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HYDROCARBONS IN BLUE MUSSELS FROM THE KIEL BIGHT

by

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Summary

Blue Mussels (Mytilus edulis) from a location east of the entrance to the Kiel Fjord have been analyzed for recent biogenic and for petroleum derived hydrocarbons. The freeze-dried animals were Soxhlet-extracted with n-pentane. Hydrocarbon fractions were obtained by column chromatography on a bed of silicagel covered by alumina. In addition, this procedure separated the hydrocarbon fractions from the lipids. Both adsorbents were deactivated to prevent the formation of artifacts from sensitive components of the lipid fraction. IR- and UV-spectroscopy were used to monitor the composition of fractions eluted from the column. Preparative TLC on activated silicagel resolved the column effluents into aliphatic, olifinic, mono-, and di- + tri-aromatic hydrocarbons. Individual components and compound types were identified from their gas chromatographic retention indices, UV-spectra, and mass spectra.

The composition of hydrocarbons extracted from the Mussels depended upon the time of sampling. Mussels sampled after the spring phytoplankton bloom contained hydrocarbons presumably derived from phytoplankton over a background of hydrocarbons whose composition is indicative of fossil origin. Mussels sampled in January before the spring phytoplankton bloom contained very little recent biogenic hydrocarbons. The altered composition of extracted hydrocarbons may be explained by the following assumptions:

aliphatic and olefinic hydrocarbons are metabolized within the mussels, but the animals lack the ability to degrade aromatics and/or

the Blue Mussels exchange hydrocarbons with the surrounding water which carries a permanent burden of aromatics, but whose content of aliphatic and olefinic hydrocarbons varies.

Introduction

Hydrocarbons in the marine environment are mainly derived from four different sources: biosynthesis by living organisms in the water, on the sea floor, and in sediments, advection through land run-offs, precipation from the atmosphere, and accidental and intentional release of fossil fuel during production, transportation, und use. Estimations of inputs of fossil hydrocarbons into the sea based on figures of production and transportation of crude oil run as high as 10 million metric tons annually (Blumer, 1970; 1972). These figures were calculated without taking into consideration atmospheric transport and precipitation and may thus well be on the low side. Therefore, the assumption does not seem to be unjustified that sea areas likely to be exposed to hydrocarbon pollution such as coastal waters and busy shipping lanes will carry a more or less permanent burden of fossil hydrocarbons.

In a previous publication (Ehrhardt, M., 1972) one of the authors has described the hydrocarbon content of oysters taken from a permanently polluted water way in the southern United States. Severe oil contamination was evident from the unusually high concentrations of alicyclic and aromatic hydrocarbons in the oysters and from the very large number of individual hydrocarbons characteristic of crude oil and many of its products.

The water of the Kiel Bight receives relatively little obvious pollution by fossil fuels. Occasional minor spills are not deemed a serious problem. On the other hand, the Baltic Sea is regarded as one of the most heavily polluted water bodies though mainly by excessive eutrophication. It was an interesting question, therefore, whether or not local shellfish contained measurable amounts of petroleum hydrocarbons.

Experimental

Blue Mussels (Mytilus edulis) were used as test organisms in this investigation. Selected animals with shells approximately 7 cm long were collected by Scuba divers in April 1973 after and in January 1974 before the spring phytoplankton bloom. The mussels were sampled from a depth of 4 - 5 m (13 - 16 ft.) at a location east of the entrance to the Kiel Fjord. The place is a recreation area devoid of any industry. The animals were returned alive within a few hours after sampling and were deep-frozen immediately after arrival at the laboratory. Work-up began less than two weeks after collection of the mussels. The animals are weighed and freeze-dried without their shells. The method has been compared experimentally with the procedure described earlier (Blumer et al., 1970; Ehrhardt, 1972; Youngblood et al., 1970). Results are the same both in terms of weight and of the chemical nature of hydrocarbons extracted from the mussels.

Freeze-dried mussel tissue is extracted in purified Soxhlet thimbles for 24 hrs with n-pentane and for an additional 12 hrs with n-pentane + 25 % benzene.

Column Chromatography

A column 30 mm wide is filled under n-pentane with approximately 100 g of deactivated silicagel (5 % H_2O) with a bed of deactivated alumina (appro. 3 cm, 6 % H_2O) on top. The column is washed with a least one column volume of n-pentane. The first fraction of hydrocarbons is eluted with appro. 400 ml of n-pentane. The first fraction is ended immediately prior to the appearance of a yellow band of carotinoids in the eluate. The second fraction is eluted with 200 ml of n-pentane. Fraction No. 3 is eluted with 200 ml of n-pentane + 10 % benzene. For a detailed analysis, fractions No. 2^{+3} (column chromatography) have been separated

on an activated silicagel TLC-plate. Increasing percentages of benzene in the n-pentane and finally pure methanol elute polar fractions from the column which contain the saponifiable lipids. IR- and UV-spectra are recorded of each fraction. Each fraction or an aliquot is weighed on the micro-balance.

Thin Layer Chromatography

Solvent: redistilled isooctane. TLC-plate: Kieselgel 60 F 254, Merck, 20 x 20 cm. After an initial purification run the plate is activated at 220°C for 30 minutes. Immediately after cooling the mixture of substances is applied dissolved in n-pentane. The separated substances are visualized under UV-light.

