

Studies on the Lipids of Coastal Waters

II. Hydrocarbons of Sediments in Hiuchi Nada

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(Figs. 1-6, Tables 1-4)

Today the pollution due to spilling of crude oil has become serious problem in the world¹⁾. It was estimated that the oil influx to the ocean was 1 million tons per year at least²⁾. The petroleum hydrocarbons are found not only in the coastal region, but also in the oceans such as the Pacific or the Atlantic³⁾. Moreover, it is recognized that such hydrocarbons are accumulated through the food chain and occasionally the marine organisms are contaminated by the hydrocarbons⁴⁾⁻⁸⁾.

Some hydrocarbons are also biosynthesized by marine organisms⁹⁾⁻¹²⁾, in which pristane and squalene are contained as major biogenetic hydrocarbons. Therefore, in order to make clear the fate of lipids and to estimate whether the marine environment is polluted by oil or not, it is very important to separate the biogenetic hydrocarbons from the petroleum hydrocarbons. The intent of this paper is to characterize the hydrocarbons of sediments in Hiuchi Nada, and to evaluate the condition around this area as of summer of 1973 and winter of 1974.

EXPERIMENTALS

The area investigated: Hiuchi Nada is located at the central part of the Seto Inland Sea. This region has been polluted or eutrophicated gradually, and recently the phytoplankton bloom occurs often during late spring to early autumn. The petrochemical industry developed at Niihama and many paper manufactories are located at Mishima and Kawano along the south coast of this area.

Survey: The surveys were carried out during 21st - 23rd, Aug., 1973 and 22nd - 24th, Jan., 1974. Mud samples were collected at 17 stations in Hiuchi Nada as shown in Fig. 5. The sediments were collected with Eckman-Berge dredge and stored at -20°C until the laboratory works.

Extraction of hydrocarbons: The procedure of extraction and the outline of the analysis of hydrocarbons are shown in Fig. 1. Lipids were extracted from sediments with chloroform - methanol (1:1, v/v), then were saponified with 5% KOH alcoholic solution. The unsaponifiable materials were extracted with petroleum ether and diethyl ether. The hydrocarbon fraction was obtained by a preparative thin-layer chromatography.

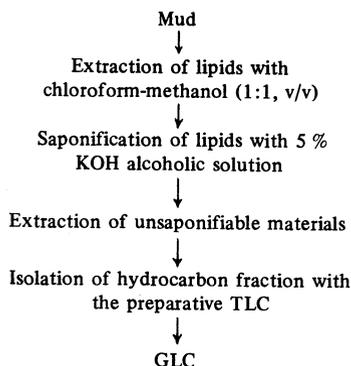


Fig. 1. The procedure for the analysis of hydrocarbons extracted from sediments.

Gas-liquid chromatography: A Hitachi 073 gas chromatograph was used. The column was 2 m in length, 3 mm in internal diameter, packed with 1.5% SE - 30 on chromosorb W (60 - 80 mesh) acid washed and siliconized. The oven temperature was programmed from 100°C to 250°C at a rate of 3°C per minute. The injector and detector were at 280°C. The carrier gas (N₂) flow rate was 40 ml/min. The detector was a flame ionization detector. The hydrocarbons were identified by the comparison of the retention time of standard samples and the relative retention time to pristane, and by the internal standard method.

RESULTS

The gas chromatogram of authentic hydrocarbons is shown in Fig. 2. The retention time and relative retention time of these hydrocarbons are shown in Table 1. The relation between the retention times and the carbon chains of n-alkane is shown in Fig. 3. Although the retention times of n-tetradecane, n-pentadecane, n-hexadecane, n-octadecane, n-nonadecane, n-eicosane, n-docosane, pristane and squalene were determined from the gas chromatogram, the retention times of other n-alkanes were estimated from Fig. 3. Under these gas chromatographic conditions previously described, the peaks of n-heptadecane and pristane, and of n-heptacosane and squalene are not separated completely.

