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GAS CHROMATOGRAPHIC SEPARATION OF DIASTEREOMERIC ISOPRENOIDS AS MOLECULAR MARKERS OF OIL POLLUTION

F. BERTHOU* and M. P. FRIOCOURT
Laboratoire de Chromatographie, Service de Biochimie ERA CNRS No. 784, Faculté de Médecine, B.P. 815, 29279 Brest Cédex (France)

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SUMMARY

By means of high-performance glass capillary gas chromatography (GC), diastereomeric isoprenoids were resolved into double peaks. The retention indices on three liquid phases and the mass spectra of the diastereoisomers were almost similar. The leading GC peaks represent the isoprenoids of fossil origin, while the rear peaks correspond to those of recent origin. Computerized gas chromatography–mass spectrometery was used for fingerprinting isoprenoids in different samples. The mass fragmentation patterns were characteristic of the branched alkanes.

Hydrocarbon mixtures from four crude oil spills in the sea and from polluted and oil-free oyster tissues were investigated. The relative ratios of *n*-alkanes/pristane or phytane were shown to be strongly dependent on the chromatographic resolution of the isoprenoid peaks. It is suggested that the double GC peaks in the isoprenoid series are an unmistakable sign of oil pollution.

INTRODUCTION

High-performance glass capillary gas chromatography (GC) is recognized as the most powerful tool for determining individual hydrocarbons (HCs), especially when coupled to mass spectrometry (MS). However, it is of primary importance that glass capillary GC is carried out by use of high-efficiency chromatographic system. The purpose of this paper is to show the need of high efficiency glass capillary GC for differentiating fossil HCs from biogenic HCs in the isoprenoid series.

EXPERIMENTAL

Oil samples

Four oil samples were analysed. Three of them were collected in the marine environment polluted by the grounding of oil tankers on the Brittany coast: Gino in 1979, 40,000 tons of carbon-black; Amoco-Cadiz in March 1978, 230,000 tons of crude oil; Tanio in March 1980, 28,000 tons of No. 2 fuel. The fourth sample was obtained from the Ekofisk oil field in the North Sea by courtesy of Dr. J. E. Portmann[1]. The oil samples were purified and fractionated into aliphatics and aromatics as previously described[2].

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Oyster samples

Flat oysters (*Ostrea Edulis*) were collected at four different commercial sites of the Brittany coast. They were collected on June 1978 from the Aber-Benoit estuary (site A) which was heavily polluted by oil spilled from the Amoco-Cadiz. The three others sites were recognized as oil-free: Binic (site B) near Saint-Brieuc and the Crach river (site C) in the Morbihan department on the southern Brittany coast, and Lézardrieux (site D) on the northern coast.

Approximately 10 g (dry weight) of oyster tissues were analysed. The preliminary clean-up steps consisted of Florisil and silica gel chromatographies as previously described[2]. The aliphatic fraction after concentration was redissolved in 0.1 ml hexane. A 2-μl aliquot of the final solution was injected into the gas chromatograph.

Gas chromatography

GC was carried out on a Carlo Erba (Milan, Italy) Model 2150 AC instrument equipped with a flame ionization detector (FID). Injection was made via a split–splitless injector heated at 275°C while the column was maintained at 40°C. After elution of the solvent the oven temperature was rapidly raised to 60°C and then at 0.8°C/min or 0.5°C/min to 280°C. Hydrogen was used as carrier gas with the flow-rate adjusted according to the Grob test[3].

Glass capillary columns

Glass capillary columns (50 or 25 m × 0.3 mm I.D.) were laboratory made according to the Grob procedures. One column, 50 m × 0.3 mm, was prepared by the barium carbonate procedure followed by Carbowax 20M and Triton X-305 deactivations at 280°C[4]. It was coated by the static procedure with a liquid OV-1 film thickness of 0.15 μm. The other columns were persilylated at high temperature according to the Grob procedure[5] and coated with a SE-52 or OV-73 liquid film thickness of 0.12 μm. The straightened column ends were deactivated by rinsing with the silylating mixture and then expelling the liquid plug with a microflame at moderate temperature[6].

The retention indices (I) of the acyclic isoprenoid alkanes were derived from linear interpolation between the *n*-alkanes in temperature programmed runs.

