Lipids in Sea Water

LELA M. JEFFREY, Oceanography Department, Texas A&M University, College Station, Texas

Abstract

Acidified and filtered sea water samples which were extracted with petroleum ether and ethyl acetate have been shown to contain a variety of lipid compounds in trace amounts. Concentrations of these solvent-soluble substances ranged from 0.5 to 6.0 mg/liter, the lower concentrations being found in offshore waters. The solvent extracts of the sea water were separated into eight lipid classes by column chromatography on silicic acid. The fractions eluted with solvents of increasing polarity were characterized by thin-layer chromatography, infrared and ultraviolet absorption and gas chromatography. These techniques revealed a complex mixture of alkanes, alkenes, fatty acids, steroids, phospholipids and many as yet unidentified components. Twenty to thirty alkanes were present as indicated by gas chromatography. No aromatic hydrocarbons were detected. Chromatography of the methyl esters of the fatty acids indicated the presence of acids with chain lengths varying from 14 to 22 carbons, both saturated and unsaturated. In many samples the unsaturated fatty acids containing 18 to 22 carbons predominated. The lipid components varied somewhat in composition as well as concentration from location to location and with season and depth.

Introduction

Significance of Organic Solutes of Sea Water

THE CONCENTRATION OF dissolved organic compounds in the open ocean is in the range 0.22 to 2.7 mg/liter (as carbon) with higher values 3.3–8.0 mg/liter in landlocked areas (1). The distribution of organic solutes is not homogeneous, either horizontally or vertically and varies in the surface layers (0–200 meters) with biological productivity, the highest organic carbon values being found in water masses of recently high phytoplankton productivity (1). The distribution of organic carbon in the oceans is summarized in Table I. Duursma (1) presented evidence to show that decomposition of dead organisms is the primary source of soluble organic compounds and also showed that the amount of dissolved organic carbon always exceeds that of particulate matter by a factor of 8 to 1000.

TABLE I

Summary of Organic Carbon, Nitrogen and Phosphorus Distribution in the Oceans [a]

Locality	Organic carbon (mg/l)	Organic nitrogen (mg/l)	Organic phosphorus (mg/M³)
North Atlantic	0.20–1.30	0.04–0.40	0.0– 9.0
North Atlantic	1.40–1.97	———	———
Subtropical Atlantic	———	———	2.0– 9.0
Atlantic (near Bermuda)	2.28–2.42	0.16–0.32	———
Atlantic	2.40–2.48	0.24–0.26	1.0–21.0
Pacific	0.6 –2.7	———	———
Pacific	0.98–2.68	0.07–0.11	———
North Pacific	———	0.09–0.32	———
North Sea	0.50–1.80	0.80–0.54	0.0–19.0
North Sea	2.40–4.16	———	———
Baltic	3.52–6.63	———	———
Baltic	2.0 –4.6	———	———
Wadden Sea	1.0 –8.0	0.10–0.60	6.0–27.3
Norwegian Sea	0.45–1.38	0.10–0.21	———
English Channel	———	———	2.0– 8.0
Black Sea	2.83–3.36	———	———
Caspian Sea	12.0	———	———
Sea of Azov	4.63–6.02	———	———
White Sea	2.3 –4.3	———	———
Greenland Sea	2.0 –2.1	0.03–0.38	0.9–29.0

[a] Values given for the same ocean twice were determined by independent investigations, after Duursma (1) and Hood (9).

Despite the low concentration, the soluble organic fraction affects the growth of marine algae, bacteria fungi, larvae, protozoa and some physical responses of marine worms, echinoderms, molluscs, crustaceans and fish (2–6).

Sutcliffe et al. (7) demonstrated that soluble organic material can be removed from filtered sea water by aeration or in the sea by bubbles. Aggregates containing 30% organic material and the remainder salt with much adsorbed phosphate were of a size usable as food by zooplankton. The nutritional value was studied by feeding one group of *Artemia salina* the aggregates and another group with dried yeast. The growth rate of *Artemia* fed on the aggregates was slower than that of the group fed on yeast, but the difference was small. So, it appears that soluble organics may be converted to particulate form by natural processes and used as food directly by zooplankton and benthic organisms.

Organic compounds in sea water can also be detrimental if certain toxic compounds occur in excessive concentrations. Nutrient trace elements may be chelated by soluble organics also, thus indirectly affecting the growth of organisms.

Vallentyne (8), Hood (9) and Wangersky (10) have reviewed the field of naturally occurring organic materials in solution and their significance, both biologically and geochemically.