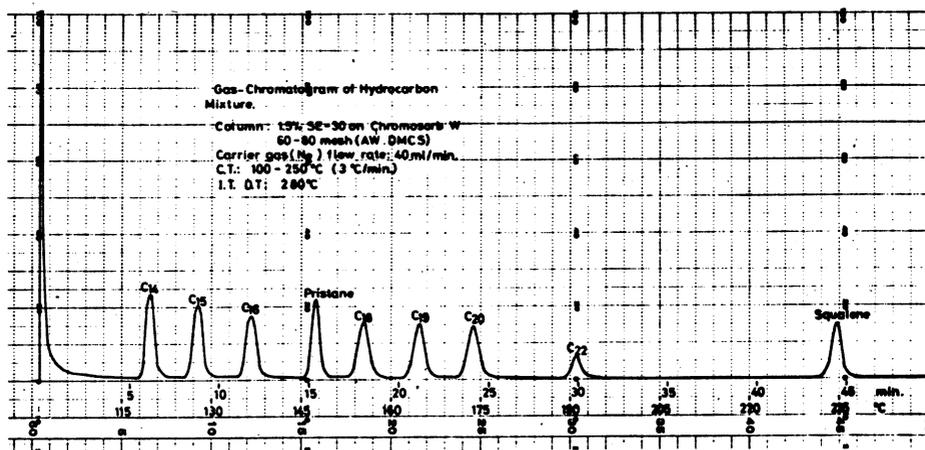


Fig. 2. The gas chromatogram of the authentic hydrocarbons.

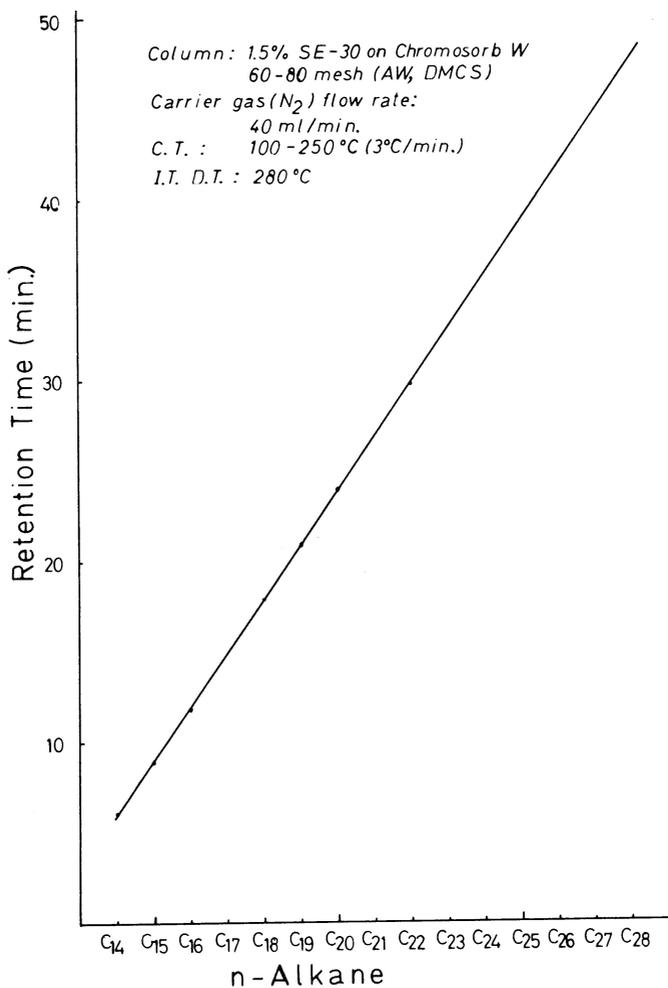


Fig. 3. The relation between the retention time and the chain length of n-alkanes.

Table 1. The retention time and the relative retention time to pristane (2, 6, 10, 14-tetramethylpentadecane) of hydrocarbons.

Hydrocarbon	Retention time (min.)	Relative retention time
C ₁₄	6.2	0.402
C ₁₅	8.8	0.576
C ₁₆	11.7	0.768
C ₁₇	14.9	0.980
Pristane	15.3	1.000
C ₁₈	18.0	1.175
C ₁₉	21.1	1.378
C ₂₀	24.0	1.573
C ₂₁	26.9	1.757
C ₂₂	29.7	1.906
C ₂₃	32.9	2.153
C ₂₄	35.8	2.343
C ₂₅	38.9	2.546
C ₂₆	41.9	2.742
Squalene	44.2	2.893
C ₂₇	44.9	2.938
C ₂₈	47.9	3.135

Column: 1.5% SE-30 on Chromosorb W 60-80 mesh (AW, DMCS).

Carrier gas (N₂) flow rate: 40 ml/min.

