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Journal of Chromatography A, 774 (1997) 311–319

JOURNAL OF CHROMATOGRAPHY A

Broad-spectrum analysis of a contaminated sediment: exemplification of a protocol

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Abstract

Chromatographic procedures have been outlined that enable isolation and analysis of structurally diverse groups of compounds from sediments. These methods have been exemplified using a contemporary sediment sample from a contaminated site in the Gulf of Bothnia that was examined for (a) selected di- and tri-terpenoids, β-sitosterol and aromatic hydrocarbons of biogenic origin and (b) chlorinated resin acids and chlorophenolic compounds. These were quantified together with the fully aromatic retene. A tetracyclic aromatic compound $C_{21}H_{22}$ was isolated and its structure deduced on the basis of its mass spectrum, high resolution MS and ^{1}H NMR and proton-decoupled ^{13}C spectra. It was identified as a des-A triterpenoid derived from lupane, or less likely as an aromatized steroid. Its concentration in segments of the sediment core was comparable to that of both lupeol and sitosterol.

Keywords: Sediments; Environmental analysis

1. Introduction

Many organic compounds are partitioned from the aqueous phase into the sediment phase. The extent of this depends both on the composition of the sediment and the structure of the compound. Structurally diverse compounds have been recovered from sediments, and include aromatic hydrocarbons, halogenated aromatic compounds, azaarenes, phenolic compounds, terpenoids and alkanoic acids. The occurrence of these compounds is of environmental concern since they may have an adverse effect on sediment-dwelling organisms and demersal fish, while their persistence is determined by anaerobic processes in the sediment, particularly when this has a high content of organic carbon that provides growth substrates for anaerobic bacteria.

A number of procedures have been described for isolating and quantifying specific xenobiotics that occur on proscribed lists of compounds —priority pollutants— though there are remaining problems for biota and sediment samples. In such samples, however, many other compounds also occur, and since neither the structure nor the environmental impact of these may be known, it is highly desirable to have access to procedures for identifying and quantifying them. A good example is provided by a study in which a variety of non-regulated compounds were isolated from Florida sediments [1].

Evaluation of their environmental impact of such compounds presupposes knowledge of their identification and an assessment of possible adverse effects.

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PII S0021-9673(97)00043-5

Since direct analysis of unfractionated extracts is virtually impossible in view of the plethora of compounds generally involved, identification and quantification necessitates the isolation of such compounds in pure form. This fractionation is critically dependent upon the application of a range of chromatographic procedures. Previous investigations from this laboratory have used a number of isolation procedures for isolating and quantifying chlorophenolic compounds from sediments [2] and a range of compounds in cyclohexane extracts from sediments [3]. In addition, they revealed the presence of a number of previously unrecognized contaminants, including both chlorophenolic compounds [4] and transformation products of terpenes [5]. There is therefore a need for a procedure that makes possible the analysis of a wide range of structurally dissimilar compounds. This will involve fractionation procedures that take into account the characteristics of functionalized compounds and avoid procedures involving concentrated H_2SO_4 that are clearly unsuitable for sensitive compounds. In addition, the procedures should be applicable to processing the large numbers of samples that are often involved in monitoring programs.

The present investigation was directed to developing chromatographic procedures for the analysis in sediment samples of a wide range of compounds that are formed during the production of bleached pulp. The analytes included are, however, not only those traditionally considered —chlorinated dehydroabietic acids and chlorinated phenolic compounds— but also a plant sterol, di- and triterpenoids and aromatized derivatives of these.

2. Experimental

2.1. Chemicals

Dehydroabietic acid was obtained from Helix (Richmond, Canada) lupeol and betulin from Sigma, fichtelite from Chiron (Trondheim, Norway), and retene from ICN Biomedicals (Costa Mesa, CA, USA). The retene was crystallized from ethanol, and the other standards analysed as received. 12-Chloro- and 14-dehydroabietic acid were synthesized and the structure of the isomers confirmed by ^1H NMR. Benzene and propan-2-ol were from J. and T. Baker, hexane from Riedel de Haën, and methyl *tert.*-butyl ether (MTBE) from Rathburn (Waterburn, UK). Sodium sulfate (Merck) was incinerated at 400°C for 17 h to remove organic impurities.

