ELSEVIER

Journal of Chromatography A, 927 (2001) 155–160

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Bile acids and sterols in urban sewage treatment plants☆

R. Chaler[a], B.R.T. Simoneit[b], J.O. Grimalt[a,*]

[a]*Institute of Chemical and Environmental Research of Barcelona, ICER-CSIC, Jordi Girona 18–26, 08034 Barcelona, Catalonia, Spain*

[b]*College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, OR 97331, USA*

Received 20 November 2000; received in revised form 26 June 2001; accepted 3 July 2001

Abstract

The composition of bile acids, sterols and sterones in water and sludge from an urban sewage treatment plant has been examined for assessment of the possible use of these compounds as pollution biomarkers. Samples were solvent-extracted, hydrolysed, and fractionated by column chromatography to separate acids, hydrocarbons, sterones and sterols. These fractions, except hydrocarbons, were methylated (acids only) and silylated for instrumental analysis. Gas chromatographic–mass spectrometric analysis was performed in the electron-impact mode, using a non-polar capillary column. Lithocholic acids (3α- and 3β-epimers), coprostanone, coprostanol, cholesterol, cholestenone, and cholestanone were found in sludge and all waters. However, the waters after secondary plant treatment contained mainly lithocholic acids epimers and coprostanone, pointing to these compounds as potential markers for urban treatment plant effluents in natural waters courses.

Keywords: Water analysis; Bile acids; Sterols; Sterones

1. Introduction

The control of urban pollution is important for health and ecological reasons. Urban sewage usually contains chemical pollutants and pathogenic microorganisms. The use of characteristic chemical markers for determining the source and the extent of this pollution is one of the objectives of environmental analysis.

Bile acids may be useful as biomarkers since they are abundant in urban sewage [1]. They originate from the conversion of cholesterol in the liver through degradation of neutral and acidic sterols to steroidal secondary products in the intestins [2]. Human feces contain more than 20 different compounds of this chemical class. In addition to these compounds, coprostanol in the intestines of higher animals results from the hydrogenation of cholesterol by the intestinal microflora [3–6]. It is used as a pollution indicator for urban sewage and waste water [3,4,8–12]. Some authors have noted that it is degradated in the aquatic environment, so that its concentration decreases with distance from the pollution source [3,4,7].

The present study is devoted to the analysis of

☆Presented at the 29th Scientific Meeting of the Spanish Group of Chromatography and Related Techniques, Alcalá de Henares (Madrid), 12–14 July 2000.

*Corresponding author. Tel.: +34-93-4006-100; fax: +34-93-2045-904.

E-mail address: jgoqam@cid.csic.es (J.O. Grimalt).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01086-X

sewage samples before and after treatment in a sewage treatment plant in order to assess the fate of bile acids and fecal sterols during the process. An analytical procedure for the identification and quantitation of these compounds in treated water, decanted water, and biological sludge has been developed.

2. Experimental

2.1. Chemicals

The solvents and reagents were from Merck (Darmstadt, Germany): residue analysis-grade *n*-hexane (reference 1.04371), isooctane (reference 1.15440), methylene chloride (reference 1.06054), methanol (reference 1.15333), water for chromatography (reference 1.15333), 37% hydrochloric acid (reference 1.00317), bis(trimethylsilyl)trifluoroacetamide (reference 1.10255), silica (Kieselgel 40, 70 to 230 mesh), and alumina (aluminium oxide, 90 active 70 to 230 mesh). Hexatriacontane and 10% boron trifluoride in methanol were from Fluka (Buchs, Switzerland). Potassium hydroxide pellets were from Panreac (Barcelona, Spain). Friedelin and 5β(H)-cholanoic acid were from Steraloids (Newport, RI, USA). A reference solution (internal standard mixture) of hexatriacontane (31 μg/ml), friedelin (24 μg/ml), and 5β-cholanoic acid (23 μg/ml) in isooctane was used.

