Final Report

An Extension of the Baseline Compositions of Hydrocarbons in Benthic Epifauna of the Outer Continental Shelf of the Eastern Gulf of Mexico

Prepared for: Bureau of Land Management
U.S. Department of the Interior

Submitted under Contract Number 08550-CT5-43

Prepared by: P. A. Meyers
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Introduction

The primary purpose of this investigation is to extend the analyses begun under Bureau of Land Management contract 08550-CT4-11 to additional sampling sites. The earlier contract, issued to the State University System of Florida Institute of Oceanography (SUSIO), involved a comprehensive environmental baseline survey study of the outer continental shelf portion of Mississippi - Alabama - Florida (MAFLA) Lease area. A subcontract to P. A. Meyers of the University of Michigan called for hydrocarbon analyses of benthic macrofauna collected prior to exploratory drilling operations. These analyses were intended to establish indigenous levels of hydrocarbons in the organisms and serve as a baseline to which later analyses could be compared. Sampling operations provided organisms from 32 to 65 areas sampled. Hydrocarbon analyses were performed on 44 samples gathered at 22 of these areas under contract 08550-CT4-11. Since samples representing ten additional areas were available, it was desirous to perform hydrocarbon analyses on these samples, thereby extending baseline data into these locations of the MAFLA area.

An additional purpose of this study is to increase knowledge of the hydrocarbon contents of animals populating relatively unpolluted areas. Most published data compare hydrocarbon compositions of organisms living in areas believed to be polluted by petroleum hydrocarbons to those of similar organisms found in areas assumed free of pollution. Such studies have been reported by Blumer et al. (1970) for the scallop Aequipecten irradians, by Farrington and Quinn (1973) for the clam Mercenaria mercenaria, and by Fossato and Siviero (1974) for the mussel Mytilus galloprovincialis. Burns and Teal (1973) report the hydrocarbon

compositions of the pelagic crab Portunus sayi and the pipefish Syngnathus pelagious collected in the Sargasso Sea. Although this region is not obviously polluted, these organisms appeared to be contaminated by petroleum hydrocarbons.

Few published reports of hydrocarbon analyses of benthos from unpolluted areas are available. The alkane hydrocarbon contents of nine hard corals and one soft coral collected from locations in the Gulf of Mexico are presented by Pasby (1965). Additional analyses of benthic macrofauna are reported by Koons et al. (1965), who list carbon preference indices for the alkane content of eight Poriferans and three Cnidarians. The n-alkane composition of only one organism, the sponge Terpios zeteki, is given by these authors.

Therefore, the present study was initiated. Samples of benthic macro-epifauna were analyzed, and a summary of their hydrocarbon compositions are herein reported.

Samples

The benthic animals analyzed in this study were collected from MAFLA lease areas during June - July 1974 under contract 08550-CT4-11. Collection was performed by SCUBA diving operations and by dredging. The samples have been stored at -20°C in glass containers since collection. A total of 24 animals are included in the present study. They represent 18 of the 65 MAFLA sampling areas. Hydrocarbon compositions of macrofauna have not been reported from ten of these previously. The ten areas include twelve sampling stations: ten dredge/trawl and two dive stations. Therefore, the present report completes baseline hydrocarbon measurements from all MAFLA locations at which organisms had been collected.

The benthic macrofauna samples are listed in Table 1, along with their SUSIO collection numbers and the MAFLA station they represent. Dive stations are indicated by a three-digit number. These stations were within a mile of the corresponding two-digit dredge/trawl stations. Members of five invertebrate and one vertebrate phyla are present. The distribution is two echinoderms, five arthropods, seven cnidarians, five poriferans, four molluscans, and one tunicate.

Each sample has been assigned a six-digit number, using the format established in the report of trace metal and hydrocarbon macrofauna data for contract 08550-CT4-11. The first digit of this number indicates the MAFLA lease area, the second and third digits the sampling area number, the fourth and fifth digits the identification of the animal, and the sixth digit the replicate analysis of that animal. The digits indicating the identifications of the animals analyzed in this study are:

10	Sponge	unidentified
24	Hydrozoan coral	*Millepora alcicornis*
31	Anthozoan coral	*Montastrea annularis*

33	Anthozoan coral	*Madracis decactis*
34	Anthozoan coral	*Porites furcata* or *divaricata*
35	Anthozoan coral	*Dichocoenia stokesii*
36	Anthozoan coral	*Scolymia lacera*
41	bivalve	*Spondylus americanus*
50	bivalve	unidentified
61	rock shrimp	*Sicyione* sp.
62	lobster	Scyllarus
64	crab	Portunid crab
70	crustacean	unidentified
72	starfish	*Astropectin* sp.
80	sand dollar	unidentified
90	tunicate	unidentified

These numbers will be used to refer to these samples in this report.

While most of these samples were selected primarily on the criterion of the MAFLA station they represent, the cnidarians were chosen because they offer the possibility of unique baseline data. These organisms include hard corals of classes Anthozoa and Hydrozoa. Advantages these corals offer with regard to determining baseline conditions are an absolutely sedentary life-style, which permits no doubt as to what area they represent, and ease of identification at the species level. Poriferans such as sponges are also totally sedentary, but species identification is quite difficult. To achieve optimum baseline data for hydrocarbon composition of organisms, it is important to know the correct species identification of each organism and the exact location at which that organism has spent its life.

Table 1

Benthic Macrofauna

Sample Number	SUSIO Collection Number	MAFLA Area	Animal Identification
164621	I - C - 4	64	*Scyllarus* sp.
164801	64 - A	64	sand dollar
163311	63 - A	63	*Montastrea annularis*
161101	61 - A	61	sponge
160721	I - E - 1	60	*Astropectin* sp.
158101	58	58	sponge
254361	54 - C	54	*Scolymia lacera*
252611	II - K - 2	52	*Sicyione* sp.
251241	51 - B	51	*Millepora alcicornis*
251331	II - S - 3	351	*Madracis decactis*
251341	II - S - 4	351	*Porites furcata*
251342	51 - D - 3	51	*Porites divaricata*
251351	II - S - 6	351	*Dichocoenia stokesii*
251411	II - S - 7	351	*Spondylus americanus*
249411	49 - A	49	*Spondylus americanus*
248411	II - Q - 1	48	*Spondylus americanus*
246101	II - C - 1	146	sponge
245901	II - M - 1	45	tunicate
244101	II - O - 1	44	sponge
243101	43	43	sponge
506701	V - C - 2	6	shrimp
505641	V - B - 1	5	Portunid crab
502641	2 - I	2	Portunid crab
501502	V - E - 2	1	bivalve

Analysis

The analytical procedure employed in this investigation is the same as that which is currently being applied to hydrocarbon analyses of benthic macrofauna under BLM contract 08550-CT5-30 to SUSIO for MAFLA outer continental shelf monitoring. The hydrocarbon extraction and isolation portions of this procedure are basically unchanged from those used in contract 08550-CT4-11, although the initial phase is different and the final analytical steps and data workup are considerably more sophisticated.

The first step of the present scheme involves obtaining a dry weight of sample tissue; the earlier procedure used a wet weight. Samples are thawed and then dried at 60°C to a constant weight. This usually requires from 20 to 60 hours. The dried organism is reduced to a granular powder with a mortar and pestle and/or a Virtis homogenizer. A weight is taken of the homogenized powder, and the material sonicated for ten minutes at 60% power using an Artek Model 300 Dismembrator. The liquid used during sonication is a saponification mixture of 0.5 N methanolic KOH/benzene, 50/50.

A modification of this first step is necessary for hard coral samples because of the massive carbonate skeleton present. Thawed samples are broken into pieces with a hammer and chisel and decalcified with 3N HCl. Coral tissue is isolated from the dissolved skeleton by filtration using preweighed filters which are then dried at 60°C and weighed to obtain the weight of dry tissue. The filters plus the tissue are inserted into a flask for the saponification step.

Samples are saponified in order to separate non-saponifiable

lipids from total lipids and from total tissue. Refluxing for one hour in a mixture of 0.5N methanolic KOH/benzene, 50/50, forms the potassium salts of saponifialbe lipids and extracts the non-saponifiable lipids from the samples. The tissue residue is removed by filtration, and the liquid phase is transferred to a separatory funnel. Distilled water is added to partition the saponifiable and non-saponifiable lipids between the aqueous and organic phases, respectively. The organic phase is isolated, and the basic aqueous phase extracted twice with petroleum ether/hexane. These extracts are combined with the original organic phase and washed once with dilute aqueous HCl to remove the trace amounts of non-lipid materials remaining. Solvents are evaporated from the organic phase on a rotary evaporator at 30°C, and the residue transferred to a pear-shaped flask. One gram of 5% deactivated alumina is added, and the solvents evaporated. The non-saponifiable lipids are now adsorbed onto alumina and ready for column chromatography. Resaponification and re-extraction of the tissue residue indicated that this procedure was 85-95% efficient in extracting lipid materials.

The classes of lipids comprising the non-saponifiable fraction are separated by chromatography on a silica gel/alumina column. The column utilized in this study consists of 2 gm 5% deactivated silica gel overlaid by 2 gm 5% deactivated alumina in a 9 mm I. D. column. The column is packed in benzene, and the benzene is washed out with multiple rinses of petroleum ether. This effectively cleans the column packing material. Non-saponifiable lipids, adsorbed on 1 gm alumina, are placed on the top of this column. Normal, branched, and cyclic alkanes and mono-alkanes are eluted from the column with 10 ml petroleum ether. Polyunsaturated hydrocarbons, aromatic hydrocarbons, and methyl ketones are eluted

with 15 ml benzene. Fatty alcohols are eluted with 25 ml benzene/methanol, 90/10. Solvents are evaporated, and the residue stored at 0°C for further analysis.