Column temperature: 100-250°C (3°C/min.).

Injector and detector temperature: 280°C.

The gas chromatogram of hydrocarbons extracted from sediments is shown in Fig. 4. The occurrence of pristane, squalene and n-alkanes is confirmed. In these hydrocarbons, pristane and squalene are main components, on the contrary, n-alkanes are minor components.

Pristane and squalene occupy about 40% in the total hydrocarbons in summer (Table 2), but in winter the amount of pristane plus squalene decrease relatively (Table 3). The ratios of pristane plus squalene/n-alkane (Table 4) are large at the south eastern part of this area, and the distribution of this ratio is coincident with that of the lipid content²⁰⁾ as shown in Figs. 5 and 6.

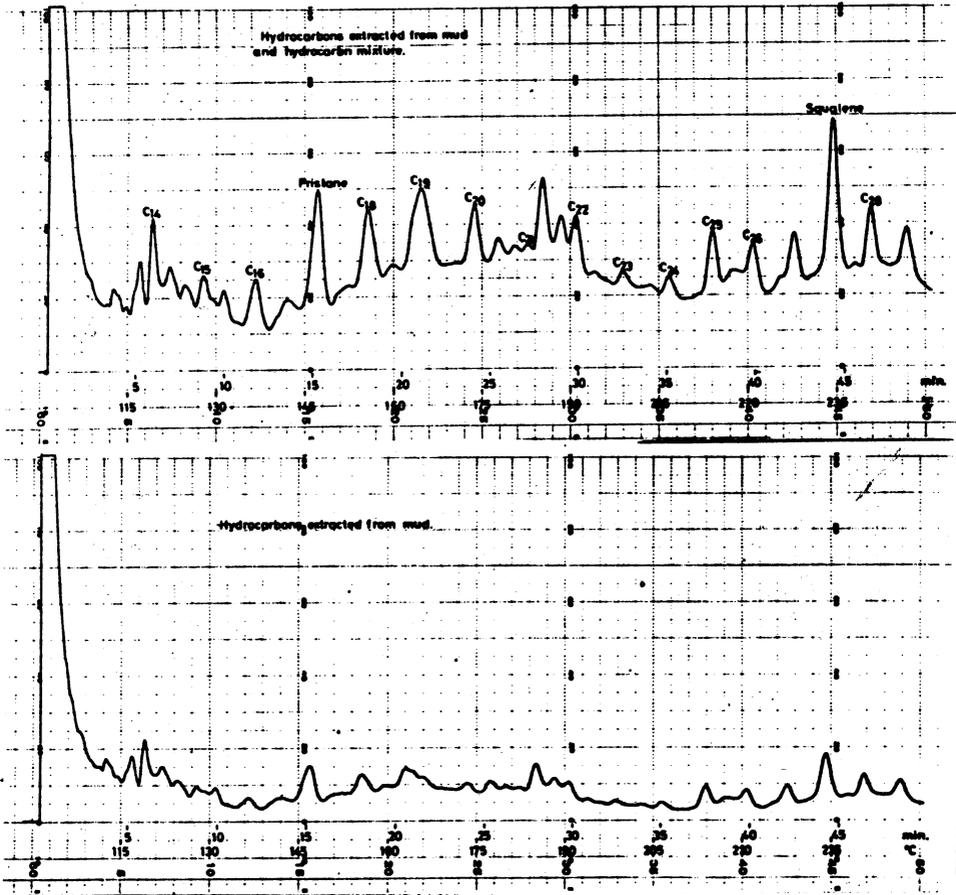


Fig. 4. The gas chromatogram of the hydrocarbons extracted from sediment.