2.2. Urban sewage treatment plant

The treatment plant considered for analysis was situated at Viladecans, near Barcelona. Water treatment firstly involved two decantation steps, one of simple sedimentation of the suspended materials and the other after addition of floculant products (primary physico–chemical treatment). A second step involved biogenic digestion for the degradation of organic matter in an oxygenation process, leading to a reduction of the oxygen chemical demand (secondary biological treatment). Finally, the biogenic sludge was decanted and part of it returned to the biodigestor in order to maintain stable concentrations of bacteria.

2.3. Preparation of samples

Water volumes of 2 l were filtered through 1-μm mesh filters. Sludge samples (ca. 1 g) were obtained from a biogenic digestor. The filter and sludge samples were freeze–dried before extraction. Extraction [3,4] was performed with methylene chloride–methanol (2:1) (4×10 ml) by agitation in a vortex mixer (1 min) and an ultrasonic bath (10 min). After stirring, the reference solution was added (100 μl) and the suspensions were centrifuged for 5 min. Extracts were vacuum-evaporated to 2 ml and to dryness under a stream of N_2. They were then hydrolysed with 6% KOH in methanol (4 ml) overnight at room temperature.

The non-saponifiable neutral lipids were extracted with *n*-hexane (4×5 ml) by vortex mixing the hydrolysis mixture. After centrifugation, the *n*-hexane solution was evaporated to near-dryness and this residue was fractionated on a chromatographic column, packed with 8 g each of 5% water-deactivated alumina (top) and 5% water-deactivated silica (bottom). Two fractions were collected: 20 ml of *n*-hexane (hydrocarbons) and 20 ml of methylene chloride–methanol (95:5) (alcohols). The organic acids were recovered from the alkaline solution by acidification with 37% HCl–water (1:1) to pH 1–2 and extraction with *n*-hexane (4×5 ml). After vacuum-evaporation to 2 ml, the acids were methylated with 10% BF_3–methanol (1 ml). The solution was kept at 60°C for 1 h and overnight at room temperature. The reaction was stopped by addition of 1 ml of saturated aqueous NaCl solution. The methyl esters were extracted with *n*-hexane (4×1 ml), which was evaporated to dryness as indicated above. The non-saponifiable esters mixtures were derivatized with bis(trimethylsilyl)trifluoroacetamide prior to instrumental analysis.

2.4. Instrumental analysis

Gas chromatography–mass spectrometry (GC–MS) analysis was performed on a gas chromatograph coupled with a mass spectrometer (MD800, Thermo Quest, Manchester, UK). The gas chromatograph was equipped with a 30 m×0.25 mm I.D. HP5-MS non-polar fused-silica capillary column (film thickness: 0.25 μm). Helium was used as the carrier gas

(1.0 ml/min). The oven temperature was programmed from 90 to 120°C at 15°C/min and then to 310°C at 4°C/min, with an isothermal hold for 15 min. Injection was in the splitless mode (hot-needle technique) keeping the split valve closed for 48 s, the injector temperature was 280°C. The ion source and transfer-line temperatures were 200 and 280°C, respectively. Mass spectra were acquired in the electron-impact mode at 70 eV ionization potential. Data were acquired in the full-scan mode between *m/z* 50–550 at 1 s/decade. Mass-Lab software (Thermo Quest) was used for data acquisition and analysis. Samples were diluted in 1500 μl of isooctane; 1 μl was typically injected.