Gas Chromatography

Gas chromatographic separations without subsequent mass spectral analyses were carried out on a Varian Model 2740 gas chromatograph with FID connected with a Varian A 25 strip chart recorder. The column was 2 m x 1/16" i.d. stainless steel packed with 3 % Apiezon L on Chromosorb W-HP, 80-100 mesh. The carrier gas was Helium. GC operating conditions were the following: The injector temperature was 200 - 210 °C. The column temperature was programmed from 80 - 290 °C at 6°/min. Samples are dissolved in redistilled carbon disulfide.

Mass Spectrometry

For GC-MS analyses the same gas chromatograph was operated under identical conditions except for the column packing and the carrier gas flow rate. The column was 2 m x 1/16" i.d.

packed with 2 % OV 101 on Chromosorb W-HP, 80 - 100 mesh. The Helium flow rate was 10.3 ml/min. The mass spectrometer was an Atlas Varian CH-7 with two stage Biemann-Watson separator and differential pump system. Mass spectra were obtained under the following conditions:

electron energy: 70 eV
acceleration potential: 3000 V
beam current: 30 and 100 uA
Source temperature: 250 ^{o}C
Scan: 19 linear
Amplifier: 0.1 - 1 V
Filter 3000 hz
Chart speed: 10 cm/sec

Results

The first batch of Mytilus was collected April 6, 1973. Upon freeze drying the mussel tissue lost 87.02 % of water. The Soxhlet-extract of the dry tissue was column chromatographed. Three fractions were obtained, the latter two of which were recombined to yield fraction No. 2.

Fraction No. 1	3 mg/kg of wet tissue
	23 mg/kg of dry "
Fraction No. 2	11.7 mg/kg of wet tissue
	90.2 mg/kg of dry "

Gas chromatograms of fractions No. 1 and 2 are shown in Figure 1. n-Heptadecane (n-C_{17}), commonly found in many phytoplankton species (Clark and Blumer, 1967) in benthic algae (Youngblood et al., 1971 lox. cit.) and in mineral oil, causes an intense peak in the gas chromatogram of fraction No. 1. It is one of a series of normal aliphatics beginning with n-tridecane and extending as far as n-heneicosane towards the end of the gas chromatogram. Pristane -2,6,10,14-

tetramethylpentadecane - (Pr) is visible closely neighboring the n-heptadecane peak. This branced aliphatic hydrocarbon is a common constituent of mineral oils, but is also found quite frequently in marine organisms (Avigan and Blumer, 1968). On the other hand, phytane, 2,6,10,14-tetramethylhexadecane, which produces a shoulder at the trailing edge of the n-octadecane peak (Ph and n-C_{18}) has not been found so far as a natural component of marine organisms. The minor signals between the peaks of normal aliphatics have been identidied by mass spectrometry as the traces of branched aliphatics. Mass spectrometry also reveals the presence of alkyl-substituted monoaromatics by intense signals at m/e 91,105,119,133,147 etc. (1.11 in Figure 2). In combination, these findings confirm the suspicion that fraction No. 1 (Column chromatography) of the Blue Mussels collected in April 1973 is composed mainly of petroleum hydrocarbons with a contribution of recent biogenic hydrocarbons (e.g. n-heptadecane).

The gas chromatogram of fraction No.2 (lower of Fig.1) appears quite different. A number of very intense signals rise over a moderately elevated background. Mass spectra indicate C_{21}-C_{24} mono, di-, tri-, and tetraenes.

The background of fraction No. 2 is composed of cycloaliphatics, alkylated benzenes, tetralenes, and naphthalenes. A characteristic spectrum taken from the background is shown in Fig.2.

The second batch of Blue Mussels collected in January 1974 at the same location has been analyzed in more detail. Upon freeze-drying the animal tissue lost 91,8 % of water. Column chromatography of the Soxhlet-extract resulted in 7 fractions whose IR-spectra are depicted in Fig.3. From the IR-spectra it is evident that fraction No. 1 and 2 consist entirely of hydrocarbons. Fraction No.3 contains traces of carbonyl compounds causing a small peak at 1740 cm^{-1}. For further analyses fractions No. 2 and 3 were recombined.

Column chromatographic fractions No. 1 - 7

		mg/kg of wet tissue	mg/kg of dry tissue
No. 1	6.16 mg	13.8	170
No. 2	4.16 mg		
No. 3	0.66 mg	10.8 (No.2+3)	133 (No. 2+3)
No. 4	29.06 mg	65.2	803
No. 5	1397.0 mg	3135	38591
No. 6	693.8 mg	1557	19166
No. 7	49.9 mg	112	1278
total	2180.74 mg		

recovery: 83.2 %

TLC-fractions No. 2.1 - 2.4

		mg/kg of wet tissue	mg/kg of dry tissue
No. 2.1	1.662 mg	5.6	68.9
No. 2.2	0.845 mg	2.8	35.1
No. 2.3	0.297 mg	1.0	12.3
Nr. 2.4	0.086 mg		

The gas chromatograms of fractions No. 1, 2.1, 2.2, and 2.3 are shown in Fig. 4. The numbered vertical bars mark the positions of mass spectra. A comparison of fractions No. 1 of the batch of mussels collected in January 1974 (Fig. 4) reveals striking differences. In fraction No. 1 of the mussels collected in January 1974 the peak intensities of normal hydrocarbons are much diminished relative to the background and relative to pristane (Pr) and Phytane (Ph). The peaks of these branced aliphatics have approximately equal intensities relative to the background in both gas chromatograms. The peak of one presumably recent biogenic hydrocarbon (fraction No. 1, Fig. 5) rises above all others. The mass spectrum (2.6 in Fig. 7) indicates a molecular weight of 344 corresponding to the general formula $C_{25}H_{44}$.