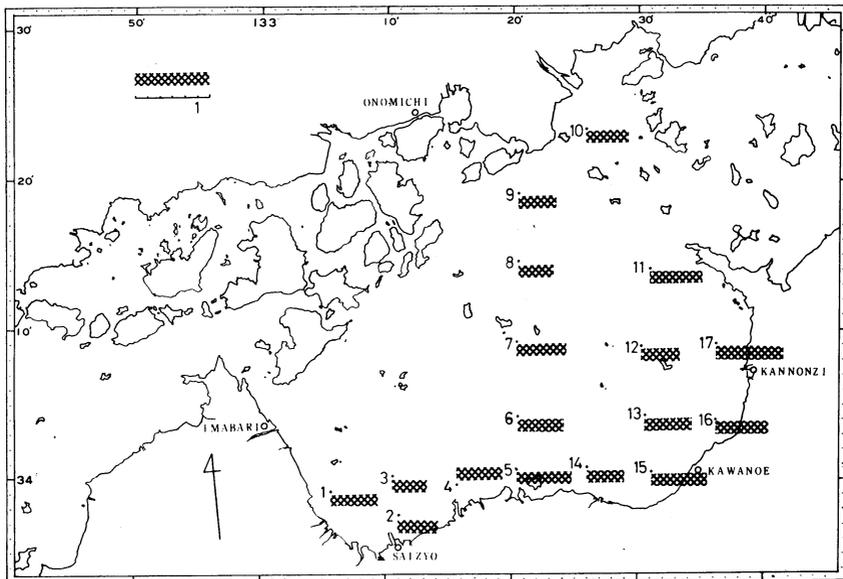


Fig. 5. The distribution of pristane plus squalene/n-alkane ratio (Aug., 1973).

Table 2. The percentage composition of hydrocarbons extracted from mud. (Aug., 1973)

Hydrocarbon	Station No.																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
C ₁₄	2.70	4.11	3.40	2.55	3.54	3.89	3.35	3.64	4.19	4.84	2.56	4.58	3.11	3.99	3.23	1.05	tr.
C ₁₅	5.40	4.11	3.40	2.92	3.14	5.01	4.71	3.02	1.41	2.76	2.39	5.15	2.75	3.99	3.23	4.07	1.94
C ₁₆	3.75	3.93	3.93	5.00	3.24	2.73	3.77	3.02	3.18	3.11	4.91	3.82	2.50	3.59	4.03	5.70	2.75
Pristane	12.38	14.05	12.52	12.40	14.05	11.13	10.99	10.63	12.71	11.05	14.74	13.60	12.44	14.02	9.68	18.62	13.25
C ₁₈	8.40	6.34	6.26	10.31	5.31	8.76	7.33	6.14	9.60	6.22	7.18	6.82	7.38	6.99	4.84	9.60	6.15
C ₁₉	6.75	4.71	8.85	5.10	7.86	7.30	5.45	12.28	14.30	7.25	11.28	8.68	8.44	9.79	8.65	8.14	3.61
C ₂₀	3.60	7.73	6.79	7.97	4.42	6.68	3.66	3.40	2.87	4.84	3.29	3.44	4.28	6.99	4.03	3.49	3.91
C ₂₁	1.50	2.11	3.62	1.25	1.75	tr.	1.57	5.29	3.09	4.15	2.56	3.58	4.89	1.50	3.03	4.65	3.61
C ₂₂	1.50	1.45	2.01	1.25	0.79	2.09	1.26	3.68	0.40	1.73	0.85	1.91	1.67	1.40	1.73	1.75	1.29
C ₂₃	1.70	2.96	2.82	3.33	2.75	1.95	2.09	2.83	2.12	1.73	2.09	1.72	2.44	3.00	2.07	2.79	1.55
C ₂₄	3.60	3.26	3.22	3.65	2.36	2.50	3.14	2.36	2.18	2.07	2.50	3.44	2.75	3.59	2.22	1.75	3.61
C ₂₅	8.55	11.81	12.92	8.44	8.26	10.01	9.53	11.24	9.53	6.91	10.17	9.30	9.16	11.68	9.51	4.07	9.29
C ₂₆	4.50	3.99	3.58	3.13	6.39	4.52	4.71	5.29	2.65	8.29	3.33	5.73	4.00	3.00	4.84	6.52	6.19
Squalene	31.52	25.80	23.46	30.63	32.58	31.29	33.30	24.80	25.60	29.02	29.72	24.81	30.32	23.97	36.71	26.06	38.58
C ₂₈	4.13	3.63	3.22	2.08	3.54	2.09	5.13	2.36	6.18	6.04	2.39	3.44	3.89	2.50	2.77	1.75	4.26

Table 3. The percentage composition of hydrocarbons extracted from mud. (Jan., 1974)

