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APPLICATION OF GAS CHROMATOGRAPHY ON GLASS CAPILLARY COLUMNS TO THE ANALYSIS OF HYDROCARBON POLLUTANTS FROM THE AMOCO CADIZ OIL SPILL

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SUMMARY

About 230,000 tons oil spilled when the Amoco Cadiz sank in 1978 and heavily polluted the northern Brittany coasts. During a 9-month period, the composition of oil on beaches near the wreck site was studied by high-performance gas chromatography (HPGC). Simultaneously, levels of aliphatic and aromatic hydrocarbons in flat and Japanese oysters were measured.

Quantification was effected by means of perdeuterated hydrocarbons which were totally resolved from the natural compounds by HPGC. Much attention has been paid to the input and fate of polyaromatic hydrocarbons in oysters. They were characterized as three-ring aromatics by gas chromatography–mass spectrometry and separation by normal-phase high-performance liquid chromatography on LiChrosorb-NH_2. Di- and trimethyldibenzothiophenes appeared to be the major persistent hydrocarbons in the environment.

INTRODUCTION

During the last 12 years the northern Brittany coasts in France have been regularly polluted by oil spills following the sinking of tankers. The total amount of crude oil spilled at sea was particularly high when the supertanker Amoco Cadiz sank near the coast on March 16th, 1978. The effects of the spill were devastating in a region heavily dependent on the quality of its coastline and inshore waters for the maintenance of an active marine economy. This ecological disaster set in motion a number of scientific activities reported elsewhere[1,2]. This paper is concerned with the use of glass capillary gas chromatography (GC) or high-performance gas chromatography (HPGC) in the characterization of hydrocarbons in the environment. The modification of the composition of oil spilled at sea by the weathering process and the low concentrations of hydrocarbons in marine biota require the use of powerful analytical techniques for monitoring studies.

In this study, detailed GC patterns of the saturated and aromatic hydrocarbons fractions obtained from oil on beaches near the wreck allowed the effect of the

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weathering process to be evaluated. Owing to the complexity of these mixtures, the influence of the selectivity of the liquid stationary phase in GC was studied.

Simultaneously, levels of aliphatic and aromatic hydrocarbons in oyster samples were measured by HPGC using perdeuterated hydrocarbons as internal standards. The characterization of non-biogenic hydrocarbons was effected by GC–mass spectrometry (GC–MS) and HPGC coupled with a flame photometric detector. The combination of separations by normal-phase high-performance liquid chromatography (HPLC) on LiChrosorb-NH_2 and by HPGC provided a powerful technique for the identification of residual polynuclear aromatic hydrocarbons (PAHs) in the environment.

EXPERIMENTAL

Materials

All organic solvents were of "pesticide residue analysis" quality from Merck (Darmstadt, G.F.R.) or SDS (Peypin, France). Silica gel 60 (70–230 mesh) was obtained from Merck. Aliphatic and aromatic hydrocarbon standards were purchased from Alltech (Arlington Heights, IL, U.S.A.) or Fluka (Buchs, Switzerland). Florisil (100–200 mesh) was obtained from Touzart et Matignon (Paris, France) and was purified by heating overnight at 600°C.

Gas chromatography

GC was performed on a Carlo Erba (Milan, Italy) Model 2150 instrument equipped with a flame-ionization detector (FID). An inlet port with built-in septum flushing, manufacturated by Carlo Erba according to Grob and Grob[3] was used only in the splitless mode. Aliquots of 2 μl of *n*-hexane were injected without stream splitting into the column at 40°C. The injection temperature was 250°C. After 40 sec, the splitting valve was opened, allowing the septum and injection port to be purged. Subsequent to the elution of the solvent the oven temperature was increased rapidly to 80 or 120°C and then at 4°C/min to 280°C. Hydrogen was used as the carrier gas with the flow-rate adjusted according to the Grob test[4]. Some samples were analysed using a flame photometric detector (FPD) equipped with a sulphur-selective filter (Carlo Erba, Model SSD 250).

Glass capillary columns (50 m × 0.3 mm I.D.) were made according to the procedure of Grob and Grob[5]. The most suitable for quantitative and qualitative work were those prepared by the barium carbonate process followed by Carbowax M and Triton X-305 deactivation at 280°C.

Gas chromatography–mass spectrometry

Mass spectral analyses were obtained with a Nermag R-10-10 B mass spectrometer (Rueil Malmaison, France). The instrument was operated in the continuous scanning mode. Data were acquired and processed on a Nermag-Sidar data system.

High-performance liquid chromatography

HPLC separations were performed on a 25 cm × 4 mm I.D. LiChrosorb-NH_2 (5 μm) column. The mobile phase was delivered by a Constametric II pumping system (LDC, Riviera Beach, FL, U.S.A.) in the constant-flow mode. The column effluent

was monitored with a Spectromonitor II UV absorption detector (LDC) at 254 nm. The injection system was a Valco valve with a 25-μl sample loop.

Preparation of oil samples

A 0.5-g oil sample containing 1 g of sodium sulphate was extracted by refluxing for 6 h with 200 ml of *n*-hexane in a Soxhlet apparatus followed by further refluxing for 6 h with 200 ml of benzene. The extracts were combined and reduced in volume on a rotary evaporator at 35°C. One eighth of the dry residue was transferred to a silica gel column. The silica gel had previously been activated overnight at 275°C and the bed volume of silica gel in the chromatographic column (300 mm × 10 mm I.D.) was 15 ml. Aliphatic compounds were eluted with 25 ml of *n*-hexane, then aromatics were eluted with 25 ml of benzene whereas polar compounds were eluted with 25 ml of methanol. The fractionated eluates were reduced in volume and finally injected into the gas chromatograph. Five oil samples spilled from the Amoco Cadiz were collected from beaches at Portsall on different dates between March 17th, 1978 (the day of the wrecking) and January 31st, 1979 (Fig. 1).

