Service of the U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, U.S.A.; PB-89-12497.
13 U. Varanasi, S.-L. Chan, B. B. McCain, M. H. Schiewe, R. C. Clark, D. W. Brown, M. S. Myers, J. T. Landahl, M. M. Krahn, W. D. Gronlund and W. D. MacLeod, Jr., *NOAA Technical Memorandum NMFS F/NWC-156*, National Oceanic and Atmospheric Administration, Seattle, WA, 1988; available from the National Technical Information Service of the U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, U.S.A.
14 J. Q. Word, in W. Bascom (Editor), *Annual Report, Southern California Coastal Water Research Project*, Southern California Coastal Water Research Project, Long Beach, CA, 1976, pp. 189–194.
15 W. D. MacLeod, Jr., D. W. Brown, A. J. Friedman, D. G. Burrows, O. Maynes, R. W. Pearce, C. A. Wigren and R. G. Bogar, *NOAA Technical Memorandum NMFS F/NWC-92*, National Oceanic and Atmospheric Administration, Seattle, WA, 1985; available from the National Technical Information Service of the U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, U.S.A.; PB-86-147873.
16 M. M. Krahn, L. K. Moore, R. G. Bogar, C. A. Wigren, S.-L. Chan and D. W. Brown, *J. Chromatogr.*, 437 (1988) 161–175.
17 W. D. MacLeod, Jr., A. J. Friedman and D. W. Brown, *Mar. Environ. Res.*, 26 (1988) 209–221.
18 R. Miller, *Anal. Chem.*, 54 (1982) 1742–1746.
19 Perkin-Elmer Corp., personal communication.
20 C. J. W. Brooks, W. J. Cole, J. H. Borthwick and G. M. Brown, *J. Chromatogr.*, 239 (1982) 191–216.
21 M. M. Krahn, D. G. Burrows, M. S. Myers and D. C. Malins, *Xenobiotica*, 14 (1984) 633–646.
22 J. E. Matusik, G. P. Hoskin and J. A. Sphon, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 994–999.
23 P. G. Hatcher and P. A. McGillivary, *Environ. Sci. Technol.*, 13 (1979) 1225–1229.
24 J. Itoh and R. Tatsukawa, *Chikyu Kagaku*, 12 (1978) 9–17.