The column chromatography procedure was developed using a solution of hydrocarbons typical of biogenic and petroleum hydrocarbons plus a fatty alcohol. This was done before any samples were analyzed. The solution consisted of 200 µgm each of cyclododecane, pristane, n-eicosene, n-tetracosane, naphthalene, anthracene, squalene, and cetyl alcohol per millililter of chloroform. These hydrocarbons were adsorbed onto 1 mgm of alumina and separated as described above for actual samples. The separations achieved with this procedure are shown in Table 2. It is evident that separation is quantitative and complete for all test hydrocarbons except for a small amount of tailing found with squalene.

Gas-liquid chromatography is performed on the petroleum ether and benzene fractions as specified in contract 08550-CT5-30 Resolution of the various components comprising each fraction is achieved using non-polar and polar columns. The non-polar column types are a 50 ft x 0.020 in I. D. (15.2 m x 0.5 mm I. D.) SE-30 SCOT column and a 4 m x 2.1 mm I.D. 3% OV-101 on 80-100 mesh Chromosorb WHP column. The polar column is 2.5 m x 2.1 mm I. D. 10% SP-1000 on 80-100 mesh Supelcoport column. Since SP-1000 is actually a Supelco trade name for high performance FFAP, the polar column will be referred to as FFAP in this report. All columns are temperature-programmed. The SCOT column is programmed from 150° to 325°C at a rate of 4°C per minute with a nitrogen carrier gas flow rate of 5 ml/minute. The OV-101 column programming rate is 4° per minute from 150° to 325°C, holding 325°C for ten minutes, with a flow rate of 15 ml N_2/minute. The FFAP column is operated at 8°C/minute from 150° to 250°C, holding the upper limit for 30 minutes. All columns are operated in dual

Table 2

Separation of Test Mixture by Column Chromatography

Mixture Component	Percent of Component in Each Fraction A	B	C
cyclododecane	100		
pristane	100		
n-eicosene	100		
n-tetracosane	100		
naphthalene		100	
anthracene		100	
squalene		99+	trace
cetyl alcohol			100

A = petroleum ether fraction (10 ml)

B = benzene fraction (15 ml)

C = benzene/methanol fraction, 90/10 (25 ml)

differential mode to minimize baseline shifting due to column bleed. The instruments used in this study are a Hewlett-Packard 5710A Gas Chromatograph equipped with a Hewlett-Packard 3380A Integrator and a Hewlett-Packard 5830A Gas Chromatograph. Both instruments use hydrogen-air flame ionization detectors.

Three different SE-30 SCOT columns were employed in this study. Representative chromatograms of these columns are displayed in Figure 1 which shows the separation of the components of the API Standard Southern Louisiana Crude Oil. The operating conditions of these columns were from 90° to 325°C at 4° per minute using a carrier gas flow rate of 5 ml N_2/minute. None of these three columns satisfactorily separated pristane from n-heptadecane, and only marginal separation of phytane from n-octadecane was achieved. Therefore, data from these columns concerning both isoprenoid and normal alkanes would be suspect.

Satisfactory separation was attained using the packed OV-101 column, as shown in Figure 2. Also shown in this figure is the separation obtained from the packed FFAP column. Excellent resolution of isoprenoid and alkane hydrocarbons is achieved with this latter column, but long-chain hydrocarbons (>n - 28) are difficult to detect because of extremely long times and peak broadening caused by the lower maximum temperature limit of this liquid phase material.

The separation characteristics of the columns used in this study are summarized in Table 3. The theoretical plates calculated for these columns show that the non-polar column types are better suited for hydrocarbon analysis than is the polar FFAP column. The calculations for isoprenoid/normal alkane resolution indicate that the polar FFAP column separates these hydrocarbons best, although the non-polar OV-101