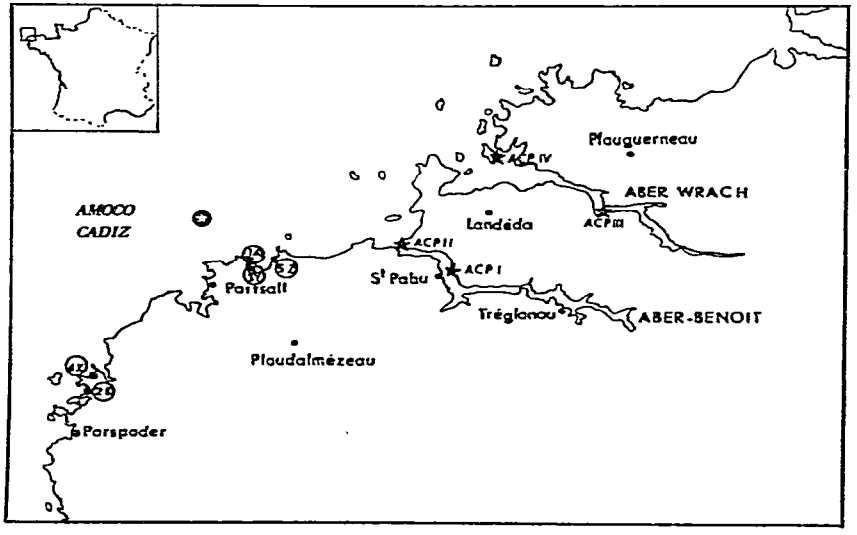

Fig. 1. Collection locations for oil and oysters samples. Oil was collected on March 17th (1A), April 8th (2B), April 14th (3Y) and September 25th, 1978 (4X) and on January 31th, 1979 (5Z). Oysters were collected serially at four sites (ACP I and ACP IV for flat oysters, ACP II and ACP III for Japanese oysters).

Preparation of oyster tissue samples

Flat oysters (*Ostrea edulis*) and Japanese oysters (*Crassostrea gigas*) were collected serially at four commercial sites in l'Aber-Wrach and Aber Benoit (Fig. 1). Approximately 15 g (dry weight) of oyster tissue were extracted with three 100-ml aliquots of acetone–*n*-pentane (1:4) in a separating funnel. The combined extracts were evaporated using a Buchi Rotavapor. In this study two types of internal standards were used. In the first part of work, two radioactive compounds were used:

n-[10(16)-^{14}C]pentacosane (Radiochemical Centre, Amersham, Great Britain), specific activity 305.2 MBq/mmole, and [12-^{14}C]benz[*a*]anthracene (Radiochemical Centre), specific activity 1.813 MBq/mmole. They were both added at the 50-Bq level in order to establish the losses during all the purifications steps. Subsequently, when the work was in progress, perdeuterated hydrocarbons from Prochem (London, Great Britain) were used: *n*-[$^{2}H_{50}$]tetracosane and [$^{2}H_{10}$]pyrene. They were both added to each sample prior to extraction at the 5-μg level in *n*-hexane solution.

The saturated and aromatic hydrocarbons extracted from biota were purified on a 21 × 1.1 cm I.D. glass column packed with 10 g of Florisil activated overnight at 150°C. Before use, the Florisil was deactivated with 5% (v/w) of water. Hydrocarbons were eluted with 60 ml of *n*-pentane.

The hydrocarbons were then fractionated into aliphatics and aromatics on a 21 × 1.1 cm I.D. glass column packed with 15 ml of silica gel activated overnight at 150°C. A few grams of dry sodium sulphate were added to the top of the column in order to dry the sample. The aliphatic hydrocarbons were eluted with 15 ml of *n*-hexane and 5 ml of dichloromethane–*n*-hexane (1:4) and the aromatics with 25 ml of dichloromethane–*n*-hexane (2:3). Each fraction was reduced in volume with a stream of nitrogen and dissolved in a known volume of *n*-hexane prior to GC analysis.

When perdeuterated standards were not used, internal standards were added prior to injection (pyrene to the aliphatic fraction and *n*-tetracosane to the aromatic fraction).

The total amount of hydrocarbons present in each fraction was integrated. The estimated relative response factor based on the FID response of hydrocarbon standards was used to calculate the concentration of hydrocarbons in each sample by an internal standard method.

RESULTS AND DISCUSSION

The monitoring of the marine environment for pollution by hydrocarbons of petroleum origin is difficult for many reasons. For example, the weathering process alters the distribution pattern of the hydrocarbons and it is therefore necessary to investigate the composition of oil deposited near oyster beds in order to obtain a better understanding of the input and the fate of hydrocarbons in marine biota. Further, the analytical methods used must permit the identification of individual hydrocarbon components in order to be able to monitor the components or group of components that provide evidence of contamination by spilled oil.