Hydrocarbon	Station No.																
	1	2	3	5	6	7	8	9	10	11	12	13	14	15	16	17	
C ₁₄	10.51	8.86	10.24	14.60	15.94	11.27	14.54	12.36	9.61	11.38	12.99	12.10	14.94	11.46	6.12	13.53	
C ₁₅	3.28	2.95	3.49	4.46	6.25	4.02	4.18	3.15	3.09	4.47	7.43	3.94	4.91	3.42	3.22	4.48	
C ₁₆	2.82	2.95	3.49	2.70	5.51	2.85	4.25	3.21	2.40	4.29	4.69	4.60	4.87	4.09	4.51	4.59	
Pristane	14.78	12.19	13.10	19.41	19.32	10.73	13.61	12.13	12.97	16.25	13.47	16.77	17.54	13.16	16.34	15.08	
C ₁₈	8.45	7.09	6.29	7.10	7.95	9.66	9.74	7.35	5.35	10.84	9.02	11.83	10.80	9.42	9.02	8.41	
C ₁₉	9.15	10.04	5.12	4.26	tr.	10.73	8.66	7.96	6.24	10.11	5.05	6.31	6.82	11.57	10.95	9.17	
C ₂₀	2.35	2.66	4.89	3.31	1.67	4.36	1.62	1.46	12.08	6.86	2.17	8.28	4.06	3.80	2.82	1.78	
C ₂₁	4.22	0.74	1.92	1.62	1.60	4.83	0.77	3.15	2.88	3.79	2.11	3.16	3.98	3.27	3.86	2.85	
C ₂₂	6.19	1.03	0.93	1.08	2.34	7.24	1.55	1.60	0.41	2.03	2.11	5.06	2.92	1.75	2.17	1.53	
C ₂₃	1.13	2.22	2.56	2.03	1.07	2.68	2.71	1.17	1.65	1.47	1.20	1.71	0.65	2.46	2.82	2.50	
C ₂₄	1.88	3.25	4.54	2.84	2.01	1.85	2.78	2.45	1.89	2.26	2.53	3.16	2.03	3.16	tr.	2.85	
C ₂₅	7.23	7.98	9.08	6.63	6.55	5.03	6.50	5.31	4.12	7.90	7.16	5.52	6.82	5.52	4.83	6.11	
C ₂₆	6.71	5.17	8.15	6.63	4.41	3.82	4.18	3.50	11.73	4.88	3.97	3.29	4.02	3.29	6.76	6.78	
Squalene	15.20	24.00	17.11	18.40	22.16	18.09	21.66	32.97	20.65	11.44	22.74	12.82	13.20	12.82	23.19	16.05	
C ₂₈	6.10	8.86	9.08	4.87	3.21	2.83	3.25	2.24	4.94	2.03	3.37	1.58	2.44	1.58	3.38	4.28	

Table 4. Pristane plus squalene/n-alkane ratio at hydrographic stations.

Station No.	Pristane + Squalene / n-Alkane	
	Aug., 1973	Jan., 1974
1	0.78	0.43
2	0.66	0.57
3	0.56	0.43
4	0.75	—
5	0.87	0.61
6	0.74	0.71
7	0.80	0.40
8	0.55	0.54
9	0.62	0.82
10	0.67	0.51
11	0.83	0.38
12	0.62	0.57
13	0.75	0.42
14	0.61	0.44
15	0.87	0.43
16	0.84	0.65
17	1.08	0.45

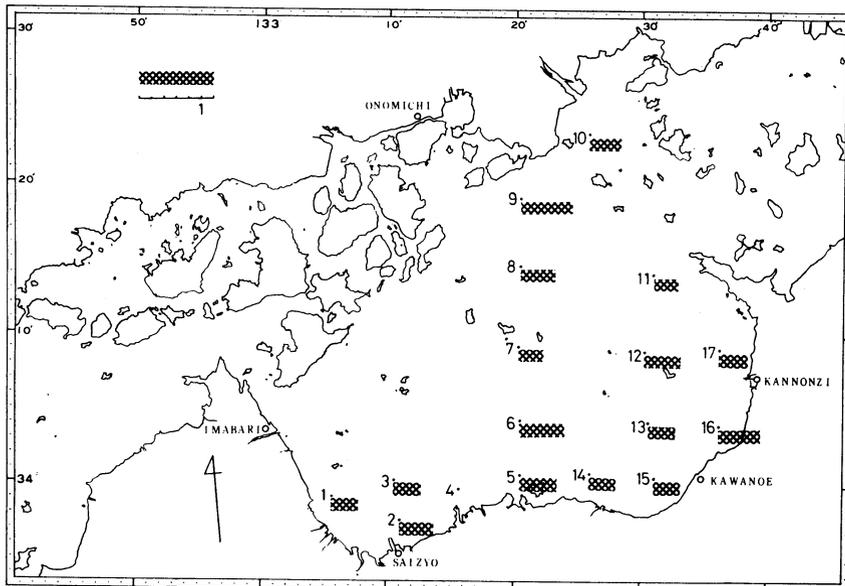


Fig. 6. The distribution of pristane plus squalene/n-alkane ratio (Jan., 1974).