Gas chromatography–mass spectrometry

MS analysis were carried out with a Nermag R-10-10 B mass spectrometer (Rueil-Malmaison, France). Samples were introduced via a Carlo Erba Model 2900 gas chromatograph equipped with a 50 m × 0.3 mm glass capillary column coated with a SE-52 liquid film. This column was directly coupled to the mass spectrometer. The apparatus was operated in the continuous mode. Data were acquired and processed on a Nermag-Sidar data system.

RESULTS

Chromatographic separation of diasteromeric isoprenoids

The aliphatic and alicyclic fractions of four oil samples were analysed by glass capillary GC on OV-1 liquid phase. The analytical conditions were such that the prominent discrete peaks between *n*-alkanes, especially pristane and phytane, were

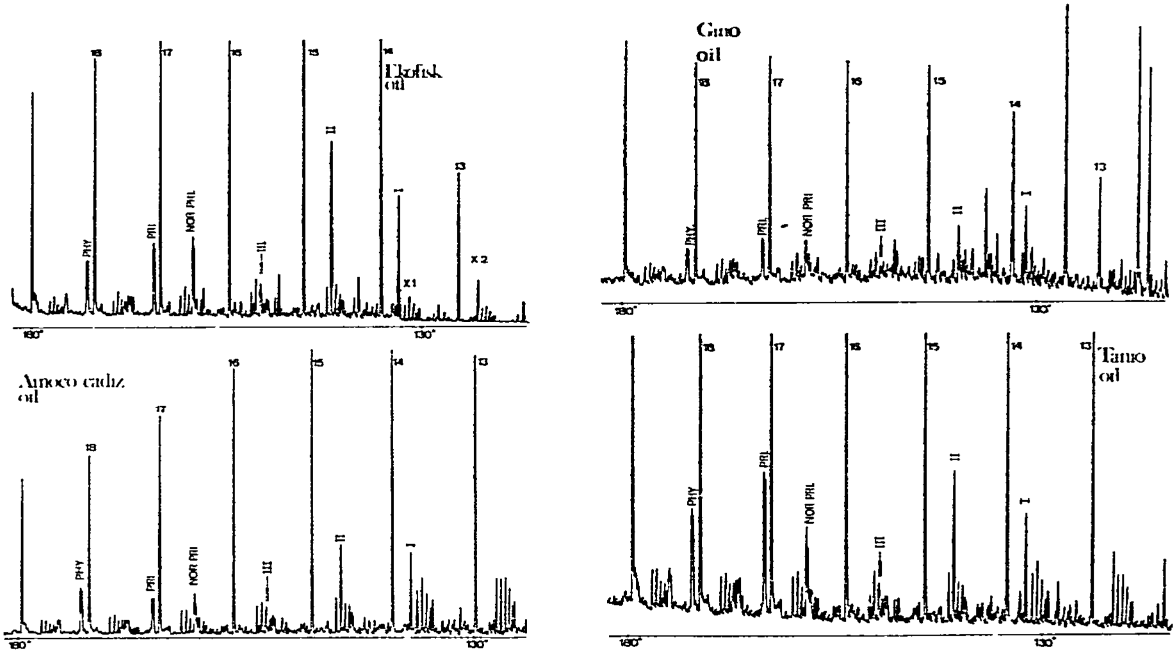


Fig. 1. Sections of gas chromatograms of the aliphatic hydrocarbon fractions of oils spilled in the sea from Gino, Tanio, Amoco-Cadiz and Ekofisk. Arabic numerals indicate the carbon numbers of the *n*-alkanes. Roman numerals refer to the isoprenoids according to Fig. 2. PHY = Phytane; PRI = pristane; NOR-PRI = nor-pristane. Conditions: column 50 m × 0.3 mm I.D., 0.15-μm OV-1; carrier gas hydrogen at 0.8 bar; splitless injection; temperature programme 0.8°C/min.

resolved into doublets. Fig. 1 shows the gas chromatograms of Gino, Tanio, Ekofisk and Amoco-Cadiz oils. They were all characterized by doublets between dodecane and nonadecane. While four double peaks were particularly prominent, two other isoprenoids (I and II) could not be further resolved on OV-1.