2.2. Isolation of compounds from sediment

The main study was carried out using a sediment core of ca. 30 cm taken at a depth of 10 m from a contaminated bay in the Baltic. As a result of dredging, the core was heterogeneous in age and covered the period since a mill producing bleached pulp came into operation at this site in 1932, although activity has existed since 1665. Samples were taken with a Benell sampler and the core was divided into segments of 2–2.5 cm. These were freeze-dried and kept in the dark at room temperature.

2.3. Analysis of non-phenolic compounds

In the following procedures, benzene may be replaced by toluene although this is less volatile, and therefore less convenient. Normal care should be exercised in the use of diazomethane. Water (50 μl) and 1 ml each of benzene and propane-2-ol were added to ca. 200 mg portions of freeze-dried sediment, followed by suitable surrogate standards: α-cholestane (30 μl, 1.55 mg/l) for saturated alicyclic compounds, 12,14-dibromodehydroabietic acid (25 μl; 1 mg/ml) for diterpene acids, and cholestanol (25 μl; 1 mg/ml) for hydroxylated steroids and triterpenoids. Samples were treated for 10 min in a sonicator bath, and then shaken overnight, centrifuged (1000*g*, 10 min) and the supernatant removed; this was repeated twice and the combined extracts treated with tetrabutyl ammonium sulfite (0.1 *M*) and Na_2SO_3 (1 *M*) to remove elementary sulfur [6]. Excess propan-2-ol and Na_2SO_3 were removed by shaking with deionized water (2×5 ml), the organic phase dried (Na_2SO_4) and the volume adjusted to 1 ml.

This was applied to a silica column deactivated with 5% H_2O (40×10 mm; Merck Kieselgel 60; 70–230 mesh), and the neutral hydrocarbons eluted with 5 ml benzene and the polar components with 5 ml MTBE. Solvent was removed under a stream of

N_2 at room temperature from the benzene eluate, the residue was redissolved in 1 ml hexane and was applied to a column of Al_2O_3 (40×10 mm; Merck Neutral Grade 2). This was eluted sucessively with 5 ml hexane for the saturated hydrocarbons (fichtelite) and 5 ml benzene for the carbocyclic aromatic hydrocarbons. Solvent was removed under a stream of N_2 at room temperature from the benzene fraction, the residue dissolved in hexane (1 ml) and 1-chlorooctane (10 μg) was added to the benzene solution as internal standard. This fraction was used for analysis of aromatic hydrocarbons.

MTBE must be removed before attempting methylation with diazomethane and was removed from the MTBE fraction of the previous fractionation under a stream of N_2 at room temperature, the residue was dissolved in benzene and the carboxylic acids were methylated with diazomethane and rechromatographed on silica gel. The methyl esters were eluted with benzene, biphenyl (20 μl; 0.5 mg/ml) added as internal standard, and this fraction was analysed for diterpene carboxylic acids. The other components were eluted with MTBE (5 ml), the solvent was removed under a stream of N_2 at room temperature and the residue acetylated overnight with acetic anhydride–pyridine. The excess reagents were removed, biphenyl added as internal standard and the sample analysed for sterols and triterpenols. The identity of the analytes was established by comparison of GC retention times and the mass spectra with those of authentic compounds. The unknown hydrocarbon (A) was isolated in a degree of purity necessary for NMR analysis as follows. Freeze-dried sediment (from ca. 500 g wet weight) was extracted twice for 15 h with a mixture of propan-2-ol and benzene (1:1, v/v) containing 1% water (200 ml). The extracts were combined, treated as above for the removal of elementary sulfur, propan-2-ol removed by shaking with water (2×30 ml), the benzene extract dried (Na_2SO_4) and solvent removed in a stream of N_2 at room temperature. The residue was dissolved in hexane (5 ml), applied to a column of silica gel (2×8 cm; Merck Kiselgel 60, (70-230 mesh) and eluted with hexane (50 ml). Solvent was removed, the residue redissolved in hexane (5 ml) and the solution applied to a column of alumina (Merck; Neutral Grade 2). Elution was carried out with hexane (25 ml) and then with benzene (40 ml). Removal of benzene gave a product that was shown by GC–MS to consist mainly of a mixture of retene and compound (A). The pure compound was obtained by preparative HPLC using a Nucleosil C_{18} column (10×250 mm: 5 μm particle size). The mobile phase was methanol, the detector was set at 280 nm, and 2 ml fractions were collected. These were analyzed by GC–MS, and those containing compound (A) were combined, concentrated and rechromatographed. The product then had a purity >99% by GC (flame ionization detection).