3. Results and discussion

The main steroidal compounds found in the waters entering and leaving the treatment plant and in the sludge are summarized in Fig. 1. Representative chromatograms of sterols, sterones, and bile acids are shown in Figs. 2 and 3. Only lithocholic acid (**I**), in its two epimeric forms (3α-hydroxy-5β-cholanic acid and 3β-hydroxy-5α-cholanic acid), was found among the bile acids. Their mass spectra exhibit the same characteristic ions (*m/z* 215, 257, 357, 372) but at different relative intensities (Fig. 4). These two bile acid epimers are found at similar concentrations in sewage, but the waters leaving the treatment plant

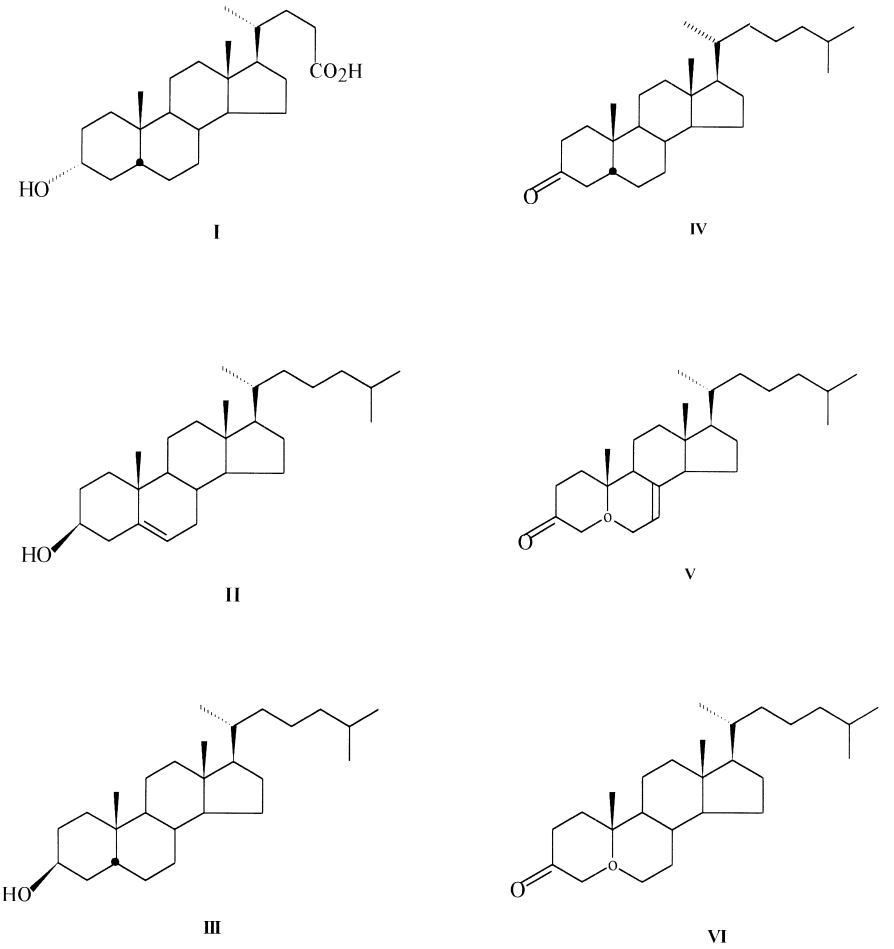

Fig. 1. Structures of lithocholic acid (**I**), cholesterol (**II**), coprostanol (**III**), coprostanone (**IV**), cholestenone (**V**), and cholestan-3-one (**VI**).