Lipids and Organic Acids in Natural Waters

Since lipids are more resistant to biological attack than amino acids, proteins and carbohydrates (11), one might expect to find them in sea water, despite their low water solubility. Wilson and Armstrong (5) and Johnson (4) were the first to note brownish waxy matter adsorbed from sea water on activated carbon and eluted by acetone or benzene. Total concentrations of this material were reported to be 2.5 mg/liter for English Channel water. Creach (12) found citric and malic acids in littoral waters off southern France at concentration levels of 0.1 mg/litter Koyama and Thompson (13) found acetic, formic, lactic and glycolic acids in Pacific surface near Seattle, Washington. Jeffrey and Hood (14) reported some unidentified lipid material containing organic acids with an average molecular weight of 400 in an ethyl acetate extract of Gulf of Mexico water. Fatty acids in sea water were observed in an ethyl acetate extract of Gulf of Mexico water by Slowey et al. (15,16). Concentrations of the methyl esters of the fatty acids ranged from 0.1–0.8 mg/liter. Williams (17) reported values of 0.01 to 0.12 in Pacific waters as well as some unidentified hydroxylated carboxylic acids with an average molecular weight of 395. The extraction solvents were carbon tetrachloride and chloroform. Both investigators found fatty acids with chain lengths up to 20–22 carbons. Slowey et al. (15,16) noted on the samples analyzed that unsaturation and chain length of the acids apparently decreased with depth in the water column. Since the

technique of Williams and Slowey involved determining the fatty acid content of the total extract, they could not provide any information as to whether or not the fatty acids existed in the free state or as esters in sea water.

Other investigations on organic acids in saline waters other than sea water include those of Cooper (18) on fatty acid constituents of oilfield brines, formation waters, and rocks, both ancient and recent. His investigation showed the presence of saturated straight chain fatty acids in recent sediments, ancient sediments and petroleum reservoir waters. Acids containing odd numbers of carbon atoms were found along with those containing even numbers, but the even number carbon chains were predominant. However, there was an increase in odd carbon acids in ancient sediments over recent sediments. Shapiro (19) found a variety of organic acids in some freshwater lakes, and Mueller (20) determined some organic acids in river water, but fatty acids were not mentioned.

This paper is concerned with partial characterization of the solvent extracts of marine waters by means of column chromatography, thin-layer chromatography (TLC), infrared analysis and gas chromatography.

Experimental

Sample Preparation

Water samples were collected in lipid-free samplers attached to a hydrographic wire or by pumping through ½ in. tubing with a jet pump system which was also lipid-free. Sample sizes varied from 8 liters to 50 liters. The water was first filtered through a 0.45 μ millipore filtering system, also lipid-free, into a clean glass container where the pH was adjusted to 2.5–3.0 with 6N HCl. The water was then transferred to 6-liter separatory funnels; redistilled petroleum ether (bp 30–60C) was added in amounts of 1:10 by volume of solvent to water. The water and solvent were shaken for 3 min vigorously and when the emulsion cleared and the phases separated, the solvent was removed and the sample extracts were combined and washed twice with a small amount of distilled water to remove residual salt. The extract was then evaporated to near dryness in rotary evaporators at room temperature, transferred to a weighed vial and taken to dryness with gaseous nitrogen. After reweighing, the extract was diluted with about 0.5 ml petroleum ether and stored in the freezer until it was separated into lipid fractions by silicic acid column chromatography.

Fractionation of Lipid Extracts

The dried solvent extract of sea water was then fractionated into eight classes of lipids on a silicic acid column by elution: I paraffinic hydrocarbons, II unsaturated hydrocarbons, III sterol esters, IV triglycerides and fatty acids, V sterols, VI diglycerides, VII monoglycerides, and VIII phospholipids. Undoubtedly other compounds of similar polarity also are eluted off the column in these fractions. The elution method of Hirsch and Ahrens (21) was used. (See Table II).

Blanks and Reagents

Analytical grade solvents and reagents were used in all operations. All solvents were redistilled in all-glass stills fitted with four-foot Vigreaux columns.