2.4. Analysis of phenolic compounds

Sediment samples were extracted as above with benzene–propan-2-ol containing EDTA (10 mg), ascorbic acid (20 mg) and 10 *M* HCl (100 μl). The propan-2-ol was removed by shaking with acidified water and this phase was re-extracted with hexane–MTBE (1:1). The combined organic phases were washed with acidified water, the organic phase removed, dried (Na_2SO_4) and acetylated by the two-stage method using acetic anhydride–sodium acetate followed by acetic anhydride–pyridine [7]. Benzene (1 ml) was added to remove traces of water and isooctane (1 ml) as "keeper", solvent was removed to a volume of ca. 100 μl under a stream of N_2 at 45°C, the residue was dissolved in hexane, and the phenol O-acetates were chromatographed as described [8]. GC analysis was carried out as descibed [9].

2.5. Gas chromatographic, gas chromatographic–mass spectrometric and nuclear magnetic resonance analysis

The GC analysis used an HP 5890A instrument with an 7673 Autosampler and a DB-5 column (J and W Scientific, Folsom, CA, U.S.A., 20 m×0.25 mm I.D., film thickness 0.25 μm) with a helium flow of 35 cm/s. The following temperature program was used: 45°C for 1 min increasing at 15°C/min to 150°C and then at 7.5°C/min to 320°C that was maintained for 5 min.

Low-resolution GC–MS was carried out using a VG Trio 2 instrument as described [9] and high resolution MS using a VG AutospecQ.

Table 1
Concentrations (μg/g organic carbon) of nonchlorinated analytes in sediment core

Depth (cm)	Abietic acid	Pimaric acid	Dehydroabietic acid	Retene	Fichtelite	Lupeol	Betulin	Des-A	Sitosterol
1	90	70	130	2.3	2.2	80	1100	0.6	62
5	280	200	350	3.0	3.6	195	1940	34	420
10	320	200	310	2.1	3.0	180	2050	190	280
15	695	535	565	6.1	3.6	350	3950	390	1530
20	190	53	190	4.0	2.6	20	133	28	150

NMR spectra were recorded on a JEOL GX400 spectrometer (399.65 MHz for protons) equipped with a standard 5 mm diameter C/H probe. The sample was prepared in deuterated chloroform at a concentration of 0.5 mg/ml. Spectra were acquired using standard pulse sequences supplied by JEOL and referenced to internal tetramethylsilane. The proton and homonuclear spin decoupled proton spectra were acquired using the following conditions: spectral width 4.4 kHz, 32 K data points, a pulse duration of 10 μs (30°) and a cycle time of 6 s. After 512 scans, the data were zerofilled once and processed using a Gaussian window (−0.2 Hz line broadening). The unoptimized NOE difference spectra were acquired using the following conditions: spectral width 4.4 kHz, 32 data points, a pulse duration of 17 μs (50°), an irradiation time of 5 s and a total time of 20 s for the NOE-normal spectrum pair. After 1024 scans, the data were zerofilled once and processed using an exponential window (1.0 Hz line broadening). Proton decoupled carbon and DEPT spectra were acquired using a Varian Inova 500 spectrometer using normal protocols.

3. Results

Analysis of the sediment segments from various depths was carried out to determine putative precursors of aromatic compounds. Analytes included the diterpene dehydroabietic acid, the triterpenes lupeol and betulin and the sterol β-stigmasterol. The concentrations of the non-chlorinated compounds are given in Table 1, and those of 12- and 14- chlorodehydroabietic acid and chlorophenolic compounds Table 2. Detailed examination of a sample from the Gulf of Bothnia locality revealed the additional presence of a compound (A) whose mass spectrum is given in Fig. 1 and whose molecular mass by high-resolution MS was 274.171851.

The acquired proton spectrum of the aromatic hydrocarbon A in deuterated chloroform exhibited the following signals (δ units); s (singlet), d (doublet), dd (double doublet), dp (double pentuplet) m (multiplet), b (broadened). The assignments (e.g., 1-*H*) refer to the Chemical Abstracts index name numbering system.

