Bromine Content of Lipids of Marine Organisms

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ABSTRACT

Lipids were extracted from a number of organisms taken in the northern Pacific ocean and the bromine content measured by neutron activation analysis. The lipids of each species studied were found to contain bromine with concentrations ranging from ten to several hundred µg/g. The major portion of the bromine was associated with the fatty acids in chromatographic separations; however, the presence of a number of different brominated compounds was indicated. When the fatty acids were prepared from the crude lipids by acid hydrolysis, partitioned into base, and then esterified, 60% of the initial bromine was recovered in the ester fraction. This would indicate the presence of brominated acids in marine lipids.

INTRODUCTION

Significant concentrations of bromine have been detected in lipids extracted from a number of marine organisms taken in the Scandinavian region (1). A small proportion of this bromine was associated with volatile constituents (2). Fractionation of the crude lipid on silica gel and analysis or detection by neutron activation established that the bromine was present in all fractions with the highest concentrations being associated with sterol esters and fractions containing triglycerides, free fatty acids, and sterols (1). This general distribution could result from the presence of brominated fatty acids, and it was observed that significant proportions of the bromine were retained in the fatty acid fraction after saponification (1). It has also been observed that the bromine content of different marine oils was not affected by storage or such processes as alkali refining or bleaching. These observations suggest that the bromine-containing compounds are reasonably stable; however, most of the bromine was lost on catalytic hydrogenation (3).

The impetus for this type of study is the concern, on the one hand, for documenting the distribution of pollutants and the possibility, on the other hand, of discovering some naturally occurring compound of pharmaceutical significance. A recent review in the pharmacognozy literature (4) lists some 50 brominated compounds identified primarily in marine organisms, such as mollusks, "red" seaweed, and sponges. These include phenols, pyrroles, indoles, and terpenoid compounds. Although naturally occurring brominated compounds have been considered a rarity, it should not be unexpected in the marine environment since sea water contains significant concentrations of bromide ion (~ 1 millimolar) and brominating enzyme systems have been observed in marine organisms (5).

In regard to marine lipids generally, it is important to

TABLE I

Bromin Concentration of Lipids of Marine Species Taken Off Newport and Astoria, Oregon

Species	Newport µg Br/ g oil	Newport mg lipid/ g tissue	Newport µg lipid Br/g tissue	Astoria µg Br/ g oil	Astoria mg lipid/ g tissue	Astoria µg lipid Br/g tissue
Chinook salmon	11.7	55.8	0.65	35.3	196	6.92
Oncorhynchus tshawytscha	17.5					
Coho salmon	24.5	64.3	1.58	49.6	30.5	1.51
Oncorhynchus kisutch	6.48					
Lingcod	31.1	9.8	0.30	60.8	31.1	1.89
Ophiodon elongatus	32.6					
Pacific halibut	42.2	38.5	1.62			
Hippoglossus stenolepis	49.1					
English sole	92.4	18.2	1.68	487	16.6	8.08
Parophrys vetulus						
Rock greenling	74.5	16.9	1.26			
Hexagrammos lagocephalus						
Pacific sand dab	64.9	27.8	1.80			
Citharichthys sordidus						
Sand sole	52.1	17.2	0.89			
Psettichthys melanostictus						
Buffalo sculpin	65.4	10.4	0.89			
Enophrys bison	65.8	12.0	0.79			
Dungeness crab	44.4	11.1	0.49			
Cancer magister						
Pacific oyster	43.4	20.1	0.87			
Crassostrea gigas						
Pacific hake				934	11.5	10.7
Merluccius productus						
Petrale sole				153	12.9	1.97
Eopsetta jordani						
Dover sole				140	6.27	0.88
Microstomus pacificus						
Rex sole				105	32.2	3.38
Glyptocephalus zachirus						
Pacific cod				126	5.92	0.75
Gadus macrocephalus						

establish how general a phenomenon this is – do they all contain bormine? Also, the identity of these compounds should be established to clarify whether they are of natural or man-made origin. This study reports the bromine content of lipids from organisms taken in the northern Pacific and provides additional evidence that marine lipids may contain brominated fatty acids.