Figure 1

Comparison of SCOT SE-30 Columns
Using Crude Oil

A = pristane/n-17 doublet
B = phytane/n-18 doublet

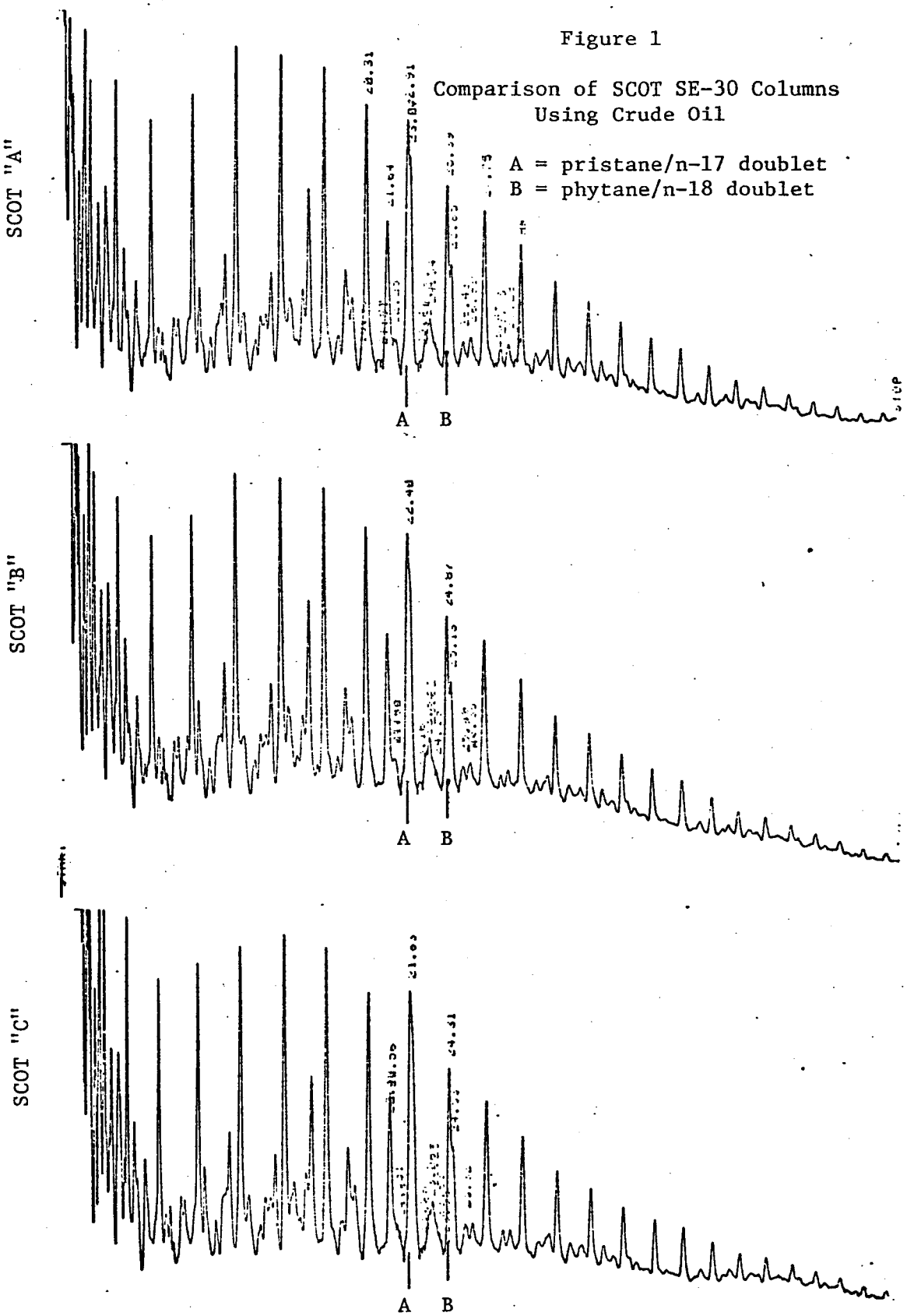

Figure 2

Comparison of Packed Columns
Using Crude Oil

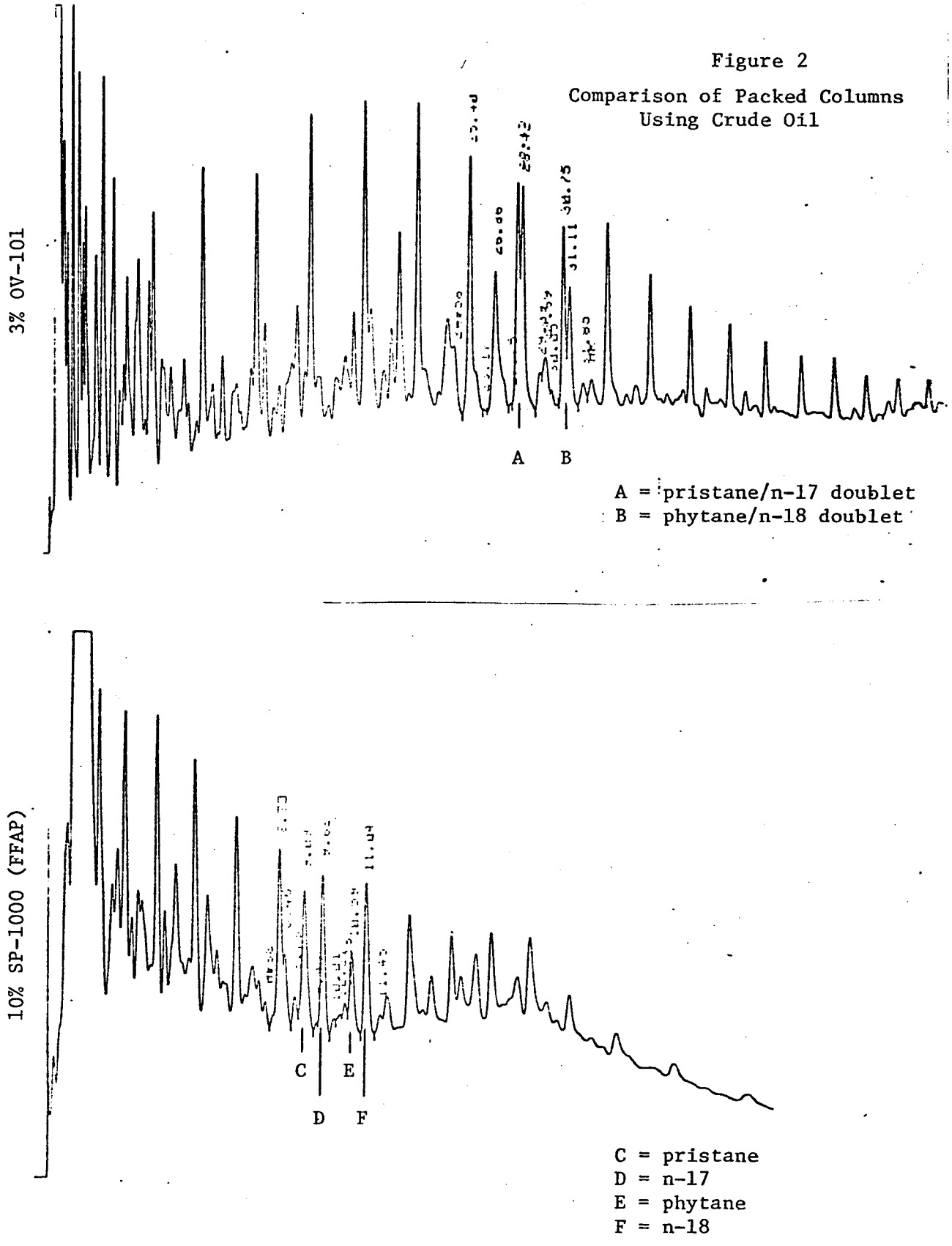

Table 3

Separation Characteristics of Gas Chromatography Columns

Column*	Theoretical Plates+ n-16	n-28	Resolution‡ Pris/n-17	Phyt/n-18
SCOT "A"	51,000	206,000	0.4	0.8
SCOT "B"	48,000	199,000	0.0	0.8
SCOT "C"	46,000	194,000	0.0	0.7
OV-101	53,000	264,000	1.0	1.2
FFAP	7,000		1.3	1.1

* See text for column description and operating conditions.

\+ Calculated from $TP = 16 \left(\frac{x}{y}\right)^2$, where x = retention distance and y = peak base width.

‡ Calculated from $R = \frac{2\,\Delta x}{y_1 + y_2}$.

is quite satisfactory. A resolution factor of unity corresponds to 98% separation.

The overall best column for separation of petroleum-type hydrocarbons appears to be the packed OV-101 column. It has good resolution of isoprenoids and normal alkanes and also has slightly more theoretical plates than any of the SCOT columns. For these reasons, most of the analytical data in this report are derived from chromatograms obtained from packed OV-101 columns. The isoprenoid ratios are obtained from FFAP chromatograms.

Both of the gas chromatograph instruments present an electronically integrated printout of each sample giving retention time in minutes, integrator counts, and area percent for every peak in that sample. Figure 3 is a copy of the printout obtained from the FFAP chromatogram of the petroleum ether fraction of sample number 251341. These data were punched onto IBM cards and entered into the University of Michigan Amdahl 470V/6 computer. A program was designed for the present study to convert the retention time and integrator count data into quantitative data for each hydrocarbon peak. Quantitation was effected using an internal quantitative standard of n-docosane added to the petroleum ether and benzene fractions after column chromatography and prior to gas chromatography. The computer program utilized the peak area of this standard, the dry weight of the organism, and the peak areas from the chromatograms to calculate the quantitative data and ratios required by contract 08550-CT5-30.

It was originally proposed to hydrogenate the benzene-eluted fraction of hydrocarbons after initial gas chromatography in order to reduce non-aromatic double bonds and thus enable better tentative

Figure 3

Sample Integrator Printout

FFAP - Pet. Ether Fraction of 251341

STOP

RT	TYPE	AREA	AREA %
2.14	M	7616	.273 1
2.39	M	1954	.070 08
2.75		143867	5.159
3.64		3116	.111 7
4.17		1979	.070 97
4.72		6193	.222
5.08	M	6123	.219 6
5.34	M	1759	.063 08
5.95		12320	.441 8
6.25	M	2743	.098 37
6.62		758	.027 18
6.91	M	913	.032 74
7.26		27655	.991 8
8.04	M	22969	.823 7
8.63		44404	1.592
9.10	M	7451	.267 2
9.31	M	12314	.441 6
9.56	M	13167	.472 2
9.99	M	37056	1.329
10.68	M	21537	.772 4
10.95	M	14275	.511 9
11.33	M	27029	.969 3
11.66	M	35072	1.258
12.06	M	39510	1.417
12.46	M	18392	.659 6
12.66	M	93881	3.367
13.07	M	21973	.788
13.31	M	38193	1.37
13.89	M	55831	2.002
14.43	M	51597	1.85
14.71	M	36304	1.302
15.11	M	114163	4.094
15.74	M	91317	3.275
16.26	M	203605	7.302
16.90	M	47454	1.702
17.41	M	517094	18.54
18.31	M	70762	2.538
19.07	M	233118	8.36
20.08	M	68541	2.458
20.98	M	160165	5.744
22.11	M	5977	.214 4
23.36		121741	4.366
25.34		34771	1.247
29.09		2170	.078
38.74		133421	4.78

identification of polyunsaturated hydrocarbons, although this procedure is not required in either contract number 08550-CT4-11 nor in contract number 08550-CT5-30. The procedure of Youngblood et al. (1971) has been employed successfully to elucidate the structure of unsaturated hydorcarbons in marine benthic algae. Hydrogenation is accomplished using Adam's catalyst (ptO_2) and hydrogen at atmospheric pressure. According to Farrington et al. (1972), olefins will be reduced but not aromatic hydrocarbons.

Prior to any actual samples, a test mixture of squalene and anthracene was hydrogenated by the procedure of Youngblood et al. (1971). Various reaction times were employed to determine optimum results. It was found that 25 minutes was the minimum time needed for complete conversion of squalene to squalene. However, at this point, 2/3 of the aromatic hydrocarbon anthracene had been hydrogenated! Unfortunately, reduction of anthracene was not complete, and a complex mixture of partailly hydrogenated products was present. In an attempt to achieve complete reduction; the procedure was continued for a total time of 48 hours. Complete conversion to perhydroanthracene was never attained; a mixture of four products remained. Column chromatography separated these products equally between the petroleum ether and benzene fractions. The results of this investigation are summarized in Table 4.

Because hydrogenation threatened to make the chromatograms of the benzene fraction more complex and harder to interpret, none of the benzene fractions of the samples were subjected to this procedure. It appears that hydrogenation is of limited usefulness in the analysis of petroleum hydrocarbons. Therefore, it is suggested that this procedure not be broadly applied to hydrocarbon analyses in general, but instead be

Table 4

Hydrogenation of Squalene and Anthracene

Time		Amount of Hydrocarbon (mgm)							
h	m	A	B	C	D	E	F	G	H
0	0	6.0	0	6.0	0	0	0	0	0
0	25	0	6.0	2.0	2.1	1.4	0.4	0.1	0
1	0	0	6.0	0.3	3.8	1.1	0.6	0.2	0
2	0	0	6.0	0.1	4.2	1.0	0.4	0.2	0.1
20	0	0	6.0	0	0	4.5	1.0	0.4	0.1
48	0	0	6.0	0	0	4.5	1.0	0.4	0.1

A = squalene

B = squalane

C = anthracene

D = partial hydrogenation product of anthracene

E = partial hydrogenation product of anthracene

F = partial hydrogenation product of anthracene

G = perhydroanthracene

H = partial hydrogenation product of anthracene

reserved as a specialized procedure intended only for certain selected samples.

Results and Discussion

A total of ninety-six gas chromatographic analyses resutled from dividing the hydrocarbons from twenty-four samples into petroleum ether and benzene fractions and analyzing each fraction on a non-polar and a polar column. The data from these analyses were transferred to the computer program. A total of 95 computer printouts were obtained. One sample, number 251412, contained only enough hydrocarbons to provide measurable peaks for three chromatographic analyses.

Because of the large amount of data contained within them, it is not practical to discuss in this report the four chromatograms plus four printouts generated for each of the twenty-four samples. Instead, the information obtained from only one representative sample will be discussed in detail. The results of all the analyses will be presented in a summary table and briefly evaluated.

The sample selected to contain data representative of this study is designated number 251341. This is a sample of the hard coral, Porites furcata, class Anthozoa, obtained from SUSIO station number 51 in the Florida Middle Grounds. Copies of the chromatograms obtained from this sample are presented as Figures 4-7. Copies of the computer printouts derived from the chromatographic data are shown as Figures 8-11.