Liquid stationary phase selectivity

HPGC seems the most suitable method for the rapid examination of oil composition. The Amoco Cadiz oil was fluid enough to permit direct injection into the gas chromatograph via the splitting system. Fig. 2 shows the GC separation of samples 1A and 2B analysed directly without previous chemical treatment. These chromatograms are complex, of course, as the samples contain many aromatic hydrocarbons in addition to normal, isoprenoid, branched and cyclic alkanes. It is obvious that the oil composition changed drastically during the first 3 weeks as a result of a major volatilization process, as demonstrated by the atmospheric odour and by damages to fields under cultivation[6].

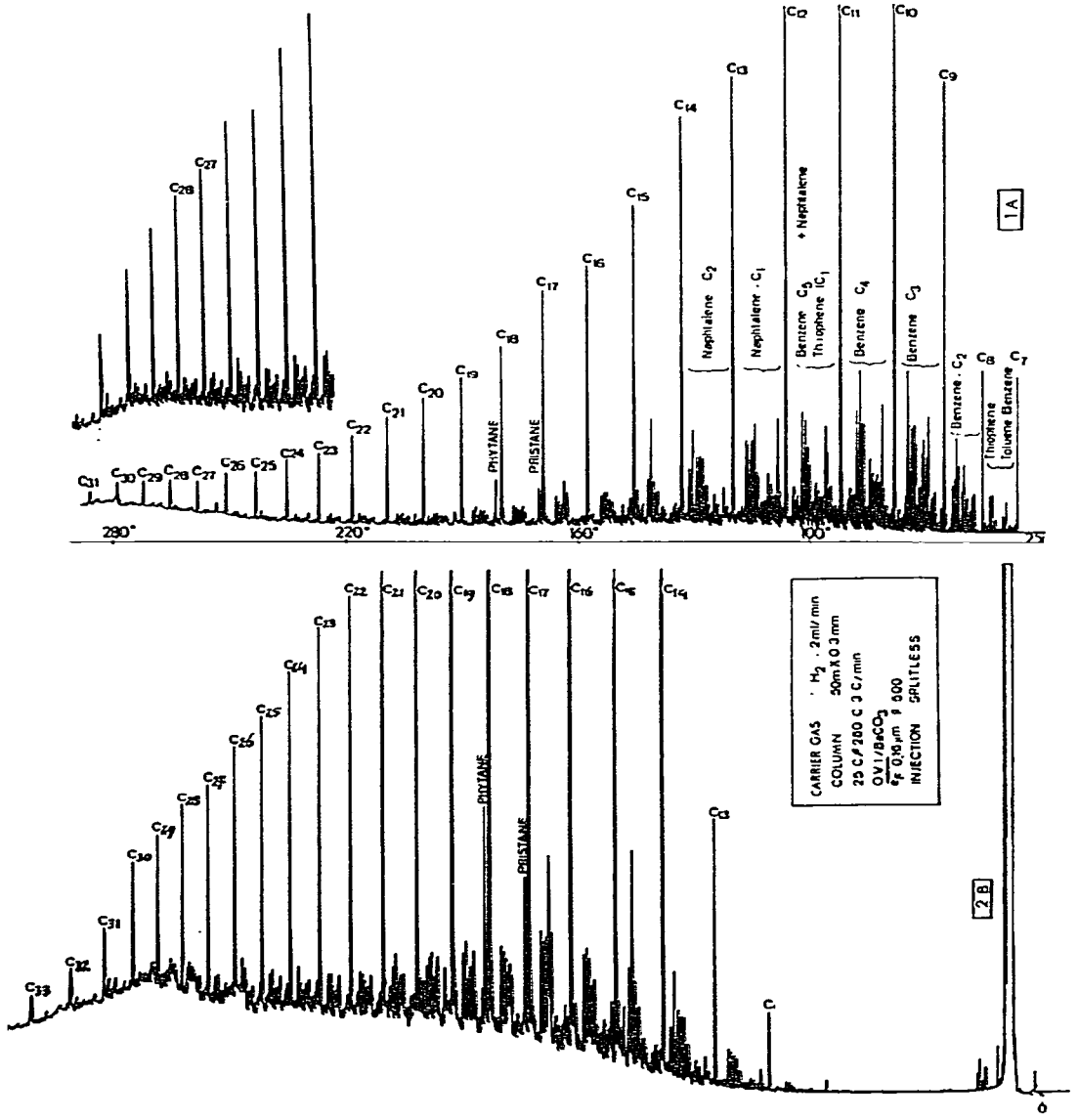

Fig. 2. Gas chromatogram of oil samples 1A and 2B. 1A was injected directly whereas 2B was first dissolved in *n*-hexane. Column, 50 m × 0.3 mm I.D., coated with OV-1; film thickness, 0.16 μm; carrier gas, hydrogen. Numbers on peaks indicate chain length of *n*-alkanes.

The loss of volatile hydrocarbons can be evaluated by inspecting the gas chromatograms of oil samples 1A and 2B. Light iso- and *n*-alkanes lost by evaporation were estimated to about 12% of original oil, whereas light aromatics represented about 20%. Accordingly, although it is difficult to establish with certainty the amount of oil evaporated near the Brittany coasts, an estimate of 60,000–70,000 tons[6] can be made. Of the volatilized compounds, the light aromatics, mainly benzene isomers,

represent about 40,000 tons. Unfortunately, the determination of volatile compounds in the atmosphere was not carried out during the first few days after the accident.

Some features of HPGC are illustrated in Fig. 2. It can be seen that the pristane and phytane peaks show an obvious shoulder. Accordingly, the ratio of the two pairs *n*-heptadecane/pristane and *n*-octadecane/phytane, currently used for the characterization of oils[7], depends on the efficiency and the selectivity of chromatography (Table I).