8.56 bd (8.5 Hz) **1**-*H*; 8.55 bd (9.0 Hz) **11**-*H*; 7.94 dd (9.5, 0.8 Hz) **6**-*H*; 7.87 d (9.5 Hz) **7**-*H*; 7.50 dd

Table 2
Concentrations (μg/g organic carbon) of chlorinated analytes in sediment core

Depth (cm)	12Cl-DHA	14Cl-DHA	4,5-CC	3,4,6-CC	Tetra-CC	4,5-CG	3,4,5-CG	Tetra-CG	5,6-CV
1	6	23	170	660	4400	14	60	20	0
5	18	58	350	5200	17 000	48	210	90	40
10	21	65	270	4500	12 900	78	150	70	50
15	24	71	375	5400	21 000	40	140	70	30
20	21	71	90	330	1200	76	17	6	0

DHA: dehydroabietic acid; CC: chlorocatechol; CG: chloroguaiacol; CV: chlorovanillin.

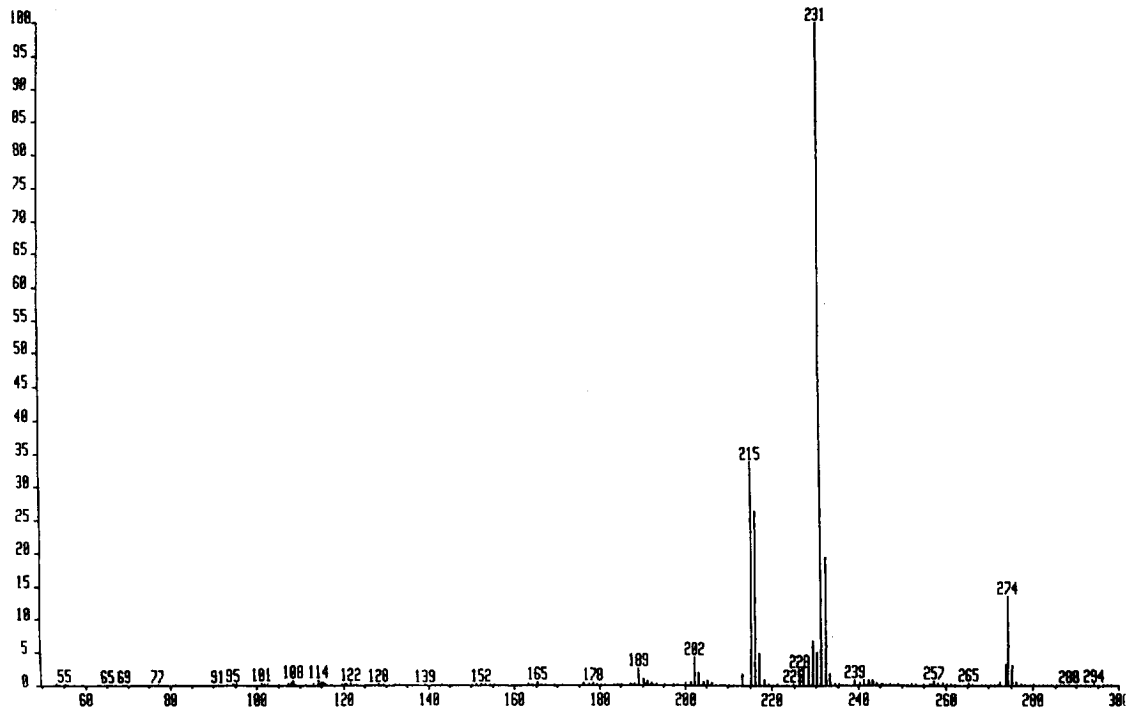

Fig. 1. Mass spectrum of compound A.

(8.5, 7.5 Hz) **2-***H*; 7.50 d (9.0 Hz) **12-***H*; 7.40 dp (7.5, 0.8 Hz) **3-***H*; 3.76 m **15-***H*; 3.14 m **17-***H*; 3.01 m **17-***H'*; 2.73 s (**4-C***H*$_3$); 2.36 m C*H*(CH$_3$)$_2$; 2.22 m **16-***H* (both protons); 1.12 d (7.0 Hz) CH(C*H*$_3$)(CH$_3$)'; 0.60 d (7.0 Hz) CH(CH$_3$)(C*H*$_3$)'.