A mass spectrum of the background positioned at 5 (2.5 in Fig. 7) shows peaks characteristic of alkanes, cycloalkanes (m/e 83, 97 etc.) monoaromatics (m/e 119, 133, etc.) tetralenes (m/e 145, 159, etc.), (Ehrhardt, M., 1972, loc. cit. and references cited therein).

Some characteristic mass spectra of fractions No. 2.1, 2.2 and 2.3 are shown in Fig. 8. The mass spectrum 2.1.3 is characterized mainly by three fragmentation series: monoaromatics with fragments at m/e 105, 119, 133 etc., tetralenes with fragments at m/e 145, 159, 173 etc., and naphthalenes with fragments at m/e 115, 141, 155. Spectrum No. 2.1.4 is characteristic for phenylcycloparaffins.

Spectrum No. 2.1.6 shows a fragmentation series produced by hydrocarbons of the general formula C_nH_{2n-14}, among them alkylated biphenyls, and of the general formula C_nH_{2n-12} denoting alkylated naphtalenes.

Spectrum No. 2.2.5 has intense peaks at m/e 165, 179, 193 and a corresponding molecular ion peak at m/e 222. This series is in good agreement with the fragmentation pattern of alkylated fluorenes. m/e 206 is the M^+ -peak of a dimethylphenanthrene, and m/e 208 denotes an alkyldihydrophenanthrene. No. 2.3.5 is interpreted as the mass spectrum of, primarily, a dimethylphenthrene (MG 206).

Conclusions

Blue Mussels (Mytilus edulis) from a location in the Kiel Bight, contain fossil hydrocarbons in concentrations somewhat above the natural background of recent biogenic hydrocarbons. The composition of the hydrocarbon fraction is not constant. Mussels collected after the spring phytoplankton bloom contain relatively large amounts of recent biogenic hydrocarbons whose concentrations in mussels collected in January are quite low. On the other hand, concentrations of cycloalkanes, mono, di, and tri-aromatics as well as mixed types of fossil origin have a tendency to rise. The experimental results may be interpreted as follows:

1) The mussels exchange hydrocarbons with the surrounding water which contains a relatively constant if not rising concentration of fossil hydrocarbons in addition to recent biogenic hydrocarbons whose concentrations vary seasonally.

2) The mussels are able to degrade recent biogenic hydrocarbons which they ingest with their food and take up from the water, but are much less efficient in degrading cyclic saturated and aromatic hydrocarbons originating from fossil fuels.

3) Some time near the start of the experiment the mussels were exposed to oil pollution. Subsequently, they exchange saturated and olefinic hydrocarbons much more rapidly with the water than cyclic and aromatic hydrocarbons.

Present data are insufficient do decide on one of the three interpretations given above or a combination thereof. As a working hypotheses it is assumed that hydrocarbons introduced as pollution and excreted by phytoplankton are predominantly injected at or near the sea surface. Subsequently, they are adsorbed onto or dissolved in particulate matter, because the partition coefficient should favour the solution of hydrocarbons in the lipids of detritus. Detritus then carries the adsorbed or dissolved hydrocarbons to the seafloor where they are ingested by filter-feeding organisms.

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References

Avigan, J., and M. Blumer (1968) The Origin of Pristane in marine Organisms.
J. Lipid. Res., 9, 350-352

Blumer, M; G. Souza and J. Sass (1970) Hydrocarbon Pollution of edible Shellfish by an Oil Spill.
Mar. Biol., 5, 195-202

Clark, R.C. and M. Blumer (1967) Distribution of n-paraffins in marine organisms and sediments.
Limnol. Oceanogr. 12, 79-87

Ehrhardt, M. (1972) Petroleum hydrocarbons in Oysters from Galveston Bay.
Environ. Pollut., 3, 257-271

Youngblood, W.W; M. Blumer; R.L. Guillard and F. Fiore (1971) Saturated and unsaturated Hydrocarbons in Marine Benthic Algae.
Mar. Biol., 8, 190-202

Figure-captions

Fig. 1: Gas chromatograms of column chromatographic fractions No. 1 and 2 of Mytilus sampled in April 1973

Fig. 2: Mass spectra of the unresolved background of column chromatograpic fractions No. 1 and 2 of Mytilus sampled in April 1973

Fig. 3: IR spectra of 7 column chromatographic fractions of Mytilus collected in January 1974

Fig. 4: Gas chromatograms of 4 fractions (columns chromatography and TLC) of Mytilus collected in January 1974

Fig. 5: Mass spectra from fraction No. 1 of Mytilus collected in January 1974

Fig. 6: Characteristic mass spectra of TLC-fractions 2.1, 2.2 and 2.3 of Mytilus collected in January 1974