These characteristic doublets are due to the presence of diastereoisomers. Fig. 2 summarizes the structures of isoprenoids, the number of asymmetric carbons and the number of possible diastereoisomers. All the compounds of this series have two diastereoisomeric configurations, except for phytane which has four possible diastereoisomers. Of course, liquid phases such as OV-1, OV-73 or SE-52 can resolve

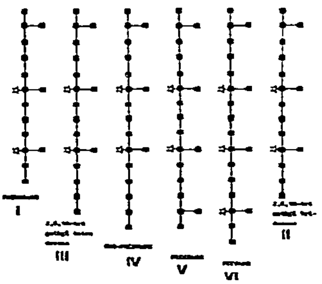


Fig. 2. Structure of isoprenoids. The symbol ☆ denotes an asymmetric carbon atom.

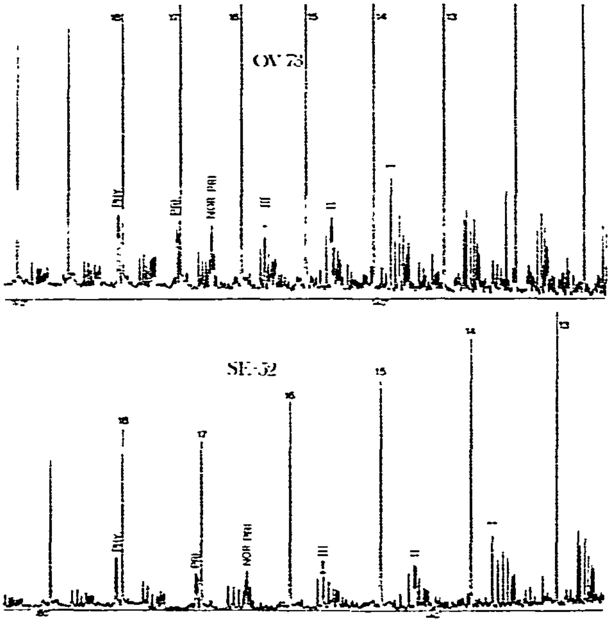


Fig. 3. Gas chromatogram of aliphatic hydrocarbon fraction of Amoco-Cadiz oil on two liquid phases. Conditions: OV-73, 25 m × 0.3 mm I.D., film thickness 0.12 μm, temperature programme 0.5°C/min; SE-52, 50 m × 0.3 mm I.D., film thickness 0.12 μm, temperature programme 0.6°C/min.

only diastereoisomeric molecules and not enantiomers. Accordingly, all isoprenoids of Fig. 2 are capable of resolution in doublets, perhaps in quadruplets for phytane.

Fig. 1 shows that hydrocarbons III, IV, V and VI were effectively resolved into two sub-peaks on OV-1 liquid phase. The second isoprenoid 2,6,10-trimethyltridecane (II) which was not resolved on OV-1 was quite well resolved on OV-73 or SE-52, both methylsilicones with 5.5% and 5% of phenyl groups respectively as shown in Fig. 3. Only the first isoprenoid, farnesane (I), was not resolved while it is capable of resolution into two diasteroisomers.

The resolution of the doublets improved with increasing retention times, *i.e.*, with molecular weight. Table I reports the *I* data of isoprenoid alkanes. The *I* differences were between 1.9 and 1.4. They were insufficient to allow a full resolution of sub-peaks. Table I shows the separation number required in these parts of chromatograms for an adequate separation of doublets. The effective number of theoretical plates, N, required for such separations calculated from[7]

$$TZ = 0.78\sqrt{N} - 1$$

must be between 390,000 and 600,000, *i.e.*, values attainable with great difficulty in routine analysis.

The *I* values of authentic samples of pristane and phytane (Alltech, Arlington Heights, IL, U.S.A.) were 1710.2 ± 0.1 and 1812.8 ± 0.3 respectively under the same analytical conditions on OV-1 liquid phase. Pristane from biological sources is composed solely of the 6(*R*),10(*S*) isomer[8], while in petroleum during the maturation process of crude oil this isoprenoid loses its stereospecificity giving two dia-

TABLE I

RETENTION INDEX (I) DATA OF ISOPRENOID ALKANES SEPARATED BY GLASS CAPILLARY GC ON THREE LIQUID PHASES

TZ is the separation number required for separating the diastereoisomers down to the baseline with not more than 1.2% relative overlapping ($R = 1.177$).