TABLE II

Scheme of Elution of a Synthetic Lipid Mixture from a Silicic Acid Column [a]

Fraction number	Solvent mixture	Types of lipids eluted	Tube number
I	50 ml 1% ethyl ether in petroleum ether	Paraffinic hydrocarbons	1–3
II	75 ml 1% ethyl ether in petroleum ether	Squalene, beta-carotene, other unsaturated hydrocarbons	4–8
III	225 ml 1% ethyl ether in petroleum ether and 60 ml 4% ethyl ether in petroleum ether	Sterol esters, alpha-tocopherol	9–22
IV	240 ml 4% ethyl ether in petroleum ether and 200 ml 8% ethyl ether in petroleum ether	Triglycerides, followed by free fatty acids, fatty alcohols	23–65
V	450 ml 8% ethyl ether in petroleum ether and 50 ml 25% ethyl ether in petroleum ether	Unesterified steroids, Vitamin D$_3$, Vitamin A alcohol	66–98
VI	200 ml 25% ethyl ether in petroleum ether	Diglycerides, tributyrin	99–112
VII	300 ml pure anhydrous ethyl ether	Monoglycerides, Vitamin A acetate, lithocholic acid, chimyl alcohol	112–122
VIII	400 ml absolute methanol	Phospholipids, monoacetin	123–150

[a] Hirsch and Ahrens (21), and Sorrels and Reiser (22).

Three liters of redistilled petroleum ether yield an almost undetectable residue, which did not show infrared absorption. The solvent residue was also found to have negligible amounts of hydrocarbons, fatty acids, and other lipid fractions. Some light hydrocarbons, less than C-8 in chain length, appeared on the gas chromatograph, but no long chain compounds were detectable.

In order to determine if the Unisil column, the redistilled ethyl ether and methanol, and the glassware used contained a significant residue, the three liter petroleum ether residue was treated as a sample extract and fractionated on the Unisil column. Each fraction was then chromatogrammed on thin-layer plates and analyzed by infrared absorption and gas chromatography. The blank fraction residues were found to be negligible in comparison with the sample fractions, but the sample weights were corrected for the blank weights.

Analysis of Fractions

Thin-Film Chromatography

Chromatography of the residues of each of the fractions and subfractions on Silica Gel G coated glass plates in toluene/ethyl acetate (19/1), petroleum ether/ethyl ether (90/10) and cyclohexane (100%) was performed in order to see if (1) the column was fractionating the extract properly and (2) to obtain R_f values for the constituents. The R_f values were used not as proof of types of compounds present, but only as an additional piece of evidence. The dried plates were developed with 2,7-dichlorofluorescein, 50% sulfuric acid, or ninhydrin. It was quite evident from the TLC results that there is a variety of lipid-like compounds in sea water and that many of these are not the ordinary lipids found in organisms.

Infrared Analysis

An infrared absorption curve was obtained for each of the eight fractions of the lipid extract of sea water on a double-beam Beckman IR-4 with standard NaCl optics. Depending on the amount of material available and the solubility of the fraction, a pair of liquid cells or a pair of KBr discs were used to obtain the absorption curves. The curves indicated that the com-

TABLE III
Concentration of Lipids of Sea Waters from Various Locations

Location	Depth, M	Date	Concentration, mg/liter
Galveston, Texas Pier	1	June, 1963	9.10
Redfish Bay, Texas	1	May, 1963	7.70
Corpus Christi Bay, Texas	1	Dec., 1964	2.95
Laguna Madre, Texas	1	Dec., 1964	0.95
Port Aransas, Texas	1	Dec., 1964	1.29
Sigsbee Deep, Gulf of Mexico	10 900 3400	Aug., 1963	2.40
Peruvian Trench off coast of Chile	10 1500 4000	July, 1962	1.60
English Channel (offshore Plymouth)	1	Nov., 1963	0.60
North Sea	1	April, 1964	0.40

pounds suspected in the various fractions may be present, but since the fractions are mixtures of compounds of similar polarity, no really definitive conclusions can be made, except that in fraction I, the major components are indeed paraffinic hydrocarbons and that methyl, methylene, hydroxyl and other groups are present in fractions V, VI, VII, and VIII.

Chemical Analyses

Elemental analyses of the lipid extracts of sea water revealed that they contain carbon, hydrogen and oxygen primarily, but some nitrogen and phosphorus was present in fraction VIII, the phospholipid fraction.

Qualitative solubility tests in concentrated sulfuric acid, water, petroleum ether and ethyl ether were made on the fractions to confirm the presence of hydrocarbons, esters and acids.

The Liebermann-Burchard test for sterols was positive for fractions III and V which supposedly contain sterol esters and sterols. Spray tests for phospholipids were made by spraying the developed thin layer chromatograms with ninhydrin and/or Dragendorf's reagent (23). Free amino groups were present only in fraction VIII and the Dragendorf test for phospholipids was also positive for this fraction.

Gas Chromatographic Studies of Fractions

Fraction I, containing saturated aliphatic hydrocarbons, was further fractionated by chromatography at both 160C and 220C on a ¼ in. × 8 ft 5% SE-30 on an Anakrom ABS 70/80 mesh column with helium flow of 60 ml/min. A GC-2 Beckman Gas Chromatograph with a flame ionization detector was used. Undiluted hydrocarbon samples (0.1–0.5 μl) were injected into the packed column. Identification of a few of the peaks was obtained from their relative retention times and those of a standard mixture of C-10 to C-18 hydrocarbons.