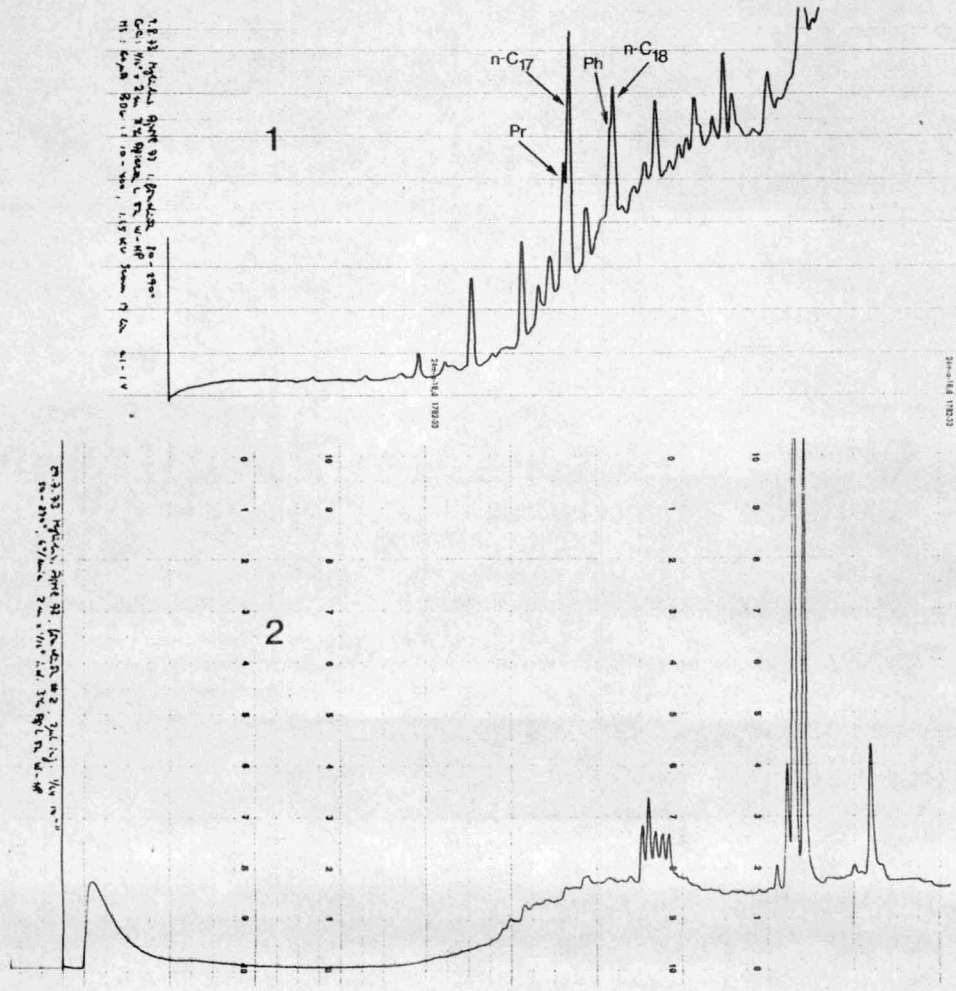

n-C17
Ph
n-C18
Pr
1
2

Fig. 1

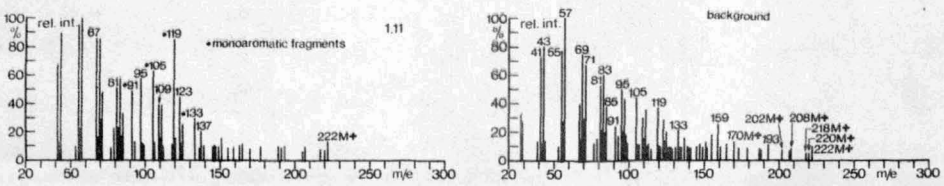

rel. int.
•monoaromatic fragments
1.11
222M+
background
m/e

Fig. 2

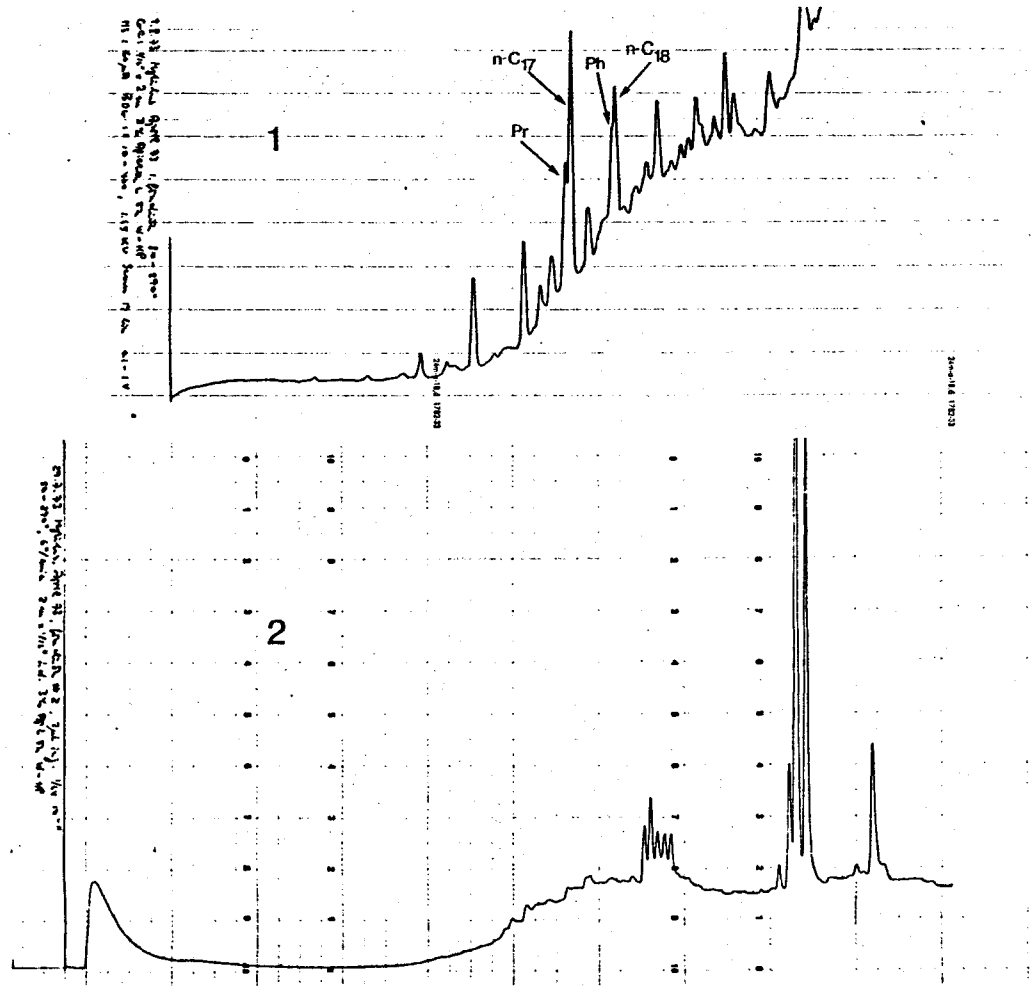

n-C17