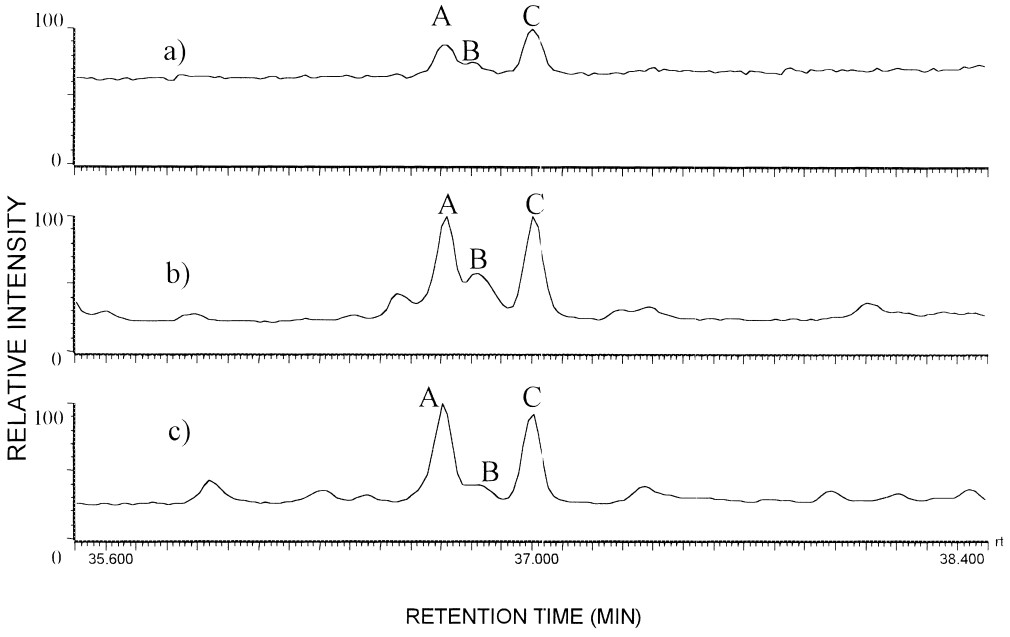

Fig. 2. Partial TIC (total ion current) traces from GC–MS data of the silylated and methylated acid fractions: (a) treated water, (b) decanted water, and (c) biological sludge. Labeled peaks: A=3β-epimer lithocholic acid, B=cholesterol, and C=3α-epimer lithocholic acid.

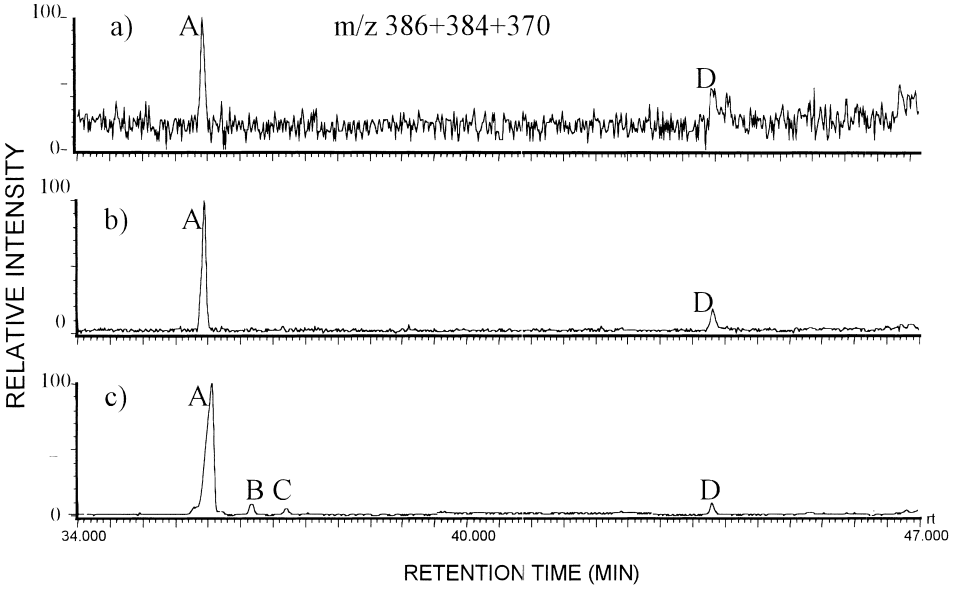

Fig. 3. Partial mass chromatograms of silylated sterolic fractions: (a) treated water, (b) decanted water, and (c) biological sludge. Labeled peaks: A=coprostan-3-one (m/z 386), B=cholestan-3-one (m/z 386), C=cholestenone (m/z 384), D=coprostanol (m/z 370).