Compound	*OV-1*		*OV-73*	*SE-52*
	$I \pm$ *S.D.*	*TZ*	I	I
Phytane	1812.5 ± 0.3	48	1808.3	1808.7
	1810.6 ± 0.3		1806.7	1806.9
Pristane	1710.2 ± 0.1	47	1705.6	1706.6
	1708.4 ± 0.2		1704.3	1705
Nor-pristane	1652.9 ± 0.3	53	1649.3	1650
	1651.5 ± 0.2		1647.8	1648.5
III	1558.2 ± 0.3	61		1564
	1556.8 ± 0.5		1563.6	1562.6
II	1464.2 ± 0.4	—	1462.8	1462.3
			1461.3	1460.8
I	1377.5 ± 0.2	—	1374.7	1374.6

stereoisomers. Accordingly, the leading peak of the pristane doublet probably consists of the two enantiomers *RR* and *SS* of fossil origin while the rear peak consists of the *meso*-form (*RS* = *SR*) of biogenic origin.

The situation with phytane is more complex, due to the possibility of four diastereoisomers. Biogenic phytane is assumed to be derived from phytol [*trans*-3,7(*R*),11(*R*),15-tetramethylhexadec-2-en-1-ol]. Accordingly, recently biosynthesized phytane has the configurations 6(*R*), 10(*S*), 14(*R*) or (*S*); it is formed by the racemic hydrogenation and dehydration of phytol in nature. While the rear peak of the doublet contains these two pairs of enantiomers, the leading peak of phytane probably corresponds to the two other diastereoisomers of fossil origin.

The configurations of the other isoprenoid alkanes could not be established experimentally. However, only pristane, phytane and nor-pristane may be useful in the characterization of oil pollution because the other isoprenoids are probably eliminated very rapidly from the marine environment by an evaporation process[1].

Isoprenoids as markers of oil pollution

The characteristic double peaks for the isoprenoids are suggested to be an unmistakable sign of fossil contamination. Fig. 4 shows a gas chromatogram (A) of the aliphatic fraction of tissues from oysters taken from an area polluted by oil spilled from the Amoco-Cadiz compared with those (B–D) of the aliphatic fraction from oysters sampled from three non-polluted areas. These chromatograms illustrate biogenic, petrogenic and anthropogenic HCs. Among these, n-C$_{16}$, n-C$_{17}$ and C$_{18}$ may have both biogenic and fossil components. The sample in Fig. 4A was collected 3 months after the spill. Three isoprenoid double peaks are obvious, and it is clear that linear alkanes such as n-C$_{17}$ and n-C$_{18}$ were preferentially degraded compared to the isoprenoids. Fig. 4B shows that the area from which the samples were taken was not totally oil-free. Indeed, the unresolved complex hump is obvious; such a profile is