Figure 4 represents the petroleum ether fraction of the hydrocarbons isolated from this organism. This fraction contains the saturated and mono-unsaturated hydrocarbons. Eight major peaks exist at retention

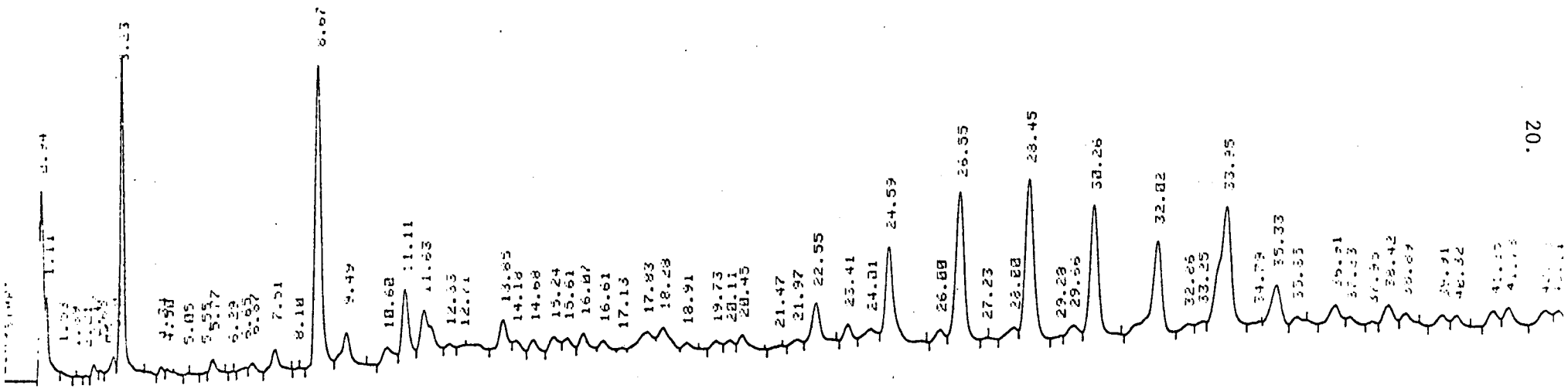

Figure 4. Chromatogram of petroleum ether fraction of hydrocarbons from sample number 251341, Porites furcata, obtained from OV-101 column.

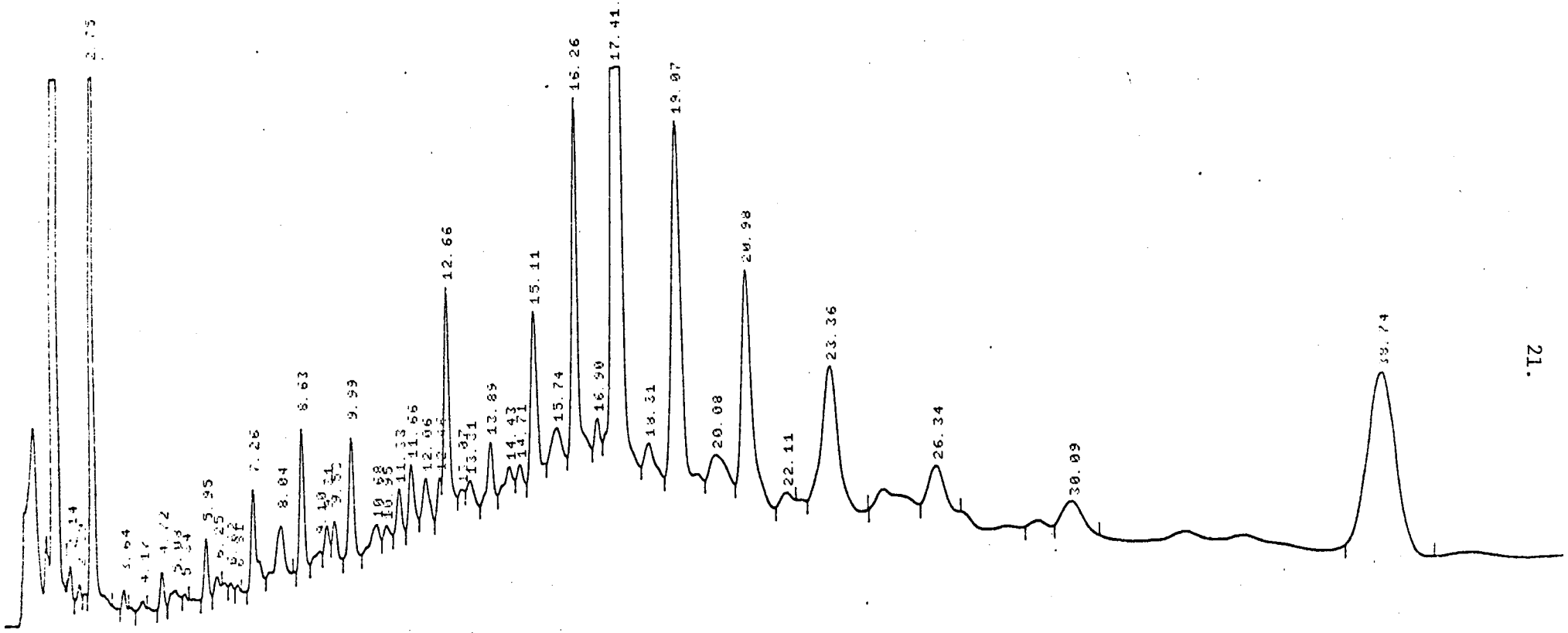

Figure 5. Chromatogram of petroleum ether fraction of hydrocarbons from sample number 251341, Porites furcata, obtained from FFAP column.

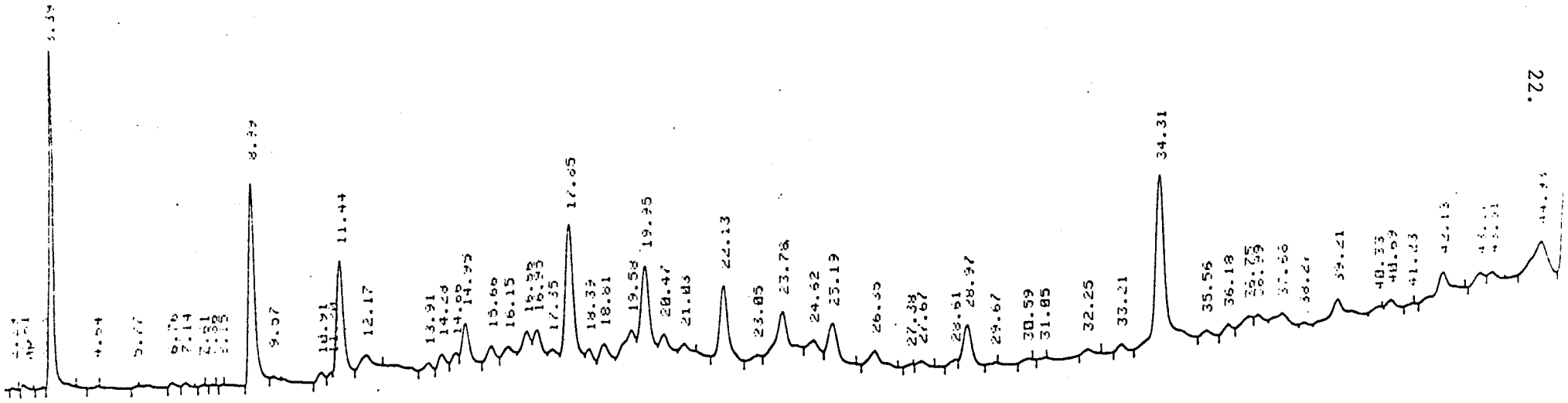

Figure 6. Chromatogram of benzene fraction of hydrocarbons from sample number 251341, Porites furcata, obtained from OV-101 column.