DISCUSSION

The occurrence of pristane in organisms was reported by many researchers. Its occurrence was reported by INOUE *et al.*¹³⁾ in salmon, trout and carp, by SHINMA *et al.*¹⁴⁾ and by KAYAMA *et al.*¹⁵⁾ in shark liver oil, and also reported by SANO¹⁶⁾ in the sperum whale blubber oil. It was also presented by CLARK JR. *et al.*¹⁷⁾ that pristane was predominant hydrocarbon in the zooplankton samples. Squalene is also a major constituent of hydrocarbons¹⁵⁾. It is recognized from these results that pristane and squalene are major biogenetic hydrocarbons.

M. BLUMER analyzed some species of phytoplankton and benthic algae, and he detected that n-alkane occurred in them^{9),11)}. In these organisms n-pentadecane or n-heptadecane were predominant. But it is considered that n-alkanes extracted from the sediments are mainly derived from crude oil or oil products.

Although BLUMER¹⁸⁾ has already reported that pristane was found in the sediment, the authors also found pristane as well as squalene. N-alkanes (C₁₄ - C₂₈) were also detected in mud samples as described by OHTA, KAYAMA and WAKIMOTO¹⁹⁾.

Observing the hydrocarbon composition, it can be concluded that pristane and squalene are major components and these hydrocarbons occupy about 40 to 50%. Thus the biogenetic hydrocarbons are predominant in the sediments.

The pristane plus squalene/n-alkane ratio ranged from 0.6 to 1.1 in summer and from 0.4 to 0.8 in winter. It is obvious that this ratio is higher in summer than in winter (Table 4, Figs. 5 and 6). It can be considered from this result that biogenetic hydrocarbons are decomposed faster than non-biogenetic hydrocarbons.

As the pristane plus squalene/n-alkane ratio was large at south eastern part of this area where the lipid content was also high²⁰⁾, it is thought that the large proportion of hydrocarbons extracted from sediments in Hiuchi Nada may be derived from organisms. As the hydrocarbon fraction occupies 30% of total lipids and n-alkane fraction is 50% of the total hydrocarbons, it is estimated from these data that n-alkane content of sediment is about 0.036%. It was ascertained by NITTA *et al.*⁷⁾ that the minimum concentration of oily substances to give the odour in fish was 0.2% in bottom mud. Therefore, it is thought that this area investigated was not too polluted to give the petroleum odour in fish as of Aug., 1973 and Jan., 1974.

SUMMARY

1. The hydrocarbons of sediments were analyzed with gas-liquid chromatography. The biogenetic hydrocarbons (pristane and squalene) as well as n-alkanes were detected.
2. Considering the distribution of pristane plus squalene/n-alkanes ratio and the

fact that this ratio was coincident with that of lipid content, it is recognized that the hydrocarbons of mud were mainly derived from the organisms in this area.

3. It is supposed that this area was not too polluted to give petroleum odour in fish as of summer of 1973 and winter of 1974.

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沿岸水域の脂質に関する研究 Ⅱ 燧灘の底土にみられる炭化水素

山田 久・鹿山 光

今日、石油の流出事故による沿岸水域の油濁は世界的に深刻な問題となっている。1973年夏期と1974年冬期時点における燧灘17測点の底土に含まれる炭化水素を検討した結果を報告する。

- (1) 底土から脂質を抽出し、その炭化水素のガスクロマトグラフ分析を行い、生物由来の炭化水素（プリスタンおよびスクワレン）とn-アルカンを検出した。
- (2) （プリスタン+スクワレン）/n-アルカンの比の分布とこの比が脂質含量と一致している事実を考え、この海域の底土中の炭化水素は生物起源のことが多いことを認めた。
- (3) この時点でこの海域は、魚に石油貝を与えるほどに油濁されていないと考えられる。