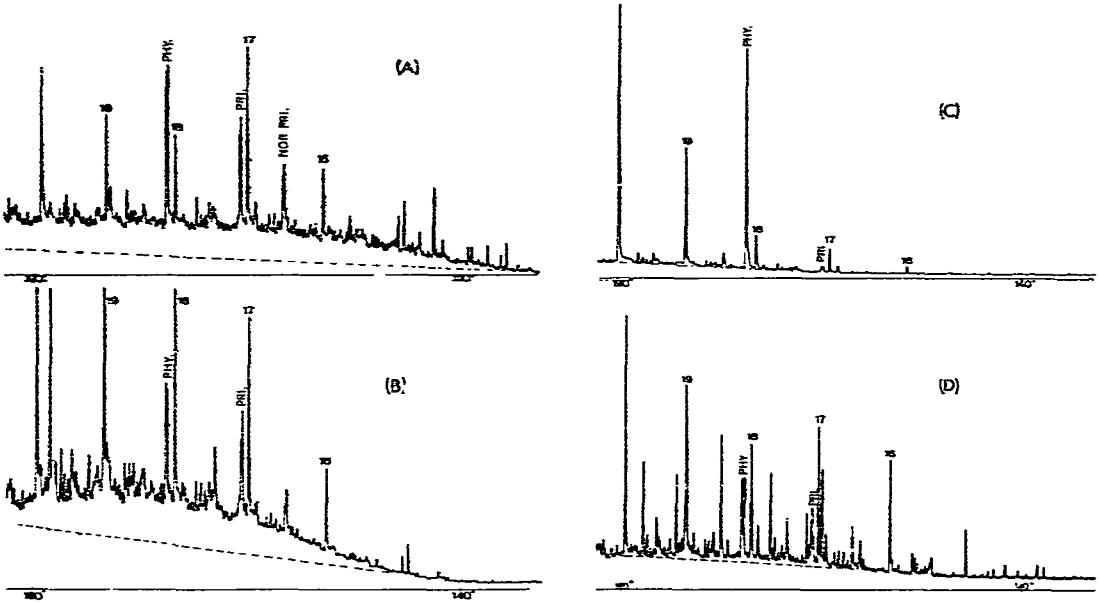


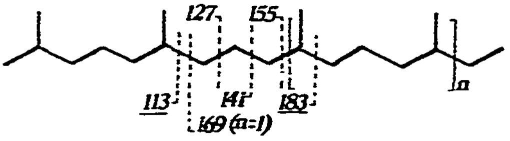
Fig. 4. Sections of gas chromatograms of four aliphatic hydrocarbon fractions extracted from oyster tissues collected from four sites (see Experimental). Conditions as in Fig. 1. The unresolved GC hump is indicated by a dotted line.

characteristic of oil pollution[9,10]. Furthermore, three double peaks of phytane, pristane and nor-pristane occurred.

On the other hand, in Fig. 4C and 4D the unresolved hump is absent. Furthermore, in Fig. 4C the isoprenoid doublets are absent. Only the phytane peak was prominent. This peak (I = 1812.4) was identified due to biogenic phytane. The little shoulder on the phytane peak is probably due to fossil phytane. The situation in Fig. 4D was more complex, due to the interference from other biogenic compounds. The phytane and pristane doublets had a peak height ratio very different from unity, with the biogenic pristane and phytane components predominant. Furthermore, a minor doublet peak of nor-pristane could be detected with I values of 1653.2 and 1651.9. The other peaks, which did not appear to be crude oil alkanes, probably originate from recent biological sources, *e.g.*, alkanes derived from phytol. Therefore, the fossil oil contamination was very low but still discernable. It was ascertained from the doublets of nor-pristane, pristane and phytane.

Fingerprinting by mass fragmentography

Mass fragmentography provides a satisfactory tool for obtaining specific fingerprints for homologous series of compounds resolved by glass capillary GC. Fig. 5 shows an example of a computer reconstructed mass fragmentogram of acyclic isoprenoid hydrocarbons from Amoco-Cadiz oil. Such molecules exhibit prominent ions at $m/z = 113 + 70\,n$, corresponding to the fragmentations induced by the regular side-chain methyl substituents according to the following scheme[11]:



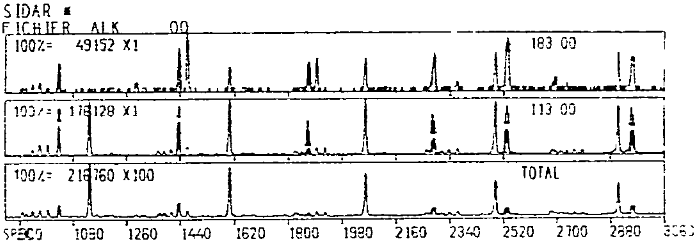


Fig. 5. Computer reconstructed mass-fragmentogram of acylic isoprenoids from an aliphatic fraction of Amoco-Cadiz oil at m/z = 113 and 183. The total ion current is also shown. Isoprenoid peaks are marked by arrows at m/z = 113.