Ph
n-C18
Pr
1
2

Fig. 1

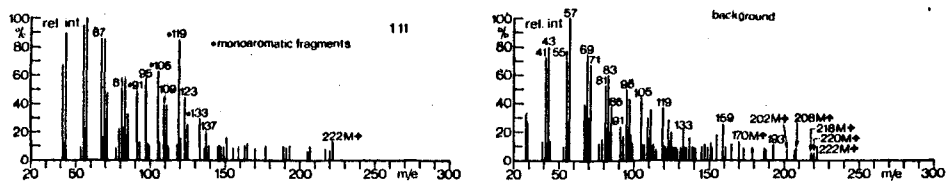
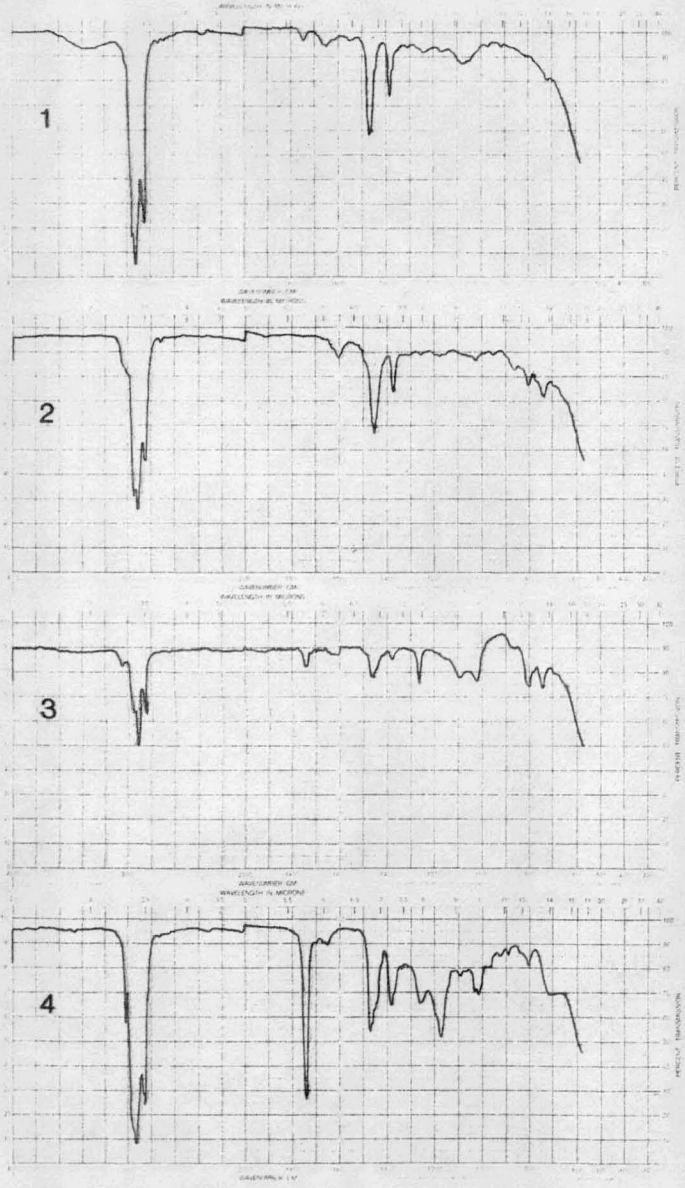

rel. int
monoaromatic fragments
222M+
background
57
43
55
69
71
83
85
105
119
133
159
170M+
202M+
208M+
218M+
220M+
222M+
m/e