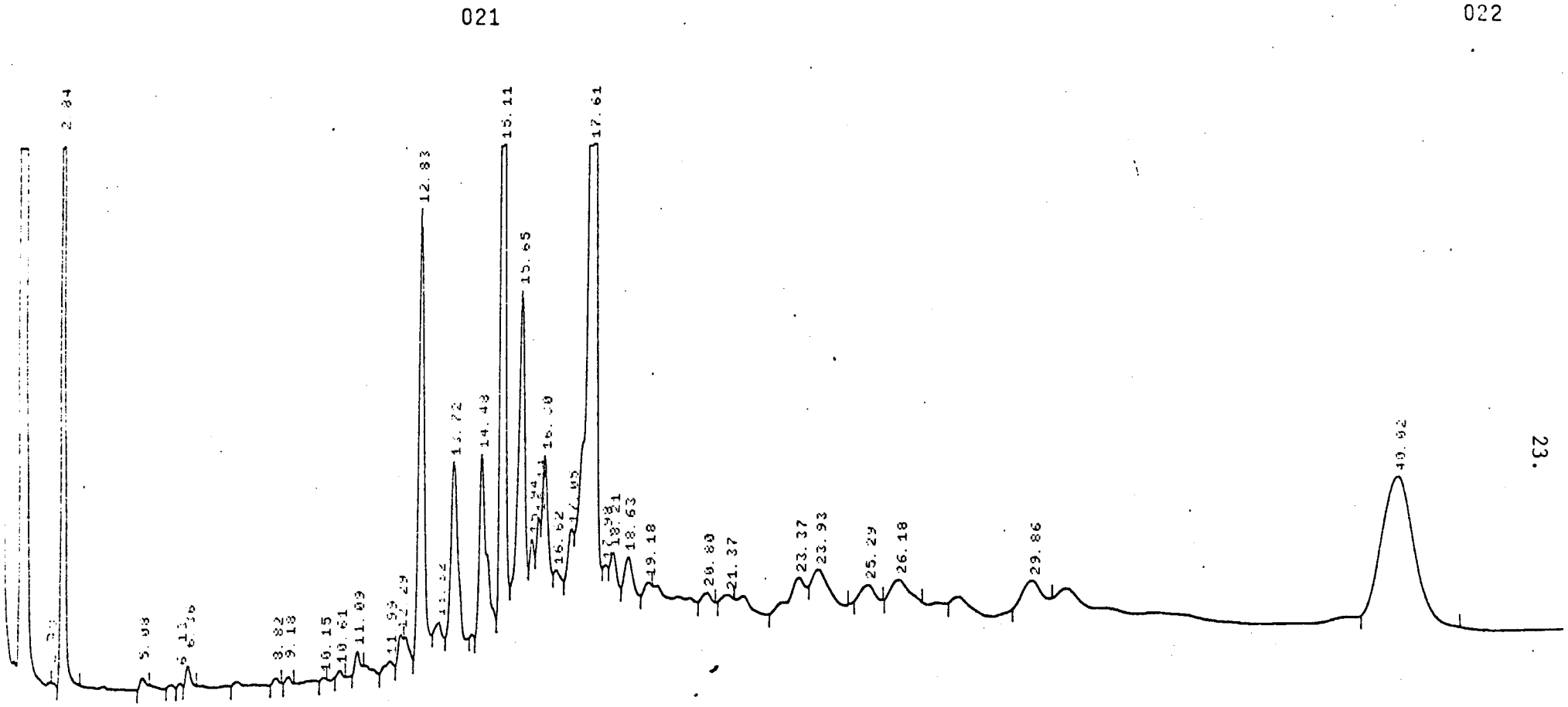

Figure 7. Chromatogram of benzene fraction of hydrocarbons from sample number 251341, Porites furcata, obtained from FFAP column.

SAMPLE NUMBER: 9[illegible] BLM NUMBER: II-3-4[illegible]

FRACTION: PET ETHER COLUMN TYPE: OV 101

NUMBER OF PEAKS: 62 SAMPLE WEIGHT: 0.334 GRAMS

ISO/N-ALK: 0.[illegible] BRANCHED/NORMAL: 0.748

ODD/EVEN: 0.776 CDD/EVEN ≤20: 0.618 ODD/EVEN >20: 0.891

N-ALK/ALL: 0.557 N-ALK/C16: 29.72 N-ALK(≤20/>20): 0.48

PRIS/PHYT: NONE PRIS/C17: NONE PHYT/C18: 0.34

MICROGRAMS OF HYDROCARBON PER GRAM OF SAMPLE: 198.412

RT	RI	AREA	UG/PEAK	PCT	UG/G
4.34	0.0	12260.	0.1079	0.16	0.3233
4.50	1311.84	5822.	0.0512	0.08	0.1533
5.05	1352.18	1315.	0.0116	0.02	0.0346
5.55	1385.20	1805.	0.0159	0.02	0.0475
5.77	1398.79	33740.	0.2964	0.45	0.8883
6.29	1431.52	1000.	0.0088	0.01	0.0263
6.66	1452.70	13490.	0.1185	0.18	0.3552
6.87	1465.09	25160.	0.2211	0.33	0.6624
7.51	1498.99	73340.	0.6417	0.97	1.9231
8.19	1531.26	1094.	0.0096	0.01	0.0288
8.47	1560.39	854400.	7.5068	11.34	22.4955
9.49	1599.10	141300.	1.2415	1.86	3.7203
10.60	1653.02	84240.	0.7401	1.12	2.2180
11.11	1675.97	259300.	2.2782	3.44	6.8271
11.63	1698.32	331600.	2.9134	4.40	8.7307
12.33	1731.72	94720.	0.8322	1.26	2.4939
12.71	1749.22	64120.	0.5634	0.85	1.6882
13.05	1768.75	100900.	0.8865	1.34	2.6566
14.16	1814.41	33860.	0.2977	0.45	0.8920
14.69	1837.77	34790.	0.3057	0.46	0.9160
15.24	1863.10	55630.	0.4889	0.74	1.4621
15.61	1878.17	54040.	0.4748	0.72	1.4228
16.07	1898.74	68360.	0.6006	0.91	1.8125
16.61	1924.14	39080.	0.3434	0.52	1.0289
17.13	1946.71	8024.	0.0705	0.11	0.2113
17.83	1979.01	116600.	1.0244	1.55	3.0700
18.20	1998.31	114500.	1.0060	1.52	3.0147
18.91	2029.44	20480.	0.1799	0.27	0.5392
19.73	2066.52	27810.	0.2445	0.37	0.7327
20.11	2083.64	32130.	0.2823	0.43	0.8460
20.45	2098.68	57530.	0.5055	0.76	1.5147
21.47	2140.36	6169.	0.0542	0.08	0.1624
21.97	2171.94	20470.	0.1798	0.27	0.5390
22.55	2199.64	156000.	1.3706	2.07	4.1073
23.41	2241.93	55980.	0.4918	0.74	1.4739
24.01	2271.33	59720.	0.5247	0.79	1.5724
24.59	2298.05	405800.	3.5654	5.38	10.6843
25.00	2321.70	44630.	0.3922	0.59	1.1754
25.55	2339.02	562300.	4.9404	7.46	14.8048
27.23	2405.52	1571.	0.0138	0.02	0.0414
28.00	2475.90	52830.	0.4642	0.70	1.3910
28.45	2498.98	647830.	5.6916	8.60	17.0559
29.28	2545.76	6820.	0.0599	0.09	0.1796
29.66	2566.79	57520.	0.5054	0.76	1.5144
30.26	2599.46	517200.	4.5441	6.86	13.6174
32.02	2700.00	470000.	4.1293	6.25	12.3983
32.86	2751.06	58990.	0.5183	0.78	1.5531
33.25	2772.87	59110.	0.5193	0.78	1.5563
33.95	2814.32	790000.	6.9409	10.48	20.7999
34.79	2865.78	69260.	0.6085	0.92	1.8235
35.31	2899.22	165000.	1.4501	2.20	4.3475
35.85	2931.69	18130.	0.1593	0.24	0.4773
36.91	2996.75	89540.	0.7867	1.19	2.3575
37.33	3020.59	28350.	0.2491	0.38	0.7464
37.95	3057.11	8674.	0.0756	0.11	0.2265
38.42	3087.43	80120.	0.7039	1.06	2.1095
38.89	3120.84	35530.	0.3122	0.47	0.9371
39.61	3169.66	49050.	0.4310	0.65	1.2914
40.32	3220.89	43150.	0.3791	0.57	1.1361
41.35	3296.66	63340.	0.5565	0.84	1.6677
41.78	3327.23	78300.	0.6879	1.04	2.0616
42.74	3397.90	70920.	0.6230	0.94	1.8667

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Figure 8. Printout of petroleum ether fraction of sample number 251341, OV-101 column.

SAMPLE NUMBER: 90 BLM NUMBER: II-S-4

FRACTION: BENZENE COLUMN TYPE: FFAP

NUMBER OF PEAKS: 28 SAMPLE WEIGHT: 0.334 GRAMS

MICROGRAMS OF HYDROCARBON PER GRAM OF SAMPLE: 210.689

RT	RI	AREA	UG/PEAK	PCT	UG/G
5.08	1425.35	2734.	0.1409	0.20	0.4223
6.13	1507.48	848.	0.0437	0.06	0.1310
6.36	1526.12	5383.	0.2775	0.39	0.8314
8.82	1707.04	1901.	0.0980	0.14	0.2936
9.18	1734.48	1957.	0.1009	0.14	0.3023
10.15	1803.94	818.	0.0422	0.06	0.1263
10.61	1839.27	2330.	0.1201	0.17	0.3599
11.09	1874.54	3558.	0.1834	0.26	0.5496
11.99	1942.85	2815.	0.1451	0.21	0.4348
12.29	1965.83	1973.	0.1017	0.14	0.3047
12.83	2006.64	120688.	6.2205	8.85	18.6410
13.32	2046.41	12575.	0.6481	0.92	1.9423
13.72	2077.81	62250.	3.2085	4.56	9.6149
14.48	2139.83	71045.	3.6618	5.21	10.9733
15.11	2191.24	241131.	12.4284	17.68	37.2441
15.65	2238.25	133184.	6.8646	9.76	20.5711
15.94	2263.46	22961.	1.1835	1.68	3.5465
16.14	2280.57	24226.	1.2487	1.78	3.7418
16.30	2294.12	60300.	3.1080	4.42	9.3137
16.62	2319.40	21066.	1.0858	1.54	3.2538
17.05	2352.10	30509.	1.5725	2.24	4.7123
17.61	2393.48	478716.	24.6740	35.09	73.9405
17.98	2418.14	11020.	0.5680	0.81	1.7021
18.21	2432.82	20839.	1.0741	1.53	3.2187
18.63	2459.17	19235.	0.9940	1.41	2.9787
19.18	2492.79	2484.	0.1280	0.18	0.3837
20.80	2577.76	4312.	0.2222	0.32	0.6660
21.37	0.0	3169.	0.1633	0.23	0.4895

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Figure 9. Printout of petroleum ether fraction of sample number 251341, FFAP column.