An NOE interaction was measured between 4-C*H*$_3$ and protons 6-*H* and 3-*H*. A separate experiment showed NOE interactions between proton 7-*H* and protons 15-*H* and C*H*(CH$_3$)$_2$. Homonuclear decoupling of the 4-C*H*$_3$ signal results in sharpening of signals assigned to 1-*H*, 2-*H* and 3-*H*. Homonuclear decoupling 1-*H* and 11-*H* simultaneously (because of the small chemical shift difference) results in modifications to the signals assigned to 2-*H*, 3-*H*, 12-*H* together with a sharpening of the signals assigned to 6-*H* and 7-*H*. The proton-decoupled ^{13}C spectrum, referenced to deuterated chloroform, showed the following resonances: 143.21 s; 142.11 s; 134.76 s; 131.07 s; 129.98 s; 129.53 s; 127.11 d; 126.03 d; 123.42 d; 122.44 d; 121.84 d; 120.98 d; 49.97 d; 33.28 t; 32.51 d; 25.65 t; 22.38 q; 19.97 q; 16.32q, where s (singlet), d (doublet), t (triplet) and q (quartet) refer to the DEPT multiplicities.

4. Discussion

Several procedures for extracting sediment samples have been described in the literature, including Soxhlet extraction, sonication and supercritical fluid extraction. Since sonication has been used for neutral priority pollutants [10] this was used since a large number of samples had to be analyzed. A mixture of benzene and propan-2-ol was used, and water was added to hinder association of aromatic compounds with the sediment [11]. Of the solvents used, a mixture of benzene and propan-2-ol was deemed satisfactory, though neither the extraction procedure nor the choice of solvent can be optimal for every type of organic compound that may be encountered. The use of a series of open-column chromatography steps successfully resolved the major fractions, and

HPLC enabled quantities of a hitherto unknown compound to be obtained in sufficient purity for NMR studies. Fractionation of the phenolic compounds by alkaline extraction was not more effective than direct solvent extraction followed by open-column chromatography in removing interfering compounds. Its omission simplified the analytical procedure, avoided hydrolysis of sensitive groups such as esters and the extraction of unwanted humic and fulvic acids.

Most of the analytes belong to groups of compounds with established ecotoxicological significance, (1) chlorophenolic compounds [12,13], (2) diterpene carboxylic acids [14] and (3) polycyclic aromatic hydrocarbons (PAHs), that may be associated with tumors in demersal fish sediments [15–17] and a range of sublethal effects [18].

With the exception of compound A, all of the compounds could be rigorously identified by comparison with authentic compounds that have been described in previous publications [3–5]. In the continuation of a previous study [5] considerable attention was directed to aromatic hydrocarbons and to terpenoids. A range of aromatized terpenoids has previously been recovered from sediment samples [19,20], and retene has been postulated as a specific marker for the discharge from slow-combustion wood stoves [21]. In a previous study [5], concentrations of dehydroabietic acid in sediment samples were in the range 250–780 mg/kg organic C and β-sitosterol in the range 1400–2900 mg/kg organic C; the concentrations of both retene and fichtelite found in this study are therefore considerably greater.

In attempting to interpret the concentrations as a function of the segment of the sediment core, a number of considerations should be borne in mind: (i) The heterogeneity of the sample and the established patchiness of sediment samples. (ii) The samples covered only some 2 dm of sediment in water with a depth of 10 m, so that this is a superficial sediment sample. (iii) The level of input into the bay is unknown and will certainly vary with production schedules and technologies.

Overall, however, there are three different patterns of the distribution of concentration with depth:

1. Concentrations of retene, fichtelite and 12- and 14-chlorodehydroabietic acid were not dependent on depth, and it appears that these compounds are stable in this system, and for the first two may represent a dismutation process.
2. For terpenoids and β-sitosterol, concentrations reached a maximum at ca. 15 cm, and then diminished strongly with depth by factors of 10–30, and it is possible that these compounds are undergoing anaerobic abiotic or biotic transformations. The possible reactions resulting in the production of compound A from lupeol or betulin are discussed later.
3. For chlorophenolic compounds there was also a maximum between 5 and 15 cm, but the relative concentrations of chloroguaiacols and chlorocatechols are complicated by anaerobic de-O-methylation [22], and the complex pattern of dechlorination of chlorocatechols [23].