TABLE IV
Variation of Lipid Concentration with Season

Location	Concentration mg/liter	Date
Port Aransas, Texas	6.10	May, 1962
10 miles offshore	1.29	Dec., 1964
Panama City, Fla.	1.40	Aug., 1963
offshore platform	0.20	Jan., 1965
11 mi'es offshore	0.30	Mar., 1965

The fatty acid content of fractions III, IV, VI, VII, and VIII was investigated by gas chromatography of their methyl esters prepared by a modified method of Stoffel et al. (24), in which 2% H_2SO_4 in absolute methanol was used instead of dry HCl for transmethylation of the esters and methylation of the free fatty acids present. The fatty acid esters were also chromatogrammed on the 8 ft 5% SE-30 column but at 220C, using the flame ionization detector with a gas flow of 60 ml/min. Identification of each methyl ester was made by comparison of their relative retention times to those of known methyl ester mixtures. The percent of each ester was determined by the ratio of its peak area to the total area of all peaks.

Before methyl esters were prepared, 0.5 μl of each fraction was inserted into the column at 160C and 220C to see if any volatile constituents were present. Except for Fractions I and II, there were no constituents volatile at these temperatures.

Results and Discussion

Concentration and Distribution

The concentration of the lipids of sea water is variable, both vertically and horizontally, and seasonally in the surface layers of the ocean (see Table III for total apparent lipid concentrations at various locations). The higher concentrations are found in nearshore waters, in bays, and surface water of the continental shelf where biological productivity is high. The lipids apparently do not accumulate in the sea, for at fixed locations the concentrations vary considerably with season, as shown in Table IV. The relative percentages of the eight lipid fractions also varies from station to station. Table V shows some typical examples.

Description of Lipid Fractions

Fraction I. From all available tests, this fraction is primarily a mixture of heavy paraffinic hydrocarbons. The material was not soluble in three volumes of water, ether, or concentrated sulfuric acid. Infrared spectra of this fraction showed absorption only at 3.42, 3.48, 6.80 and 11.20 μ. Comparison of the infrared curves of this fraction with those for marine sediments, published by Orr and Emery (25), indicated close similarities. Gas chromatographic results indicated that C-12, C-14, C-16, C-18, C-20, C-22,

TABLE V
Concentrations of Total Solvent Extracts of Sea Water and the Eight Fractions Separated by Column Chromatography

Station	Depth (m)	Concentration of Fraction in mg/liter								Total mg/liter
		I	II	III	IV	V	VI	VII	VIII	
Port Aransas, Texas Institute of Marine Science Pier	5	0.82	0.08	0.10	2.08	1.26	0.34	0.88	0.54	6.10
Galveston, Texas Jetty	5	0.40	0.60	2.30	1.90	1.99	0.51	0.40	0.99	9.09
Panama City, Fla. Offshore Platform	5	0.80	0.16	0.16	0.20	0.10	0.09	0.10	0.10	1.71
Sigsbee Deep, Gulf of Mexico	10	0.08	0.10	0.20	0.45	0.21	1.21	0.16	0.12	2.53
	900	0.26	0.31	0.42	0.26	0.37	0.95	1.06	0.24	3.87
	3400	0.17	0.29	0.25	0.16	0.13	0.19	0.53	0.42	2.14
South Pacific (open ocean off Chile)	10	0.50	0.20	0.07	0.20	0.30	0.80	0.30	0.10	2.47
	1500	0.50	0.15	0.10	0.12	0.15	0.10	0.20	0.25	1.57
	4000	0.56	0.20	0.35	0.25	0.24	0.20	0.20	0.30	2.30
South Pacific (near Chilean coast)	1000	0.16	1.12	0.70	0.32	0.47	0.11	0.14	0.93	3.90
	2000	0.32	0.45	0.09	1.90	0.12	0.15	0.13	0.38	3.50
	3000	0.28	1.20	0.13	1.45	0.09	0.11	0.23	0.32	3.80

TABLE VI

Fatty Acid Distribution with Depth at Various Locations

Station	Depth (m)	Fatty acid concentration (mg/liter)	Date of collection
Sigsbee Deep (Gulf of Mexico)	10	0.46	May, 1963
	900	0.26	
	2250	0.13	
	3400	0.16	
North Pacific P-35	10	1.35	April, 1962
	750	0.90	
	1000	0.70	
Peruvian Trench (South Pacific)	25	0.21	July, 1962
	1500	0.40	
	5500	0.51	

and C-24 paraffins were present. R_f values of this fraction on silica gel plates in both toluene/ethyl acetate (19/1) and cyclohexane were 1.0, indicating paraffinic hydrocarbons.