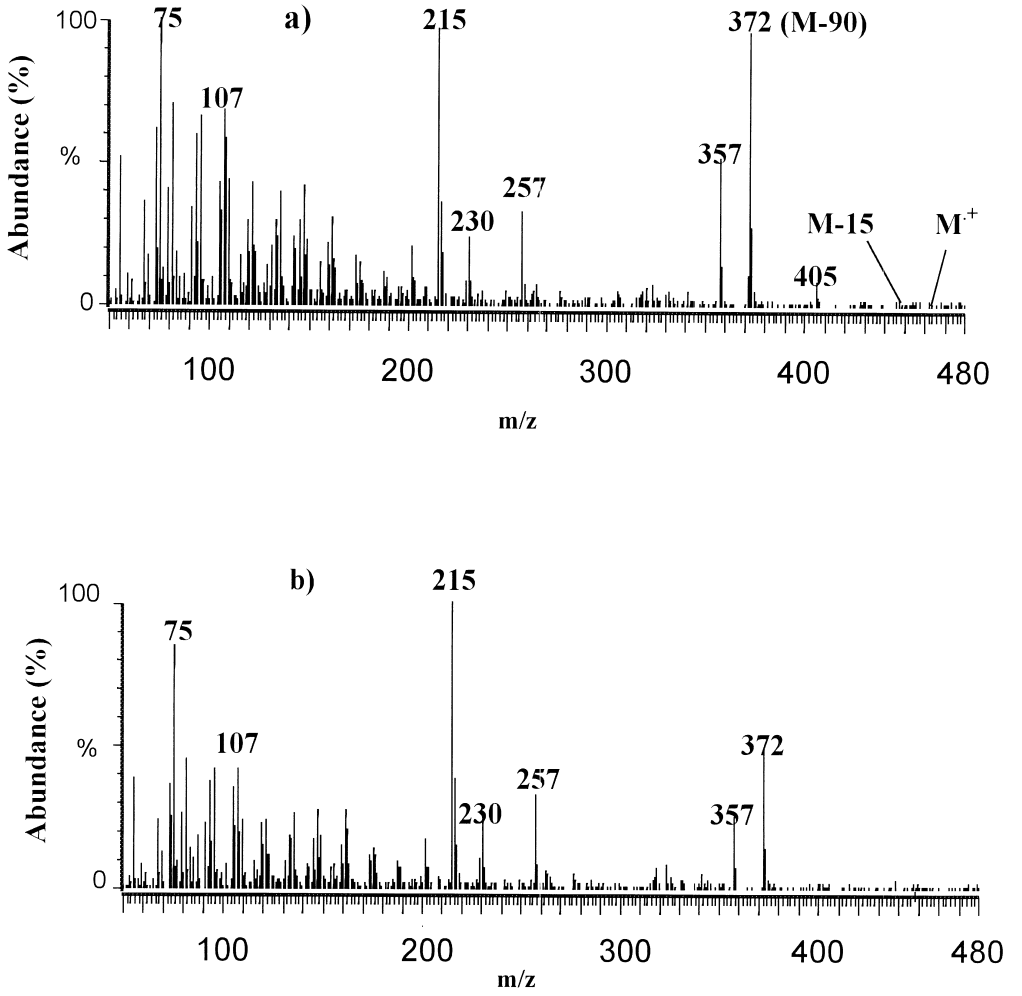

Fig. 4. Electron-impact mass spectra of trimethylsilyl ethers of 3β- (a) and 3α- (b) lithocholic acids.

are depleted in the 3β-isomer. In fact, there is a change in composition involving the predominance of this isomer in the biological sludge and decanted water. The 3α-isomer predominates in the treated water (Table 1) [13,14]. This change is paralleled by a general concentration decrease of these two acids, since they are in higher concentrations in the decanted water and biological sludge than in the waters leaving the treatment plant (Fig. 2; Table 1). Both epimers are readily degraded during sewage treatment, although the 3β-isomer is degraded to a larger extent. However, they are persistent in the aquatic environment, since they were present in the intake of the treatment plant.