SAMPLE NUMBER: 90 BLM NUMBER: II-3-4

FRACTION: BENZENE COLUMN TYPE: OV 101

NUMBER OF PEAKS: 58 SAMPLE WEIGHT: 0.334 GRAMS

MICROGRAMS OF HYDROCARBON PER GRAM OF SAMPLE: 371.914

PC	RT	AREA	UG/PEAK	PCT	UG/G
4.64	1307.77	2602.	0.0367	0.03	0.1159
5.77	1385.47	1639.	0.0254	0.02	0.0761
6.76	1445.33	12070.	0.1666	0.13	0.4993
7.14	1466.41	11170.	0.1542	0.12	0.4621
7.61	1490.99	2027.	0.0280	0.02	0.0838
7.88	1503.90	1671.	0.0231	0.02	0.0691
8.15	1519.68	1074.	0.0148	0.01	0.0444
8.99	1562.42	572300.	7.8999	6.37	23.6738
9.57	1589.65	12980.	0.1792	0.14	0.5369
10.91	1653.64	30840.	0.4257	0.34	1.2757
11.20	1666.76	20320.	0.2805	0.23	0.8406
11.44	1677.36	422700.	5.8349	4.70	17.4854
12.17	1709.74	222600.	3.0730	2.47	9.1998
13.01	1748.29	25730.	0.3552	0.29	1.0643
14.28	1804.33	55096.	0.7605	0.61	2.2769
14.66	1822.32	55170.	0.7616	0.61	2.2822
14.95	1835.74	191200.	2.6393	2.13	7.9032
15.64	1867.53	117400.	1.6206	1.31	4.8564
16.15	1888.64	124200.	1.7144	1.38	5.1377
16.65	1911.39	177900.	2.4557	1.98	7.3590
16.95	1924.95	165000.	2.2776	1.84	6.8254
17.35	1943.26	86400.	1.1926	0.97	3.5947
17.85	1965.57	593300.	8.1898	6.60	24.5424
18.34	1988.98	73500.	1.0146	0.82	3.0404
18.61	2007.83	120000.	1.6565	1.34	4.9633
19.59	2044.60	231100.	3.1763	2.56	9.5183
19.95	2061.76	459200.	6.3387	5.11	18.9953
20.47	2085.34	186400.	2.5730	2.07	7.7106
21.03	2111.26	150100.	2.2109	1.78	6.6227
22.13	2164.15	274300.	3.7864	3.05	11.3467
23.05	2207.21	26640.	0.3677	0.30	1.1020
23.78	2244.11	354300.	4.8907	3.94	14.6560
24.62	2285.19	148200.	2.0457	1.65	6.1304
25.14	2313.71	181300.	2.5026	2.02	7.4937
26.35	2373.20	76660.	1.0499	0.85	3.1463
27.35	2426.45	2436.	0.0337	0.03	0.1009
27.67	2441.86	11860.	0.1637	0.13	0.4906
28.61	2490.81	16980.	0.2344	0.19	0.7024
28.97	2511.22	154400.	2.1313	1.72	6.3869
29.67	2549.36	3445.	0.0476	0.04	0.1425
30.52	2589.46	8140.	0.1124	0.09	0.3367
31.05	2626.25	2628.	0.0363	0.03	0.1087
32.25	2684.43	19550.	0.2699	0.22	0.8087
33.21	2751.52	25670.	0.3543	0.29	1.0619
34.31	2816.89	951800.	13.1385	10.59	39.3721
35.56	2883.40	140100.	1.9339	1.56	5.7954
36.18	2932.55	150400.	2.0761	1.67	6.2214
36.75	2968.40	202600.	2.7967	2.25	8.3807
36.99	2983.32	176400.	2.4350	1.96	7.2970
37.68	3027.66	369200.	5.0964	4.11	15.2723
38.27	3066.46	137000.	1.8911	1.52	5.6671
39.21	3128.57	714400.	9.8615	7.95	29.5518
40.33	3203.48	311700.	4.3027	3.47	12.8938
41.09	3228.44	20970.	0.2895	0.23	0.8674
41.23	3265.47	2554.	0.0353	0.03	0.1056
42.13	3327.61	283400.	3.9120	3.15	11.7231
43.17	3402.01	48500.	0.6695	0.54	2.0063
43.51	3428.30	38120.	0.5262	0.42	1.5769

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Figure 10. Printout of benzene fraction of sample number 251341, OV-101.

SAMPLE NUMBER: 90 BLM NUMBER: II-S-4

FRACTION: PET ETHER COLUMN TYPE: FFAP

NUMBER OF PEAKS: 40 SAMPLE WEIGHT: 0.334 GRAMS

ISP/N-ALK: 0.036 BRANCHED/NORMAL: 1.632

ODD/EVEN: 1.090 ODD/EVEN ≤20: 0.465 ODD/EVEN >20: 1.197

N-ALK/ALL: 0.380 N-ALK/C16: 36.13 N-ALK(≤20/>20): 0.25

PRIS/PHYT: 1.74 PRIS/C17: 0.67 PHYT/C18: 0.36

MICROGRAMS OF HYDROCARBON PER GRAM OF SAMPLE: 264.038

RT	RI	AREA	UG/PEAK	PCT	UG/G
4.72	0.0	7193.	0.2410	0.27	0.7222
5.08	1430.70	6123.	0.2051	0.23	0.6147
5.34	1452.81	1759.	0.0589	0.07	0.1766
5.95	1500.83	12320.	0.4128	0.47	1.2369
6.26	1525.79	2743.	0.0919	0.10	0.2754
6.62	1553.28	758.	0.0254	0.03	0.0761
6.81	1567.19	913.	0.0306	0.03	0.0917
7.26	1598.65	27655.	0.9265	1.05	2.7765
8.04	1657.59	22939.	0.7685	0.87	2.3031
8.63	1698.66	34404.	1.1526	1.31	3.4541
9.10	1734.73	7451.	0.2496	0.28	0.7481
9.31	1750.35	12314.	0.4126	0.47	1.2363
9.53	1766.35	13167.	0.4411	0.50	1.3220
9.99	1798.63	37056.	1.2415	1.41	3.7204
10.68	1851.57	21537.	0.7216	0.82	2.1623
10.95	1871.44	14275.	0.4783	0.54	1.4332
11.33	1898.60	27029.	0.9056	1.03	2.7137
11.66	1924.85	35072.	1.1750	1.33	3.5212
12.06	1955.95	39510.	1.3237	1.50	3.9668
12.46	1986.04	18392.	0.6162	0.70	1.8465
12.66	2000.83	93881.	3.1453	3.57	9.4256
13.07	2034.40	21973.	0.7362	0.84	2.2061
13.31	2053.56	38193.	1.2796	1.45	3.8345
13.89	2098.49	55831.	1.8705	2.12	5.6054
14.43	2144.00	51597.	1.7287	1.96	5.1803
14.71	2167.04	36304.	1.2163	1.38	3.6449
15.11	2199.21	114163.	3.8248	4.34	11.4619
15.74	2254.37	91317.	3.0594	3.47	9.1681
16.26	2298.34	203605.	6.8214	7.74	20.4417
16.90	2349.02	47454.	1.5899	1.80	4.7643
17.41	2388.01	517094.	17.3243	19.66	51.9157
18.31	2449.10	70762.	2.3707	2.69	7.1044
19.07	2497.51	233118.	7.8102	8.86	23.4048
20.08	2551.72	68541.	2.2963	2.61	6.8814
20.98	2597.51	160165.	5.3660	6.09	16.0804
22.11	0.0	5977.	0.2002	0.23	0.6001
23.36	0.0	121741.	4.0787	4.63	12.2227
26.34	0.0	34771.	1.1649	1.32	3.4910
30.09	0.0	31370.	1.0510	1.19	3.1495
*****32.74	0.0	289421.	9.6965	11.01	29.0576

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Figure 11. Printout of benzene fraction of sample number 251341, FFAP column.

times of 3.23, 8.67, 24.59, 26.55, 28.45, 30.26, 32.02, and 33.95 minutes. The first peak, at 3.23 minutes, is the internal standard of n-dodecane which is used to quantitate the chromatogram and to normalize the retention times to a known compound. The retention indices of all peaks are calculated by comparing their retention times to those of authentic hydrocarbon standards. These are presented as Kovats indices (Kovats, 1965) in the tabulation in Figure 8. To four significant figures, the retention indices of the remaining seven major peaks are 1560, 2299, 2399, 2499, 2599, 2700, and 2814. Thus, the retention indices of five of these peaks correspond to n-triacosane, n-tetracosane, n-pentacosane, n-hexacosane, and n-heptacosane, and tentative identification of these hydrocarbons is possible. Other n-alkanes which can be tentatively identified on this basis and which have obvious peaks include n-docosane (2199), n-nonacosane (2898), n-hexadecane (1599), n-heptadecane (1698), and n-octadecane (1799).

The two major peaks at retention indices of 1560 and 2814 remain unidentified because no authentic standards appropriate to the petroleum ether fraction have these indices. However, the peak at 2814 appears to be an unresolved mixture of two or more hydrocarbons. One interpretation of this combination is that it represents n-octacosane, retention index 2800, overshadowed by a compound of retention index 2814. A hydrocarbon having such a retention index on OV-101 is squalene, a polyunsaturated isoprenoid. However, this should not be present in the petroleum ether fraction.