All of the compounds with the exception of compound A have already been isolated in previous studies so that effort was directed to determining the structure of compound A. The low-resolution MS of compound A had a strong parent ion at m/z 274 and suggested an aromatic structure analogous to that of retene. It was clearly different, however, since the peak at m/z 231 indicated loss of an isopropyl group from a saturated carbon atom. High-resolution MS gave a molecular mass (274.171851) that was consistent with the empirical formula $C_{21}H_{22}$ (required 274.172151). The structure was elucidated by interpretation of the proton NMR spectrum. (In the following discussion, the numbering of cyclopenta[*a*]phenanthrene is that used by Chemical Abstracts and is based on the steroid/triterpene system). Broadly, the chemical shifts and coupling patterns of the signals confirm the presence of a 1,2,3-trisubstituted benzenoid ring together with two 1,2,3,4-tetrasubstituted benzenoid rings. The NOE results confirm that the 1,2,3-trisubstituted benzenoid ring has a methyl group at either the 1 or 4 positions (ring A) and that this methyl group plays a significant role in the relaxation of a proton in one of the 1,2,3,4-tetrasubstituted benzenoid rings. NOE difference results also indicate that a proton in the second tetrasubstituted ring plays a sugnificant role in the relaxation of the protons in the isopropyl-substituted cyclopentene ring. In addition to expected interactions, the homonuclear decoupling experiments indicate long range coupling (0.8 Hz) between protons

1-*H* and 6-*H*. An unresolvable long range coupling is also suggested by an increase in peak height of the signal assigned to 7-*H* when the 11-*H* proton is irradiated. If it is assumed that the only long-range spin–spin coupling operating is the well- established $^{5}J_{HCCCCH}$ case, the spectra are consistent with 16,17-dihydro-4-methyl-15-(1-methylethyl)-15H-cyclopenta[*a*]phenanthrene. If, however, other long-range spin–spin couplings are in operation, the alternative 17-(1-methylethyl) structure could not be excluded. The proton-coupled ^{13}C data enabled further definition in favour of 4-methyl-15- isopropylcyclopenta[*a*]phenanthrene. The spectrum referenced to deuterated chloroform had signals at 22.38 d, 19.97 q and 16.32 q, one of which belongs to the aromatic methyl group. Data for 4- methylphenanthrene [24] show a chemical shift for the methyl group at δ 27.2, whereas that for 1-methylphenanthrene occurs at δ 19.70 [25]. Of the three possibilities, the methyl group of compound A is therefore that having δ 19.97, with the methyl groups of the isopropyl group at δ 22.38 and δ 16.32. This together with the absence of a methyl signal at ca. δ 27 strongly supports the proposed 4 - methyl - 15 - isopropylcyclopenta[*a*]phenanthrene structure.

The two possible structures of compound A may be rationalized on the basis of either of three plausible transformations (Fig. 2):

1. Degradation of the C-17 side chain of a sterol to isopropyl and aromatization of the cyclohexane rings with rearrangement of the C-10 angular methyl group to C-4: this has already been documented. This would produce a 1-isopropylcyclopenta[*a*]phenanthrene (C-17 isopropyl).
2. Degradation of the A-ring of hopane and aromatization without further rearrangement to produce a 1-isopropylcyclopenta[*a*]phenanthrene (C-17 isopropyl).
3. Degradation of the A-ring of a lupeol-series triterpene with aromatization of the cyclohexane rings: the isopropyl group derived from C-19 group of a lupane would then remain as 3-isopropylcyclopenta[*a*]phenanthrene (C-15 isopropyl).

The ^{1}H and ^{13}C NMR results strongly support the last of these, but cannot totally exclude either of the first two. The 15-isopropyl structure has been reported as trace component of a tertiary brown coal from China [26] and would correspond, in the lupane series, to a similar compound already isolated and unambiguously identified in the oleane series [27]. This would be consistent with the presence of lupanes in the sediment. The results showed that betulin was dominant at all depths increasing by a factor of 3.6 between the surface sample and 15 cm. Between the samples from the same depths, however, the ratios for lupeol were 2.3 and for the A hydrocarbon 240. Compound A may be the result of transformation reactions —possibly microbiological— in the sub-surface sediment. The transformation of a-amyrin to a des-A product has been tentatively established as microbiological [28], while hypothetical reactions for accomplishing this and other transformations of steroids and terpenoids have been proposed [29].