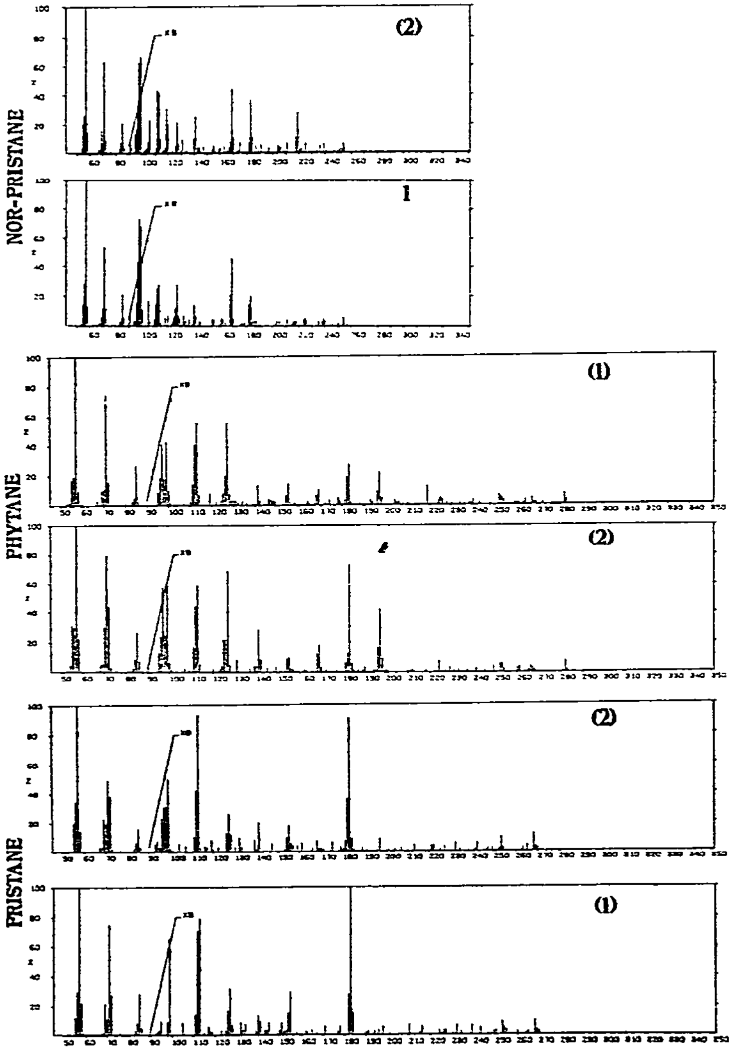


Fig. 6. Mass spectra of the double GC peaks of nor-pristane, pristane and phytane. 1, Leading peak; 2, rear peak of doublet.

The fragmentations at m/z = 113 and 183 allowed us to recognize the double peaks of isoprenoids resulting from a thermally induced racemization during maturation and genesis of petroleum. The double peaks of phytane, pristane, nor-pristane and hydrocarbon III are prominent in Fig. 5.

The chemical identity of the doublets was confirmed by glass capillary GC–MS. Fig. 6 presents the mass spectra of phytane, pristane and nor-pristane diastereoisomers. The spectra of the two pristane diastereoisomers were practically identical with a base peak at m/z = 57, significant fragment ions at m/z = 253 (M – 15), 197 ($M - C_5H_{11}$), 183 ($M - C_6H_{13}$) 155,141, 127,113 ($M - C_{11}H_{23}$) and the molecular ion at m/z = 268. The two phytane diastereoisomers also have similar mass spectra. However, the relative intensity of the fragment at m/z = 183 was higher for the rear peak of the doublet (see Fig. 5). The two nor-pristane diastereoisomers gave a classical fragmentation for branched alkanes. However, a fragment ion at m/z = 169 was characteristic and resulted from the elimination of C_6H_{13}. Any noticeable difference in the relative intensities of ions could be detected. Thus, the doublet peaks probably consist of diastereoisomers.

Finally, the acyclic isoprenoid hydrocarbons could be characterized by mass fragmentography, recording m/z = 113, 169, 183 and molecular ions.

TABLE II

PEAK HEIGHT RATIOS OF HEPTADECANE/PRISTANE, OCTADECANE/PHYTANE AND PRISTANE/PHYTANE

Four spilled oils were analysed under different analytical conditions (see Experimental unless given otherwise).