TABLE I

C_{17}/PRISTANE AND C_{18}/PHYTANE RATIOS ON THREE LIQUID PHASES OF OIL SAMPLES 1A AND 2B WITHOUT (a) OR WITH (b) SEPARATION INTO ALIPHATICS AND AROMATICS

Stationary phase	*Sample 1A*				*Sample 2B*			
	C_{17}/*pristane*		C_{18}/*phytane*		C_{17}/*pristane*		C_{18}/*phytane*	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
OV-1	3.4	5.2	4.7	3.8	4.2	5	3.3	3.6
SE-52	3.2	5.4	4.7	3.9	5	5.3	3.5	3.7
OV-225	3.1	5.5	3.4	4.3	—	—	—	—

GC "fingerprinting" by means of a capillary column coated with an apolar stationary phase is very useful for rapid screening, and can be much refined by using a polar stationary phase such as OV-225. The gas chromatograms showed an important shift of polyaromatic hydrocarbons with respect to *n*-alkanes. For instance, dibenzothiophene (DBT) had retention indices of 1690, 1760 and 2240 on OV-1, SE-52 and OV-225, respectively. Further, pristane and phytane were eluted before *n*-heptadecane and *n*-octadecane, respectively, on OV-225. The shifting of aromatics and branched-chain alkanes could be a useful criterion of identification.

Oil weathering

Oil spilled in the marine environment is subject to a number of physical, chemical and biological processes. These weathering processes can alter the original hydrocarbon composition of spilled oil in a such a way that recognition can be difficult, as shown in Fig. 3. The gross effects of weathering were evaluated by comparing the gas chromatograms of the saturated and aromatic fractions from samples collected within 9 months of the accident. In the aliphatic fraction, rapid loss of the lowest boiling components within 1 month led to stabilization of hydrocarbon distribution for about 6 months. Thus, 9 months after the accident *n*-nonacosane was the dominant *n*-alkane, suggesting that evaporative losses above octadecane were negligible. The pristane/phytane ratio remained relatively stable (Table II), but this did not occur with the *n*-heptadecane/pristane and *n*-octadecane/phytane ratios, which was indicative of the faster microbial degradation of *n*-alkanes. Further, a chromatographic envelope appeared, consisting of homologous and isomeric branched and cyclic hydrocarbons not resolved by GC. By inspecting the gas chromatogram carefully, this unresolved complex mixture appeared to be bimodal with two maxima, one at the C_{18}–C_{19} range and the other at the C_{30} range. The latter maximum probably consisted of polycyclic aliphatics[8].