Fig. 2



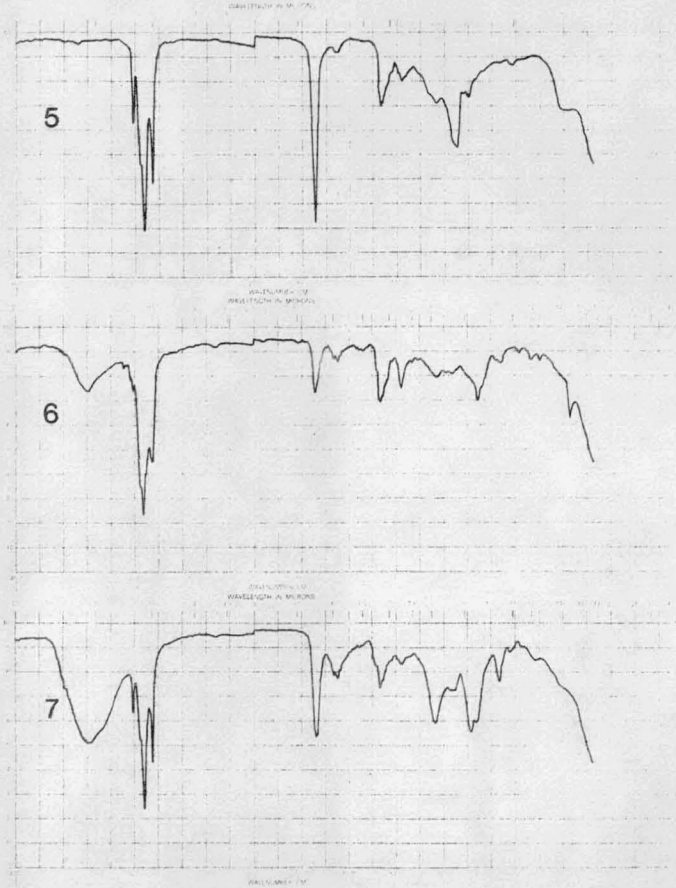


Fig. 3

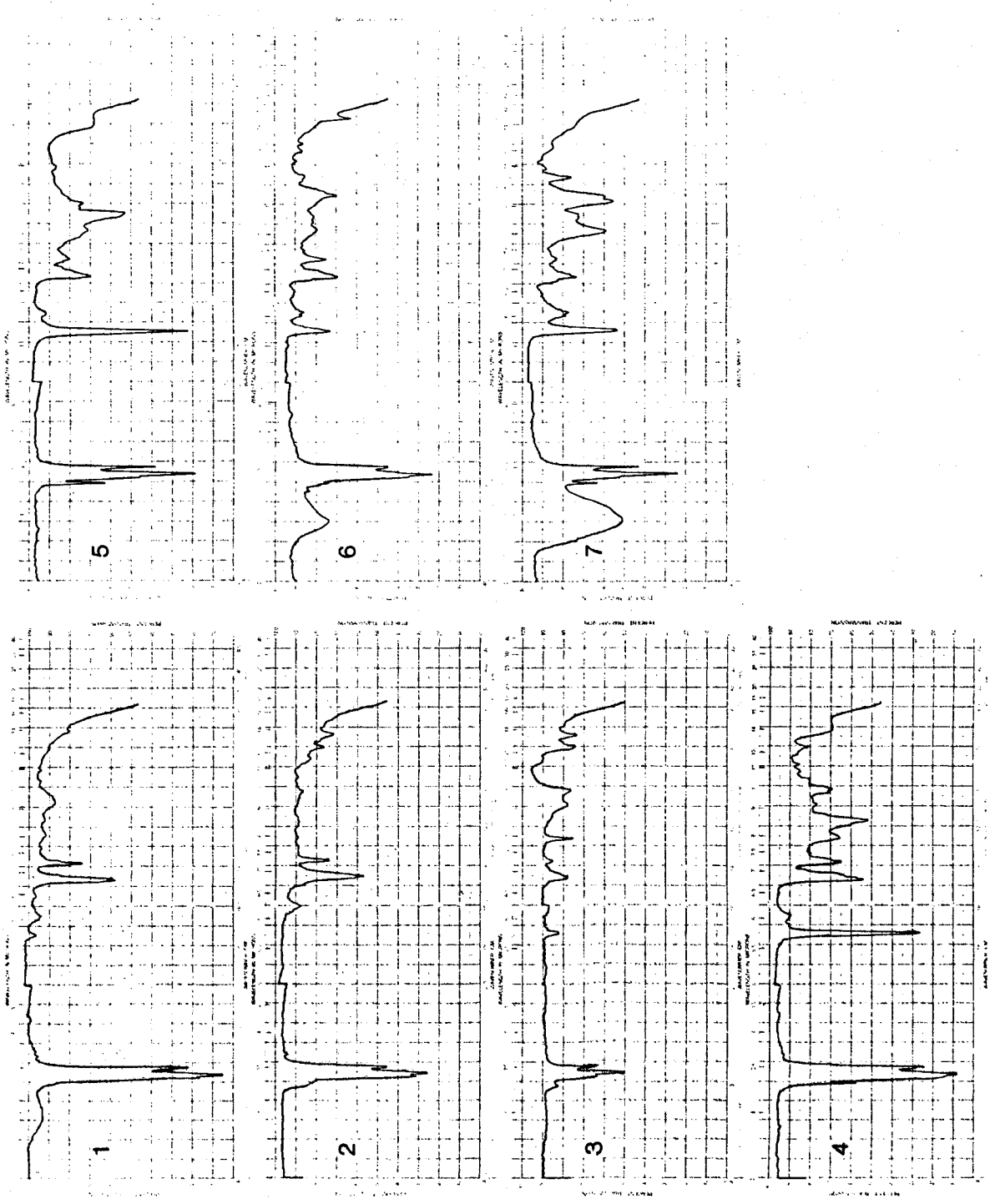


Fig. 3