	C_{17}/*Pristane*	C_{18}/*Phytane*	*Pristane/phytane*
Amoco-Cadiz oil			
OV-1	6.13	3.97	0.79
OV-73	5.46	4.04	0.81
SE-52	4.93	3.37	0.64
SE-52*	3.2	4.7	0.87
OV-1**	2.0	2.35	1
Tanio-oil			
OV-1	2.81	4.80	1.44
OV-1***	2.64	3.76	1.44
OV-73	2.77	5.18	1.59
Ekofisk oil			
OV-1	3.87	4.89	1.37
OV-1***	3.27	4.55	1.80
OV-73	4.31	5.06	1.45
Gino oil			
OV-1	5.45	6.73	1.27
OV-73	5.20	4.42	1.43

* Column: 50 m × 0.3 mm. Temperature programme: initial 40°C, then raised to 110°C at 4°C/min.

** Column: 25 m × 0.3 mm. Temperature programme: 5°C/min.

*** Column: 50 m × 0.3 mm. Temperature programme: initial 40°C then raised to 115°C at 4°C/min.

Heptadecane/pristane and octadecane/phytane ratios

By using high efficiency GC, the pristane and phytane peaks were shown to comprise two sub-peaks corresponding to the diastereoisomers of these molecules. The *n*-heptadecane/pristane and *n*-octadecane/phytane ratios have long been used as indices of degradation of oil spills during the weathering process[12,13], and it is clear that such ratios may be very dependent on the resolution of the isoprenoid peaks. Thus, a recent intercomparison[1] of HC determinations showed that peak height ratios of straight chain to branched isomers varied greatly between laboratories, *e.g.*, C_{17}/pristane varied from 1.73 to 3.01 and *n*-C_{18}/phytane from 2.07 to 8.14 for Ekofisk oil. Such results are not surprising when the peak heights of isoprenoids are known to be dependent on the separation power of the GC system.

Table II summarizes the peak height ratios for four oils spilled in the marine environment. Different chromatographic conditions were used: three liquid phases, two glass capillary lengths and different temperature programmes. The pristane/phytane ratios showed less variation as a function of analytical conditions than C_{17}/pristane or C_{18}/phytane. Indeed, when the diastereoisomers of pristane were resolved this was true also for the isomers of phytane.

DISCUSSION

In order to estimate oil contamination, the analyst must be able to differentiate recently biosynthesized HCs from petroleum fossil HCs. Indeed, the measurement of environmental hydrocarbons is too often carried out as a whole including the inputs from geochemical, biological and human activities. Many criteria have been suggested for distinguishing between fossil and biogenic HCs[9]. Dastillung and Albrecht[14] proposed the use as molecular markers of pentacyclic triterpanes which occur as diastereomeric isomers and can be separated by GC. The members of this series occurred as 1:1 mixtures of diastereoisomers in geochemical sources. Only one of the two possible diastereoisomers was found in recent marine sediments, while fossil fuels contained a second isomer formed during oil maturation. The same concept is applied in this study for the C_{15}–C_{20} isoprenoids.

Biogenic molecules tend to exhibit a very definite configuration via stereospecific reactions, *e.g.* 6(*R*),(10(*S*) for pristane[8], while this stereospecificity is lost during the maturation process in crude oil. Accordingly, the initial biological substances are racemized giving many diastereoisomers[8,14]. Insofar as such diastereoisomers can be resolved by high-efficiency glass capillary GC it will be possible to differentiate biogenic (one GC peak) from fossil (double peaks) isoprenoids.

This study demonstrates the need of high-performance glass capillary GC which permits the resolution of diastereomeric pairs in the isoprenoid series. Although the double peak shape of pristane and phytane is well known[2,15], the implications of this were never reported, until recently[16,17].

Although the hydrocarbons I–VI are useful for characterizing the degree of geological maturity of oils, only pristane, phytane and to a lesser extent nor-pristane may serve for determining the fossil part of an environmental hydrocarbon mixture. Indeed, hydrocarbons I–IV spilled in the marine environment would be eliminated almost immediately by evaporation.

Thus, nor-pristane, pristane and phytane may serve as molecular markers of oil pollution in the environment. These molecules are normal constituents of all crude oils and are present in relatively high concentrations. Furthermore, they are persistent in the environment because of their great stability to bacterial degradation. In addition, such recently biosynthesized isoalkanes are assumed to be derived from phytol, retaining its configuration and thus are present in a non-polluted environment as a single configuration. Their stereospecificity is lost during maturation of oil. Thus, the presence of diastereoisomers in the environment can be used as an unmistakable sign of fossil contamination.

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