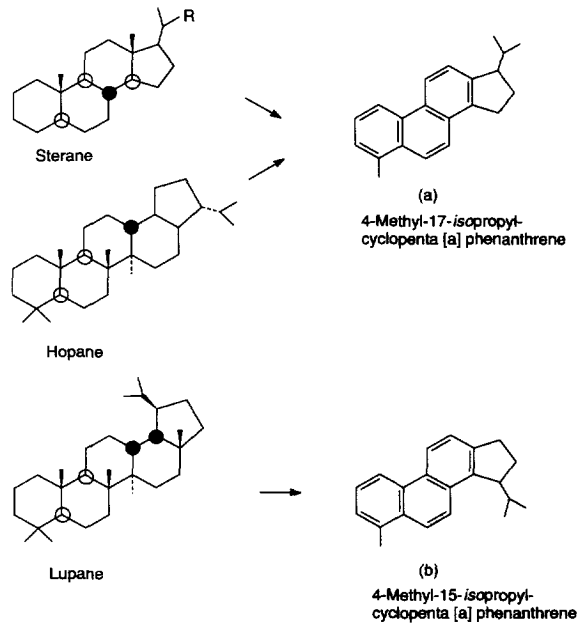

Fig. 2. Hypothetical reactions for the formation of possible alternative structures of compound A: (a) 4-methyl-17-isopropyl-cyclopenta[*a*]phenanthrene and (b) 4-methyl-15-isopropyl-cyclopenta[*a*]phenanthrene.

Although many PAHs are produced during combustion processes and are bound to particulate material that may enter the sediment phase of both freshwater and marine habitats, there is extensive

evidence that in addition to such compounds, aromatic compounds that are formed from alicyclic precursors including steroids and di- and triterpenes exist in recent lake sediments [19] and in geologically recent sediments [26]. It is suggested that aromatic hydrocarbons occur in contemporary sediments that have received discharge from pulp mills and that these are structurally related to —and probably derived from— established plant di- and triterpenoids or sterols in the raw material. (To avoid confusion with the established geological meaning of the term "recent", the term contemporary has been used for the sediments examined in this study). Previous investigations [5] revealed the presence of a number of diterpene-derived aromatic compounds, and this has now been extended to demonstrating the presence of steroid- or triterpenoid-derived aromatic compounds.

The sediment concentrations of those compounds may be high compared particularly with those of organochlorine compounds that have aroused so much concern. By comparison with traditional aromatic compounds produced by thermal reactions, however, little is known about the bioconcentration potential and toxicity of these biogenic aromatic hydrocarbons. It is worth noting the strong carcinogenicity of several alkylated aromatic hydrocarbons compared with weak activity of the parent compounds. Examples include methyl cholanthrene, 5-methylchrysene and 7,12-dimethyl-benz[*a*]anthracene. The occurrence of retene and compound A are therefore worth underscoring.

In a wider context, attention is directed to two straightforward extensions of the procedures.

1. Inclusion of thermally produced PAHs and polychlorinated biphenyls would present no problem, and the range of analytes can readily be extended to include compounds for which procedures have already been given [3]: (a) lipid-bound alkanoic and alkenoic acids by transmethylation in MTBE and (b) associated phenolic compounds by alkaline treatment.
2. They are also applicable to the analysis of solid waste. Although for industrial waste, only a structurally limited range of compounds will generally be involved, including PAHs, polychlorinated biphenyls, organohalogens and aromatic nitro compounds, a very much wider spectrum of compounds may be encountered in municipal waste [30]. At all of these sites, the initially deposited compounds may also have undergone extensive transformation —both abiotic and biotic— and identification and quantification of these is an obligatory part of an environmental impact assessment of the site.

5. Conclusions

1. A procedure involving open-column chromatography using a number of sorbents and eluting solvents was suitable for fractionating a wide range of compounds in a sediment sample. For isolating unknown compounds in a degree of purity sufficient for NMR structure determination, semi-preparative HPLC proved successful.
2. The results underscore the occurrence of hitherto unsuspected compounds even in contemporary surface sediments, and the structure of one of these has been tentatively established and a hypothesis for its formation proposed. It is therefore suggested that routine MS analysis of sediment samples be carried out to reveal the presence of unsuspected contaminants.

Acknowledgments

We thank the Knut and Alice Wallenberg Foundation for the purchase of instrumentation in Stockholm, Ann-Sofie Allard for producing Fig. 2 and Östen Ekengren for his interest.

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