Using retention indices of the petroleum ether fraction as determined from retention times, ratios of certain individual hydrocarbons and classes of hydrocarbons were calculated. These are presented in Figure 8 for data from the OV-101 column and in Figure 9 from the FFAP column. A

window of ±5 Kovats units was allowed in selecting retention indices for these calculations. Because of the column characteristics summarized in Table 2, it is felt that the OV-101 column data are more reliable for ratios involving n-alkanes and that the FFAP data are preferable for ratios involving isoprenoids.

For sample number 251341, the ratio of odd-to-even n-alkanes, or carbon preference index (CPI), is 1.289. Such a value is generally believed to be low for recently biosynthesized hydrocarbons and is considered more typical of ancient oils. However, Koons et al. (1965) reported similarly low CPI values for a number of marine invertebrates, and Clark and Blumer (1967) found CPI values close to and less than unity for marine benthic algae. It may well be that a low CPI is characteristic of marine organisms in general.

The ratio of branched-to-normal hydrocarbons for this sample is 0.794 on the OV-101 column indicating that n-alkanes comprise more than half the total peaks. This column does not resolve mono-alkenes from their corresponding alkanes, so virtually all the peaks have retention indices of saturated hydrocarbons. The dominance of n-alkanes in this coral is further indicated by the value of 0.557 for the ratio of total n-alkanes to total peaks.

Isoprenoid ratios are obtained from the FFAP column data tabulated in Figure 9. The pristane-to-phytane ratio is 1.74, the pristane-to-n-heptadecane ratio is 0.67, and the phytane-to-n-octadecane ratio is 0.36. It is interesting to note the difference in response to these hydrocarbons for the two different columns. Pristane is probably present in the OV-101 chromatogram (Figure 4) as a shoulder on the rear of the n-heptadecane peak (retention time 11.63 minutes) but is poorly resolved. Therefore,

no pristane is indicated in the integrator data given to the computer, and no data for a peak having a retention index corresponding to pristane (1710) appear in the tabulation in Figure 8. However, phytane (retention time 14.18) is resolved from n-octadecane (retention time 13.85). Therefore a phytane-to-octadecane ratio of 0.34 is calculated from the OV-101 data. This agrees well with the FFAP-derived ratio of 0.36.

Other comparisons can be made between the petroleum ether fraction chromatograms using OV-101 and FFAP columns. The n-alkanes which dominated the OV-101 chromatogram (Figure 4) appear in the FFAP chromatogram (Figure 5) as major peaks with retention times of 12.66, 15.11, 16.26, 19.07, 20.98, 23.36, 26.34 and 30.09 minutes. The first four times correspond to retention indices of 2001, 2199, 2298, 2498, and 2598, respectively, and most liekly represent n-alkanes having carbon chains 20, 22, 23, 25, and 26 atoms in length. Authentic standards were not available to compare with the last three retention times for this chromatogram, but comparison with a crude oil sample strongly suggests these times correspond to n-heptacosane, n-octacosane, and n-nonacosane. The peak at retention time 17.41 has a calculated retention index of 2388. This may represent n-tetracosane (2400) plus some highly branched hydrocarbon which has been shifted to a retention index that is different on the FFAP column than on the OV-101 column. A possible candidate is the peak with retention index 2814 on the OV-101. Such a retention index shift corresponds to that of pristane, from 1706 on OV-101 to 1656 on FFAP

Two other important changes in peak distributions can be seen in the FFAP chromatogram. First, the peak size at retention index 2001 is

much larger than in the OV-101 chromatogram, suggesting another hydrocarbon is co-eluting with n-eicosane. Second, n-octacosane appears as a single, well-resolved peak. Whatever compound was overshadowing this n-alkane on the OV-101 column is evidently of a different polarity and has a different retention time than n-octacosane on the FFAP column.

The changes in retention times exhibited by the two chromatograms of the petroleum ether fraction of this sample illustrate the usefulness of using several columns of different polarities in separating and making tnetative identifications of petroleum-like hydrocarbons. It is possible to conclude from the chromatograms in Figures 4 and 5 that a homologous series of n-alkanes ranging from tridecane to triacontane is present. The most abundant n-alkanes in this sample of Porites furcata are found between C_{22} and C_{29}. Both pristane and phytane are present at about a two-to-one ratio, and n-alkanes dominate the petroleum ether fraction of hydrocarbons. Both the dominance and the distribution of n-alkanes in this sample are similar to the hydrocarbon distributions reported by Pasby (1965) for several Anthozoan corals from the Gulf of Mexico.

The chromatogram of the benzene fraction of hydrocarbons obtained from the OV-101 column is shown in Figure 6. As indicated in the copy of the computer printout in Figure 10, the retention indices of the major peaks are 1562, 1677, 1966, 2062, 2164, and 2817. Because virtually no authentic polyunsaturated hydrocarbon standards are commercially available, tentative identification of most of these peaks is impossible. The only available standard is the triterpenoid hydrocarbon, squalene, which has a Kovats index of about 2815 on OV-101. The largest peak in

this fraction has a retention index of 2817 and is probably squalene.

While identification is not possible, the three peaks with retention indices 1966, 2062, and 2164 form an intriguing series not found elsewhere in this chromatogram. They may represent a homologous series of three polyunsaturated hydrocarbons. These could be of the isoprenoid type or derived from polyenoic fatty acids and therefore straight-chain. However, none of these retention indices coincide with those given by Youngblood et al. (1971) for polyunsaturated n-alkenes isolated from marine algae. Therefore, it is unlikely that any of these compounds are n-alkenes.

This fraction chromatographed on the FFAP column produces a different distribution of peaks, as shown in Figure 7 and tabulated in Figure 11. The retention indices of the major peaks are 2007, 2191, 2238, and 2393. The retention times are longer on this column, indicating the relatively more polar nature of the hydrocarbons in the benzene fraction. The retention time of the squalene peak shifts from 34.31 minutes on OV-101 to 40.02 minutes on FFAP.

The three compounds which may be a homologous series may again be present at retention indices 2191, 2238, and 2294. The intervals between the peaks have been reduced from 96 and 102 Kovats units on OV-101 to 47 and 56 units on FFAP if these are the same compounds.

In general, the benzene fraction chromatograms of this and other samples have fewer peaks than the petroleum ether chromatograms. The presence of major peaks with Kovats indices around 2000 to 2400 on FFAP plus squalene is often found. Until authentic polyunsaturated hydrocarbon standards become available, further identification of the components of this fraction by gas chromatography or mass spectrometry will be difficult.

Quantitative information for each peak is displayed in the computer program printouts in Figures 8-11. This is presented both as weight percent of total hydrocarbon composition and as micrograms of each hydrocarbon per gram of dry organism weight. Because of the method used to obtain the quantitative data, differences exist between a hydrocarbon fraction chromatographed on the OV-101 column and the same fraction re-chromatographed on the FFAP column. Considering the small amounts of hydrocarbons present, most of these differences are minor.

The weights of total hydrocarbons per unit of dry weight for sample 251341 for the petroleum ether fraction are 198 µgm on OV-101 and 264 µgm/gm on FFAP. For the benzene fraction; the weights are 372 µgm/gm and 211 µgm/gm for the same columns. These weights, based on the quantitative internal standard, are a function of the column retention and separation characteristics and how long the chromatographic analysis is allowed to continue. As more peaks accumulate, a greater total weight of hydrocarbons will accrue. For this reason, the higher value from the two columns is probably the better indication of total hydrocarbon weight for that fraction. This number will be used to report total weights.

Within a fraction, rather good agreement is obtained for compounds which can be tentatively identified on the basis of retention indices and which appear to be well resolved. For instance, the concentration of n-pentacosane on OV-101 is 17 µgm/gm and on FFAP is 23 µgm/gm. The concentration of n-heptacosane is 12 µgm/gm on both columns. Phytane is present at 0.9 µgm/gm on OV-101 and 1.3 µgm/gm on FFAP, and the phytane to n-octadecane ratio on these two columns is virtually identical.

Similar extensive examinations of each sample can be made but are

beyond the scope of this report. A summary tabulation of some key hydrocarbon ratios and of quantitative data for each sample is given in Table 5.

The ratio of odd-to-even n-alkanes ranges from a low of 0.20 for sample 506701, a shrimp, to a high of 5:26 for sample 251412, Spondylus americanus. The range of odd-to-even ratios of the seven coral samples is from 0.54 to 3.39. These ranges are similar to those of Koons et al. (1965) and Clark and Blumer (1967) and strongly suggest that a CPI close to unity is to be expected in marine organisms.

Total n-alkanes rarely comprise even half of the total aliphatics in these samples. This ratio ranges from 0.01 for a sand dollar, sample 164801, to 0.56 for the corals Madracis decactis and Porites divaricata, samples 251331 and 251341. The n-alkane content of most organisms usually is one-quarter or less of total aliphatics, but in four organisms is between 44% and 56% of the total. Three of these four are corals.

Pristane-to-phytane ratios for these twenty samples range from 1.01 to 3.38, indicating that in all organisms containing pristane, the former is more abundant. Nineteen of the twenty-four samples do contain pristane, while twenty-two have phytane. The amount of pristane is always less than that of n-heptadecane and ranges from 39% to 95% of this n-alkane in concentration. Phytane concentrations range from 27% to 60% of those of n-octadecane.