Cholesterol (**II**) was also found in the same acidic fractions, but in minor quantity. Coprostanol (**III**), coprostanone (**IV**), cholestanone (**VI**), and cholestenone (**V**) were found in the silylated sterol fraction. Their concentrations are given in Table 1. Coprostanone is the major constituent in silylated sterol fraction of all samples (Fig. 3). The occurrence of this sterone in urban sewage pollution has been reported elsewhere [4,8], but its use for monitoring urban pollution has rarely been considered. Thus, the results in Table 1 and Fig. 3 show that this compound survives after biological treatment in urban sewage plants, pointing to its utility as a tracer.

Detection limits of these compounds are given in

Table 1
Concentrations of steroidal compounds detected in all samples

	Concentration (μg/kg)						
	3β-Lithocholic acid	3α-Lithocholic acid	Cholesterol	Coprostanone	Coprostanol	Cholestenone	Cholestanone
Treated water[a]	680	800	210	140	1	n.d.	n.d
Decanted water[a]	5200	4600	2300	830	10	n.d.	n.d.
Biological sludge[b]	30 000	20 000	5000	5000	10	20	200

n.d.=Not detected.
[a] Indicates μg of compound per litre of filtered water.
[b] Indicates μg of compound per kg of freeze–dried sludge.

Table 2
Limits of detection of steroidal compounds detected in all samples

	3β-Lithocholic acid	3α-Lithocholic acid	Cholesterol	Coprostanone	Coprostanol	Cholestenone	Cholestanone
Limit of detection (μg/kg)	70	80	20	15	1	15	15

Table 2. Recoveries were between 75 and >80% with mean reproducibilities better than ±10%.

4. Conclusions

Lithocholic acids, cholesterol, coprostanol, cholestenone, and coprostanone were found in urban sewage samples. Both epimers of lithocholic acid and coprostanone are still found in the water leaving the treatment plant, the former showing an enrichment in the 3α-isomer. These three steroidal compounds may be used for tracing contamination from effluents of urban treatment plants in river and marine waters. They are also useful as tracers of raw sewage inputs.

References

[1] M.M. Elhmmali, D.J. Roberts, R.P. Evershed, Environ. Sci. Technol. 31 (1997) 3663.
[2] S.M. Grundy, E.H. Ahrens Jr., T.A. Miettinen, J. Lipid Res. 6 (1965) 397.
[3] J. Danielsson, in: R. Paoletti, D. Kritchevsky (Eds.), Advances in Lipid Research, Vol. 1, Academic Press, New York, 1963, p. 335.
[4] J.O. Grimalt, P. Fernández, J.M. Bayona, J. Albaigés, Environ. Sci. Technol. 24 (1990) 357.
[5] P.G. Hatcher, P.A. McGillivary, Environ. Sci. Technol. 13 (1979) 1225.
[6] R.W. Walker, Ch. Wun, W.K. Litsky, CRC Crit. Rev. Environ. Control 12 (1982) 91.
[7] D.V. McCalley, M. Cooke, G. Nickless, Bull. Environ. Contam. Toxicol. 25 (1980) 374.
[8] D.V. McCalley, M. Cooke, G. Nickless, Water Res. 5 (1981) 1019.
[9] M.I. Venkatesan, I.R. Kaplan, Environ. Sci. Technol. 24 (1990) 208.
[10] J. Laureillarnd, A. Saliot, Mar. Chem. 43 (1993) 247.
[11] P.D. Nicholls, R. Leeming, G. Cresswell, Water 19 (1992) 32.
[12] C.J. Kirchmer, Ph.D. Thesis, University of Florida, FL, 1971.
[13] C. Maldonado, M.I. Venkatesan, C.R. Phillips, J.M. Bayona, Mar. Pollut. Bull. 40 (2000) 680.
[14] M.I. Venkatesan, F.H. Mirsadeghi, Mar. Pollut. Bull. 25 (1992) 328.