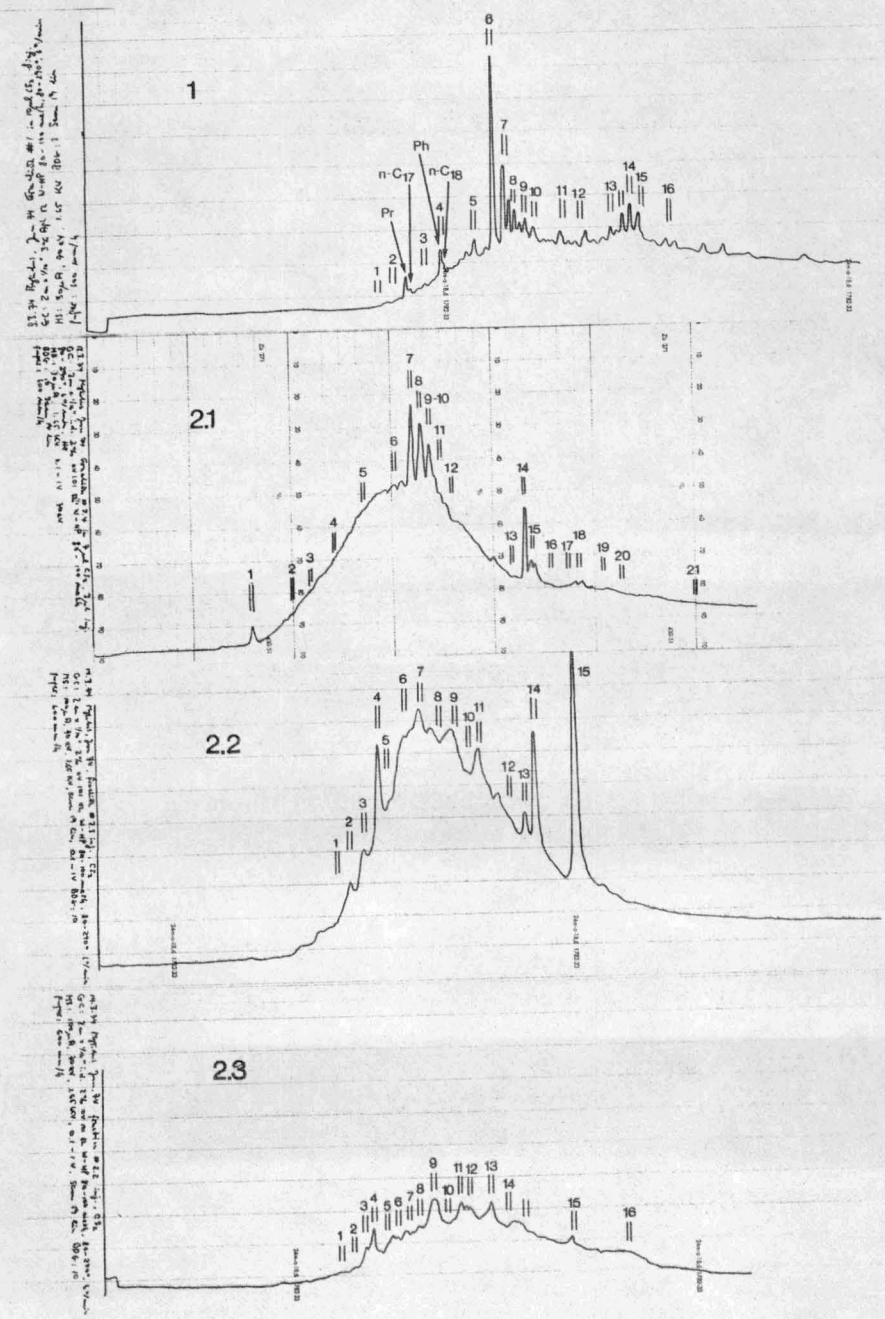


Fig. 4

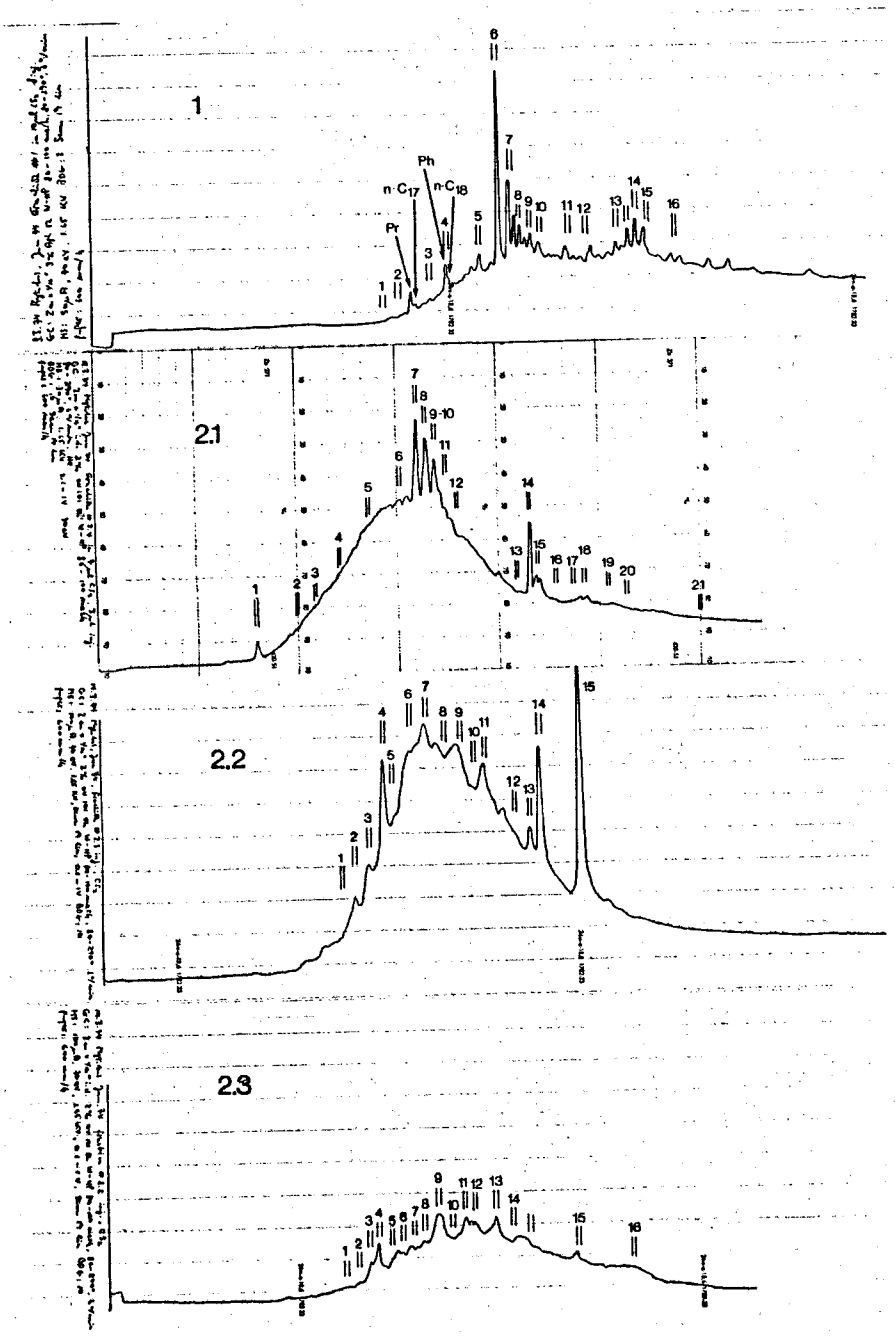
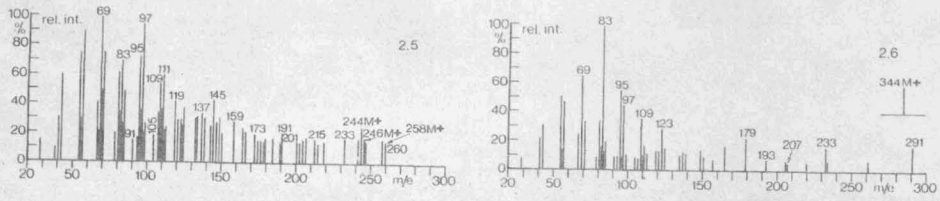