The concentrations of hydrocarbons in the petroleum ether and benzene fractions was usually in the parts-per-million range, although three coral samples had benzene fraction concentrations at the parts-per-thousand level. Samples in which only tissue was analyzed generally indicated higher concentrations than those in which the entire organism was dried and analyzed. Tissue-only organisms were the corals M. annularis,

Table 5
Summary Tabulation of Hydrocarbon Analyses

Sample Number	Analysis Number	Organism	A	B	C	D	E	F	G
164621	164	Scyllarus sp.	0.57+	0.06	0.90	0.85	1.53	3.16	492.18
164801	79	sand dollar	2.13	0.01	195.69	0.00	0.00	8.2	9.0
163311	94	Montastrea annularis	1.18	0.05	1.27	2.02	0.70	58.7*	3139.6*
161101	87	sponge	0.93	0.01	40.78	1.20	0.56	15.0	120.8
160721	83	Astropectin sp.	1.14	0.01	6.70	1.06	0.39	0.7	47.6
158101	88	sponge	1.12	0.01	8.47	1.38	0.64	6.2	20.0
254361	78	Scolymia lacera	0.65	4.02	53.49	0.00	0.00	7.1*	237.4*
252611	80	Sicyione sp.	0.32	0.01	7.14	1.09	0.41	252.6	51.6
251241	92	Millepora alcicornis	1.45	0.10	2.58	1.20	0.54	31.6*	243.8*
251331	89	Madracis decactis	1.08	0.01	0.80	0.00	0.00	214.1*	773.3*
251341	90	Porites furcata	1.28	0.01	0.79	1.74	0.67	264.0*	371.8*
251342	93	Porites divaricata	3.39	0.04	2.13	1.01	0.53	18.3*	4043.4*
251351	91	Dichocoenia stokesii	0.54	0.01	10.29	1.51	0.51	49.0*	15944.6*
251412	77	Spondylus americanus	5.26+	0.07	0.88	3.00	0.61	0.8*	542.4*
249411	97	Spondylus americanus	0.59	0.01	1.13	2.87	0.89	27.2*	3844.0
248411	84	Spondylus americanus	1.10	0.01	2.88	1.33	0.46	16.0*	213.2*
246101	75	sponge	2.33	0.01	3.08	2.17	0.43	25.4	36.2
245901	86	tunicate	4.60	0.01	8.72	2.14	0.41	2.2	56.3
244101	85	sponge	1.91	0.01	2.88	3.38	0.68	5.5	143.9
243101	76	sponge	2.44	0.01	3.48	2.07	0.95	4.8	10.6
506701	96	shrimp	0.20	0.05	0.33	1.31	0.87	40.6	3730.9
505641	81	crab	0.80	0.01	14.40	0.00	0.00	9.3	4.8
502641	82	crab	2.39+	0.01	173.70	0.00	0.00	12.8	50.2
501502	95	bivalve	0.58	0.11	2.12	1.19	0.74	3.6*	60.0*

A = odd/even n-alkane ratio
B = isoprenoid/n-alkane ratio
C = branched/n-alkane ratio
D = pristane/phytane ratio
E = pristane/n-heptadecane ratio
F = total aliphatics/dry weight of organism (µgm/gm)
G = total unsaturates/dry weight of organism (µgm/gm)

* Weights based upon dry weight of tissue after removal of skeletal material.

+ Odd/Even ratio obtained from FFAP column data.

S. lacera, M. alcicornis, M. decactis, P. furcata, P. divaricata, and D. stokesii, the bivalve S. americanus, and the unidentified tunicate. Sponges had minimal skeletal material, but the Echinoderms and Arthropods had substantial amounts. Generally, the benzene fraction total hydrocarbon weights were greater than the petroleum ether fraction totals. This is similar to the gravimetric data obtained for these fractions in contract 08550-CT4-11 last year.

Comparison of the data from the analyses performed under this contract with those from contract 08550-CT4-11 are difficult. The quantitative data this year are based on dry weights, while last year they were on wet weights or on total lipid contents. Chromatographic analyses last year were performed only on 2 m 3% OV-101 columns. This did not offer the resolution and identification achieved this year using 4 m OV-101 columns plus FFAP columns. Therefore, no pristane or phytane data exist from 08550-CT4-11. Electronic integration was not available last year, so measurement of individual peaks was not done. Obviously, odd-to-even ratios could not be calculated, nor could any other ratios like those in Table 4 be obtained. It is intended eventually to re-chromatograph selected samples from last year's analyses on the present system in order to permit comparison with the present data and data being generated under contract 08550-CT5-30. However, demands on instrument time presently make this impossible.

Conclusions

From the hydrocarbon analyses of these twenty-four samples of benthic macrofauna, the following generalizations can be made:

1. The carbon preference indices of these organisms are close to unity. Since these organisms include representatives from six marine phyla, low CPI's may be typical of oceanic fauna.

2. Homologous series of n-alkanes occur in natural distributions of faunal hydrocarbons. The most abundant n-alkanes occur in the range of C_{20} to C_{30}.

3. Phytane and pristane are common to most marine animals, but usually at low levels.

4. Squalene is an important constituent of the unsaturated hydrocarbon fraction of marine lipids.

5. The concentration of total unsaturated hydrocarbons is usually considerably higher than that of saturated hydrocarbons in natural populations. This may serve as an indicator of non-polluted populations in the Gulf of Mexico area where native crude oils are high in paraffinic hydrocarbons.

6. These samples do not appear to be contaminated by petroleum or petroleum products.

Summary

Twenty samples of benthic macrofauna collected during the MAFLA baseline survey under contract 08550-CT4-11 were analyzed for indigenous hydrocarbons. The procedures used are specified in contract 08550-CT5-30 and involve separating the extracted hydrocarbons into aliphatic and unsaturated fractions and analyzing each fraction by gas chromatography on two different columns. Data obtained from these analyses shows that the ratio of odd to even alkanes is close to unity in all the samples. This appears to be a natural characteristic of marine organisms. A homologous series of n-alkanes peaking around C_{25} to C_{27} is found in many of the samples. In most samples, the unsaturated fraction of hydrocarbons is at a greater concentration than the aliphatic fraction. These organisms display no obvious evidence of oil contamination.

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Page	Line	Correction
1	12	". . . 32 of 65 areas. . ."
4	8	"Scyllarus sp."
7	3	". . . saponifiable. . ."
8	9	". . . onto 1 gm of alumina. . ."
16	5	". . . hydrocarbons in. . ."
16	6	". . . (PtO_2). . ."
16	13	". . . squalene to squalane."
16	15-16	". . . of partially. . ."
19	1	". . . resulted. . ."
25 27		Figures switched, Figure 9 should be Figure 11, and vice versa.
30	13	". . . likely. . ."
31	9	". . . tentative. . ."
38	1	"Twenty-four. . ."

Appendix I: Summary Tabulation of Hydrocarbon Analyses

Sample Number	Analysis No.	Organism	H	I	J	K	L	M
164621	164	Scyllarus sp.	3.18	1.85	0.53	11.61	4.21	0.71*
164801	79	sand dollar	8.95	0.44	0.01	15.76	1.71	0.37*
163311	94	Montastrea annularis	1.55	0.99	0.44	8.33	3.48	0.46*
161101	87	sponge	1.18	0.42	0.02	4.91	2.11	0.38*
160721	83	Astropecten sp.	1.14	n.d.	0.13	4.56	1.10*	0.31*
158101	88	sponge	0.55	4.55	0.11	5.37	2.60	0.40*
254361	78	Scolymia lacera	0.65	n.d.	0.02	18.71	1.09	0 *
252611	80	Sicyione sp.	0.16	1.00	0.12	16.04	1.38	0.28*
251241	92	Millepora alcicornis	1.39	1.62	0.28	12.83	1.61	0.40*
251331	89	Madracis decactis	1.32	0.89	0.56	36.80	0.34	0.36*
251341	90	Porites furcata	1.62	1.12	0.56	29.72	0.48	0.36*
251342	93	Porites divaricata	1.18	11.17	0.32	26.04	1.10	0.54*
251351	91	Dichocoenia stokesii	0.54	n.d.	0.09	3.73	0.73*	0.28*
251412	77	Spondylus americanus	0.21*	0.88*	0.53*	20.2 *	6.38*	0.27*
249411	97	Spondylus americanus	1.74	0.34	0.47	17.93	0.58	0.39*
248411	84	Spondylus americanus	1.39	n.d.	0.26	8.03	2.31	0.31*
246101	75	sponge	1.38	2.61	0.25	n.d.	0.42	0.28*
245901	86	tunicate	6.16	3.39	0.10	77.75	1.65	0.49*
244101	85	sponge	2.49	1.72	0.26	182.64	0.50	0.32*
243101	76	sponge	3.02	1.82	0.22	n.d.	1.54	0.60*
506701	96	shrimp	1.04	0.13	0.75	137.41	0.27	0.52*
505641	81	crab	0.92*	0.67	0.29*	49.16*	0.25*	0.39*
502641	82	crab	0.99*	n.d.	0.03*	n.d.	0.19*	0 *
501502	95	bivalve	1.11	0.15	0.32	8.69	0.97	0.77*

H = odd/even n-alkane ratio, eicosane and less
I = odd/even n-alkane ratio, greater than eicosane
J = total n-alkanes/total resolved aliphatics
K = total n-alkanes/n-hexadecane
L = n-alkanes/n-hexadecane
M = phytane/n-octadecane ratio

* ratio obtained from FFAP column

n.d. = not determined

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.