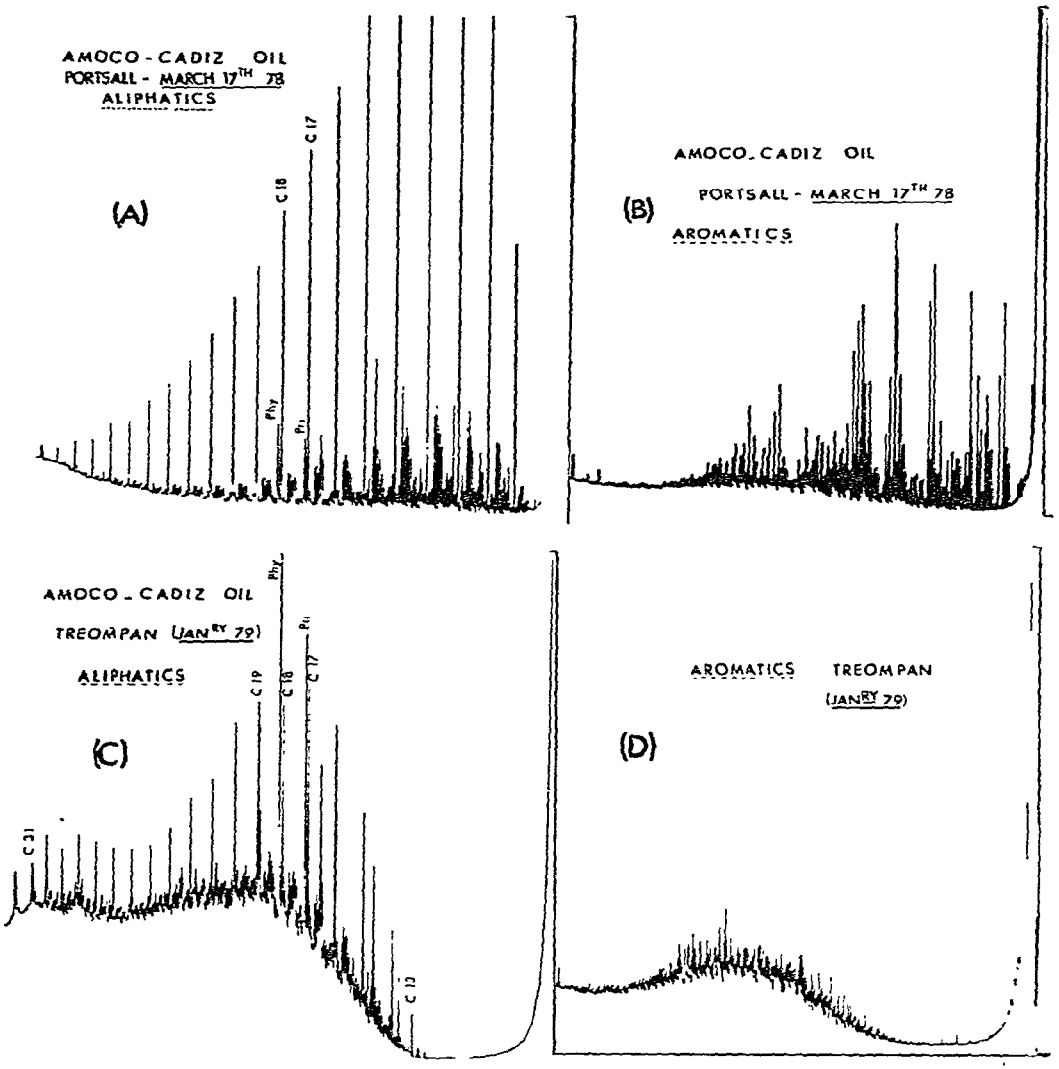

Fig. 3. High-resolution gas chromatogram of saturated and aromatic hydrocarbons of original oil (A and B) and oil weathered for 9 months (C and D). Column, 50 m × 0.3 mm I.D. coated with SE-52.

TABLE II

EFFECTS OF WEATHERING PROCESS ON DIFFERENT RATIOS OF HYDROCARBONS

Hydrocarbon ratio	*Sample*				
	1A	*2B*	*3Y*	*4X*	*5Z*
C_{17}/pristane	5.4	5.3	4.2	1.9	0.5
C_{18}/phytane	3.9	3.7	2.9	1.3	0.8
Phytane/pristane	1.3	1.3	1.3	1.3	1.4

Additional evidence of the considerable environmental modification of spilled oil was clearly displayed in the aromatic fraction. The original oil was high in naphthalene and its alkylated homologues, but these compounds, which were mostly responsible for the acute toxicity of the oil during the first few days after the spillage, weathered rapidly. The weathering process could not be demonstrated to be dependent on the number of alkyl substituents. The PAHs most resistant to physical, chemical and biochemical weathering were the alkylated organosulphur aromatics.

Determination of hydrocarbons in oysters

One area of concern in the development of an analytical method for the determination of pollutants in marine biota was the choice of a primary internal standard, which could be added at the beginning of the sample work-up. In the first step of this study, radioactive compounds were added to the sample, enabling us to correct for losses that might occur during the clean-up procedure. Recovery data for radioactive *n*-pentacosane and benz[*a*]anthracene were $94 \pm 2.9\%$ and $95 \pm 6.4\%$, respectively. Although these standards were not incorporated in the tissue matrix of oysters, they could be estimated to be recovered to the same extent as compounds incorporated into live oysters[9]. When using radioactive standards, secondary standards had to be added prior to GC injection in order to minimize variations during GC analysis and to calculate concentrations from the relative responses of hydrocarbons *versus* internal standard.

The best results, however, were obtained by using analogues labelled with stable isotopes[10]. Such compounds could be added to the sample prior to the extraction. By virtue of their almost identical physical and chemical properties they accompanied the compounds to be measured throughout all the procedure. Using perdeuterated hydrocarbons, no mass spectrometer was required to differentiate between such labelled compounds, indeed they were completely separated by HPGC alone from the natural compounds, as shown in Fig. 4. The retention indices of deuterium-labelled hydrocarbons were lower than those of the unlabelled species by 0.5 index unit per deuterium atom. As the number of deuterium atoms was less for PAHs than for *n*-alkanes, the GC separation of deuterated and natural PAHs was incomplete. For instance, Fig. 4 shows the separation between pyrene-d_{10} and normal pyrene. Hence a glass capillary column with a high efficiency was necessary.

Hydrocarbons in marine biota are usually incorporated into very complex matrices of lipids. Therefore, in trace analysis great care must be taken to separate compounds of interest from the matrix. Interfering apolar biogenic components such as fatty materials were removed by two successive liquid chromatographic steps on Florisil and silica gel. A substantial background could interfere in the identification of saturated and aromatic fractions without a clean-up procedure. This background could consist of glycerol lipids, fatty acids, waxes and carotenoids, as reported by several workers[11–13]. Preliminary results suggested that purification of hydrocarbons from marine biota may be advantageously performed by normal-phase HPLC (see characterization of pollutants).

The use of high-efficiency glass capillary columns provided an excellent approach to the rapid analysis of complex mixtures of petroleum origin. The major advantage of such columns was their superior separation power compared with packed columns. Typical chromatograms of the aliphatic and aromatic fractions of

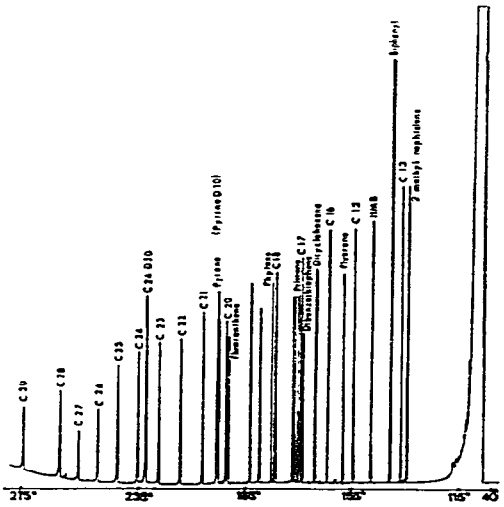

Fig. 4. Gas chromatogram of a standard mixture including perdeuterated hydrocarbons ($C_{24}D_{50}$ and $C_{16}D_{10}$). Column coated with OV-1; film thickness, 0.15 μm. Numbers on peaks indicate chain length of *n*-alkanes.