Fraction II. Presumably this fraction contains unsaturated hydrocarbons, but this has not been substantiated. This fraction from sea water had an R_f value of 0.9 in all cases, but its infrared absorption spectrum varied considerably from station to station.

Fraction III. There is evidence to show that sterol esters occur in this fraction. Development of a bluish-purple spot on thin-film silica gel plates with a 50% sulfuric acid spray corresponded to the R_f range of known sterol esters. This fraction also gave a positive Liebermann-Burchard test for sterols. When this material was transmethylated, fatty acid esters were detected by gas chromatography (see Table VII for a typical example).

Fraction IV. This fraction contained fatty acids and triglycerides and possibly other compounds. Gas chromatography of the fatty acid esters of this fraction indicated the presence of a large number of fatty acids with oleic and palmitic acids generally predominant (see Table VII). Fraction IVA indicates the predominantly free fatty acid portion and IVB the predominantly triglyceride portion of fraction IV. This fraction has a range of R_f values in toluene/ethyl acetate (19/1) of 0.10 to 0.90. The infrared spectra indicated acid and ester groups in addition to the usual methyl and CH_2 groups. Table VI shows the variations of fraction IV (fatty acid) concentrations at various depths and locations. Generally the concentrations decrease with depth, but in some cases, i.e., in areas of upwelling, they increase with depth.

Fraction V. Evidence that free sterols are present in this fraction consists of a positive Liebermann-Burchard test and development of a purple spot with an R_f value of 0.25 in toluene/ethyl acetate (19/1) with 50% sulfuric acid as a spray. Infrared spectra were not conclusive for sterols since other materials were also present.

Fraction VI. This fraction appeared to contain diglycerides from its R_f values, and infrared absorption. Fatty acids were also present, but in esterified condition. Table VII shows a typical fatty acid content of this fraction. Fraction VI generally had more long-chained fatty acids, i.e., C-18 to C-22 than fractions III and IV.

Fraction VII. Monoglycerides and some unknown acidic compounds occurred in this fraction. Infrared analysis showed hydroxyl, acid and ester groups. Gas chromatography of methyl esters indicated the presence of the usual fatty acids (Table VII) and some unidentified long chain acids. TLC of this material in toluene/ethyl acetate (19/1) yielded low R_f values of 0.0 to 0.10.

TABLE VII

Percentage Fatty Acid Composition of Fractions III, IVA, IVB, VI, VII, and VIII of an Extract of a 15-Gallon Sample Taken in Surface Offshore Water at 27° 47'N, 96° 56'W Near Port Aransas, Texas

Carbon No.	Fraction No.					
	III	IVA	IVB	VI	VII	VIII
	Percent					
<10:0			1.80	5.10	2.21	36.74
10:0		0.09	0.49	0.71	0.71	
11:0				1.23	0.45	
12:0		0.32	3.71	6.76	5.25	4.79
13:0	3.95		0.38	0.78	0.89	
14:0	4.36	3.91	2.38	1.69	1.61	8.62
14:1		0.26				0.76
15:0	1.65	3.13	0.42			0.96
16:0	21.48	17.14	5.41	8.51	4.03	11.51
16:1	7.67	0.16	1.42			
16:2	3.38	0.32				
16:3		4.52	0.66			
17:0	4.95		0.14		2.32	
18:0	5.95	10.00	7.09	2.12	8.92	7.18
18:1	14.06	52.68	26.60	11.36	10.87	7.82
18:3	3.95	1.42	1.81	2.60		3.96
18:4		3.67	0.73			
19:0	21.14				2.88	
20:0	4.10	1.06				
20:2		0.98	15.20	27.18		
20:3		0.16	0.36	2.82		3.20
20:4	3.30	0.16	1.82		6.0	
20:5			8.24	3.37	17.86	4.83
22:4				3.14		
22:5						9.50
22:6				2.70		
<20 unidentified			21.40	19.94	36.02	

Fraction VIII. Positive indications that phospholipid-like substances are present in this fraction is the phosphorus and nitrogen content, the R_f value of 0.0 on thin layer silica gel plates in toluene/ethyl acetate (19/1), a positive ninhydrin test, a positive Dragendorf test, and the presence of esterified fatty acids as indicated by gas chromatography (Table VII). The infrared spectrum of this fraction was rather complex but was rather uniform from sample to sample. Some neutral nonphospholipid material was also present as evidenced by TLC of this fraction in chloroform-methanol (10/1).

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