Fig. 4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2.5
69
97
83
95
111
109
119
145
137
159
173
191
201
215
233
244M+
246M+
258M+
260
91
m/e
rel. int.
2.6
83
69
95
97
109
123
179
193
207
233
291
344M+
m/e

Fig. 5

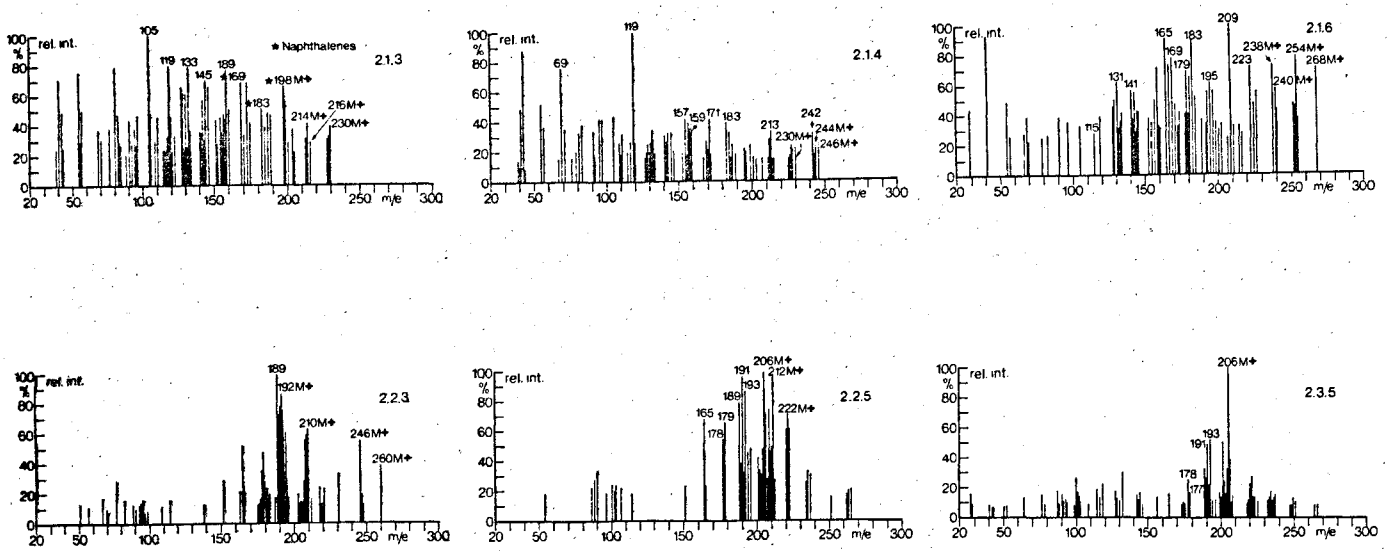
rel. int.
* Naphthalenes
2.1.3
105
119
133
145
169
189
*198M+
183
214M+
216M+
230M+
2.1.4
119
69
157
158
171
183
213
230M+
242
244M+
246M+
2.1.6
209
165
183
169
179
131
141
195
223
238M+
254M+
240M+
268M+
115
2.2.3
189
192M+
210M+
246M+
260M+
2.2.5
206M+
191
212M+
193
189
222M+
165
179
178
2.3.5
206M+
191
193
178
177
m/e
20
50
100
150
200
250
300

Fig. 6