oysters samples are shown in Fig. 5. They show a large unresolved chromatographic envelope in the C_{18} to C_{30} range for the aliphatic fraction and in the three-ring PAH range for the aromatic fraction. This envelope consisted of complex and overlapping

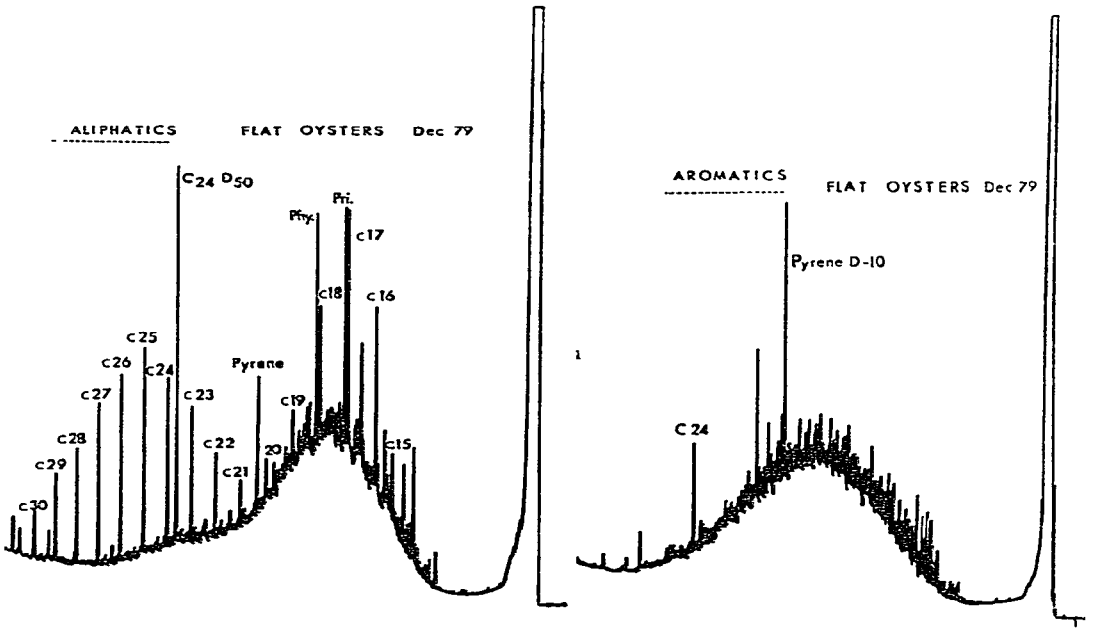

Fig. 5. Glass capillary gas chromatogram of aliphatic and aromatic fraction from contaminated flat oysters collected from l'Aber-Wrach in December 1979. Two internal standards were added to aliphatics: pyrene (secondary) and $C_{24}D_{50}$ (primary). For the aromatic fraction *n*-tetracosane and pyrene-d_{10} were added as internal standards. Column, 50 m × 0.3 mm I.D. coated with OV-1.

series of homologous, isomeric branched and cyclic hydrocarbons which could not be resolved, in spite of the use of a high-performance chromatographic system. With packed columns, the envelope showed a very uninformative profile.

Identification of long-term markers of pollution

Fig. 6 shows a typical chromatogram of the aromatic fraction extracted from oysters collected at l'Aber Wrach 2 years after the oil spillage. On comparing this chromatogram with that of the original oil (Fig. 3C), it appeared clear that organo-sulphur compounds derived from the dibenzothiophene ring were the most persistent components in the environment.

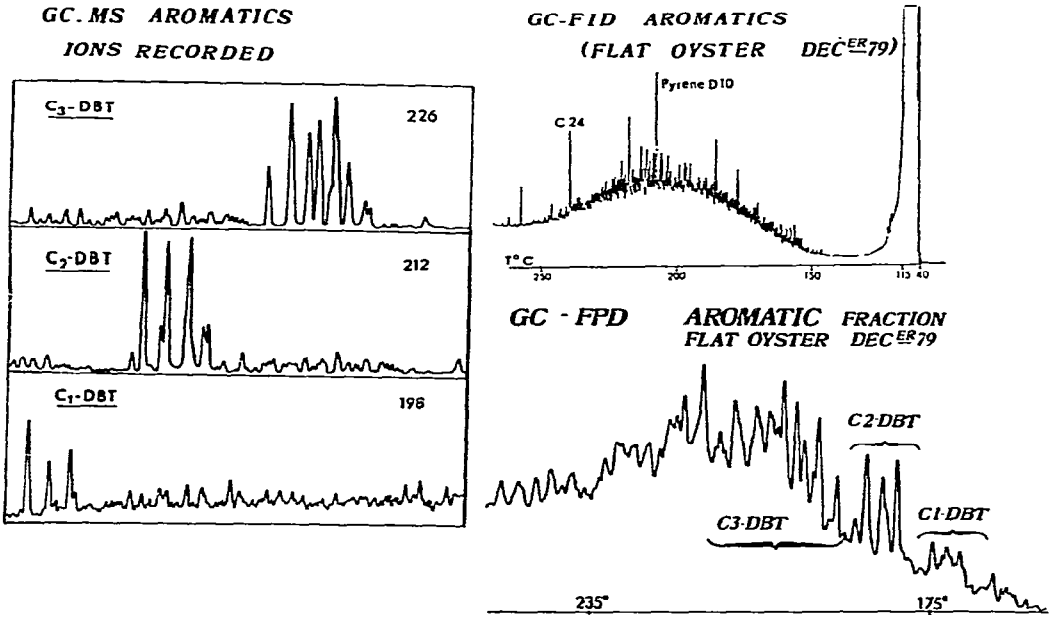

Fig. 6. Gas chromatographic profiles of aromatic fraction from contaminated flat oysters in December 1979. Three modes of detection were used: FID, FPD and MS. Ions displayed were representative of alkyl homologues of dibenzothiophene (DBT).

Chromatographic analysis of the same sample using the FPD (sulphur mode) confirmed the persistence of alkylated dibenzothiophenes. These alkyl homologues consisted principally of C_2- and C_3-DBT (two and three methyl substituents). Further, the mass chromatogram of the aromatic fraction extracted from contaminated flat oysters showed the presence of these sulphur hydrocarbons. The ions displayed were indicative of C_1-(m/e = 198), C_2-(m/e = 212) and C_3-(m/e = 226) dibenzothiophenes. Also, the similarity between the sulphur-specific chromatographic profile and the mass fragmentogram of the oyster aromatic fraction was evident. Identical analyses of weathered oil samples, not shown in these figures, exhibited essentially similar behaviour. However, the parent dibenzothiophene and C_1 alkyl homologues were degraded at a much greater rate than the C_2- and C_3-alkyl homologues. These obser-

vations were consistent with similar studies on the distribution of aromatic hydrocarbons after oil spillage[14–16]. Some results suggested that di- and trimethyl-DBT could represent up to about 50% of the total aromatic fraction.

The three-ring PAHs persistent in the environment were in addition characterized by combining separations first with HPLC then with GC. These two chromatographic separations appeared to be complementary. Normal-phase HPLC with a chemically bonded aminosilane packing material achieved a PAH fractionation on the basis of the number of condensed aromatic rings[17], whereas the separation of the alkyl homologues was obtained by GC. This combination was applied to the analysis of oyster aromatic fraction.

Fig. 7 shows the liquid chromatogram obtained on LiChrosorb-NH_2. Four fractions (f_1–f_4) were collected for additional GC analysis. The largest peak in Fig. 7 (fraction f_2) was in the three-ring PAH region. GC and GC–MS indicated that the compounds in this fraction were primarily C_1-, C_2 and C_3-substited dibenzothiophenes. In addition, fraction f_1 was found to contain almost all of the compounds corresponding to the unresolved complex mixture of the chromatographic envelope. These compounds have not yet been characterized.

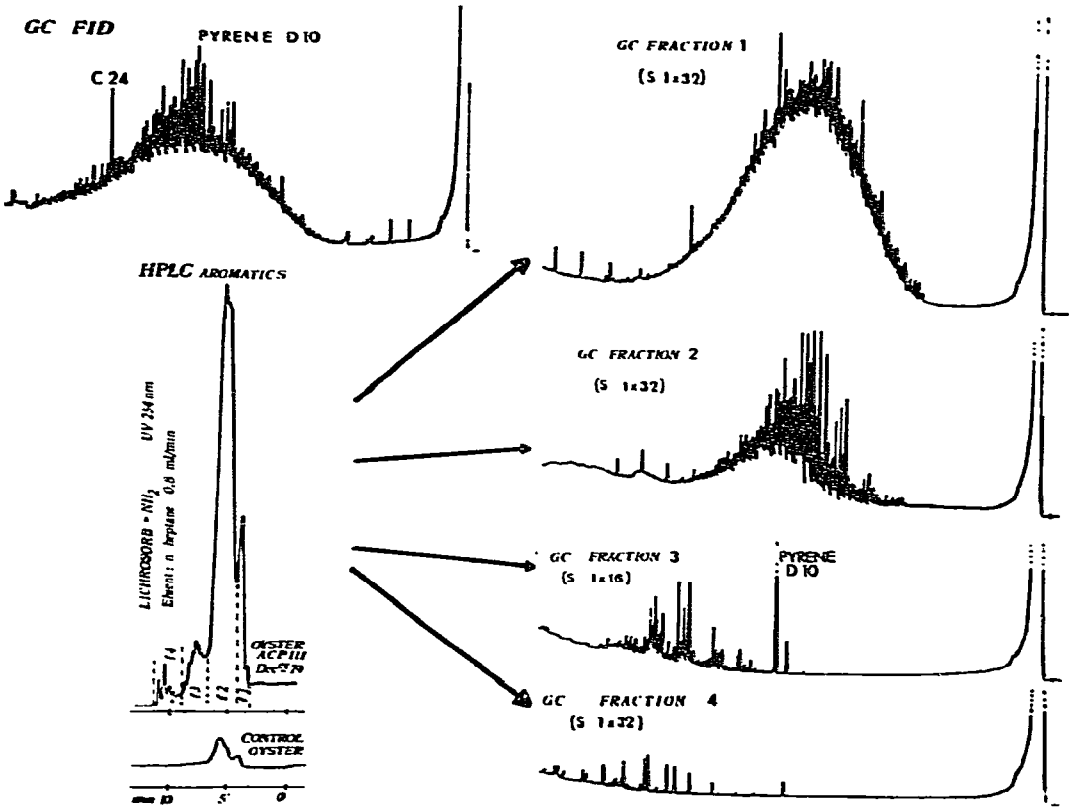

Fig. 7. HPLC of aromatic fraction from contaminated and control oysters. Fractions f_1, f_2, f_3 and f_4 were collected and analysed by GC. HPLC conditions: column, 25 cm × 4 mm I.D., packed with LiChrosorb-NH_2, 5 μm; eluent, *n*-heptane at 0.8 ml/min; 0.64 a.u.f.s. GC conditions as in Fig. 4.

HPLC using an aminosilane silica packing can provide a versatile and convenient method for the rapid measurement of PAHs present in marine biota, as shown by the chromatograms of contaminated and control oysters in Fig. 7.

Organosulfur compounds are bioaccumulated in oysters polluted by oil spillage or industrial sewages[15]. Accordingly, such compounds may be useful as markers of oil pollution in marine biota[18].

Accumulation and release of hydrocarbons in oysters

The oyster beds of l'Aber Wrach and l'Aber Benoit (Fig. 1) were heavily polluted during the first few days after the oil spillage. In order to monitor the spontaneous depuration of these sites, the release of total hydrocarbons accumulated by oysters was studied.

Fig. 8 shows the levels of total aliphatic and aromatic hydrocarbons in two types of oysters. The data were derived by adding the amounts of all saturated and aromatic hydrocarbons obtained by GC–FID. As expected, the levels of these hydrocarbons decreased substantially within 2 years. Control flat oysters collected at five unpolluted commercial sites had levels of total aliphatics and aromatics of 6.2 ± 4.3 and 4.6 ± 4 ppm (dry weight).

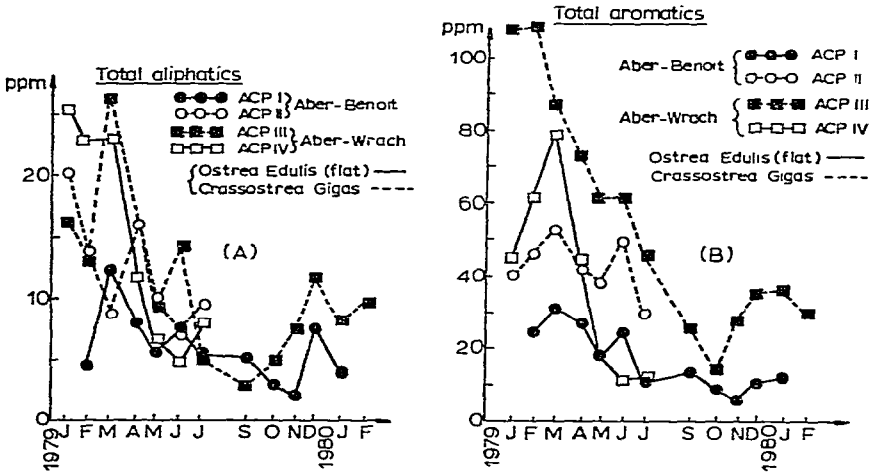

Fig. 8. Evolution of the total saturated (A) and aromatic hydrocarbon (B) concentrations (ppm, dry weight) in cultured flat (solid line) and Japanese (broken line) oysters from l'Aber Benoit (● and ○) and l'Aber-Wrach (■ and □) which were collected between January 1979 and February 1980.

Aliphatic hydrocarbons were encountered at nearly normal concentrations and distributions in both flat (6.5 ± 1.6 ppm) and Japanese oysters (8.8 ± 1.4 ppm) during the winter of 1979. In contrast, the release of PAHs by flat and Japanese oysters was noticeably different. Whereas the concentration of PAHs in flat oysters decreased to 14 ± 3 ppm within 2 years of depuration (PAH maxima were 100 ppm), Japanese oysters showed a small decrease in PAH concentration from 100 to 39.7 ± 10.2 ppm. Comparing the same two sampling intervals, the aromatic hydrocarbon levels in Japanese oysters decreased by a factor of 2.5 and those in flat oysters by a factor of 8.

This study confirms that oysters could be used as a test organism for monitoring long-term pollution because of their capacity for bioconcentration of pollutants[19] and their low detoxification metabolism[20].

CONCLUSION

The complexity of environmental and biota samples requires the combination of several chromatographic techniques in order to achieve suitable separations. For studying the fate of pollutant oil, HPGC is the most suitable method. For monitoring the weathering of oil spilled at sea, additional information can be obtained by using polar stationary phases in addition to the usual non-polar phases.

The determination of trace levels of hydrocarbons in oyster samples requires their extraction from a lipid matrix and an efficient clean-up procedure. The accuracy of quantitation is greatly improved by using perdeuterated hydrocarbons as internal standards and GC on a glass capillary column.

In the monitoring of petroleum pollution in marine culture regions, particular emphasis has to be placed on the fate of polyaromatic derivatives, which are health hazards and can be present in marine food.

Trace amounts of organosulphur compounds in oyster samples were characterized by GC with flame photometric detection and by mass spectrometry. In addition, normal-phase HPLC provided a versatile and convenient method for the isolation of hydrocarbon classes prior to analysis by other techniques such as GC or MS. This study suggests that the dibenzothiophene derivatives can be recommended as long-term markers for pollution in marine biota because they are persistent in the environment.

Finally, for the assessment of the biological and ecological effects of an oil spillage, only GC or/and GC–MS can provide reliable data because accurate quantitation of individual compounds is needed for this purpose.

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