EXPERIMENTAL PROCEDURES

Samples

Most samples were obtained from Newport or Astoria on the Oregon Coast. Samples were obtained fresh, transported in ice and extracted as soon as possible after returning to the laboratory. Tissues from the marine mammmals were frozen prior to transporting to the laboratory.

Extraction Procedures

All tissue samples were extracted by conventional chloroform-methanol procedures (6).

Bromine Analysis

Lipid samples (100-200 mg.) were sealed under nitrogen in a ½ dr. vial. The samples were activated for 1-2 hr in the O.S.U. TRIGA reactor operating at one megawatt. After standing for 1-2 days, the samples were counted at 777 keV for 20-80 min, depending on the bromine concentration. Methyl dibromostearate added to olive oil was used as a standard.

To avoid contamination the vials were thoroughly washed in nitric acid, distilled water, and then acetone. During irradiation the sample vials were placed inside a larger vial to minimize the possibility of contamination in the reactor. The vials were found to contain small quantities of bromine. Since it was not possible to remove the sample from the vial prior to counting (some of the sample adsorbs to the vial), the endogenous level in the vial was taken into account in subsequent calculations. Bromine levels in the extracting solvents and reagents were also checked. It was possible to detect < 0.05 μg of bromine with good reproducibility using these procedures.

Base Partitioning

To establish how much of the bromine was associated with the fatty acid fraction, it was necessary to separate the fatty acids from the nonsaponifiable material. One cannot use a simple base-catalyzed hydrolysis because at the elevated temperatures required bromine could be lost (up to 50% with an ester of dibromostearate) by substitution and elimination reactions. The procedure outlined below has been followed to accomplish this separation: (a) the crude lipids were hydrolyzed at 80 C for 40 min with concentrated hydrochloric acid in acetone; (b) free fatty acids were extracted with hexane-ether and the solvent removed; (c) the soaps were formed by treating the fatty acids at room temperature with 60% potassium hydroxide in methanol; (d) an aqueous solution of the soaps was extracted several times with chloroform to provide the nonsaponifiable fraction; (e) the chloroform extract was extracted with aqueous methanol to remove any of the soaps that may have been carried over, and this fraction was returned to the aqueous solution containing the soaps; (f) the aqueous solution containing the fatty acid soaps was acidified and extracted with hexane-ether; (g) the fatty acids were then methylated with methanolic HCl; (h) the bromine content of the different fractions was then determined.

TABLE II

Bromine Concentration of Lipids of Marine Mammals

Species	µg Bromine/g Muscle	µg Bromine/g Fat
Bearded seal *Erignaphus barbatus*	10.1	—
Ringed seal *Phoca hispida*	46.0	—
Walrus *Odobenus rosmarus*	31.7	—
Elephant seal *Mirounga angustirostris*	50.8	9.4
Dolphin *Delphinus delphinus*	62.4	61.4
California gray whale *Eschrichtius gibbosus*	18.5	28.5
Harbor seal *Phoca vitulina*	20.9	—
	Liver	
California sea lion *Zalophus californianus californianus*	495	

RESULTS AND DISCUSSION

Bromine Content of Marine Lipids

Bromine was detected in the lipids of all organisms analyzed (Tables I and II) with the concentrations varying widely among the different samples. These observations would complement those of Lunde (1), and it would seem reasonable to conclude that the presence of bromine in marine lipids is a general phenomenon. Concentrations observed in lipids from different species cannot be considered typical of that species or the region where it was taken. In this exploratory study, the sampling was not comprehensive enough on a temporal or geographical basis to allow such conclusions.

Marine mammals such as the seal or porpoise occupy the higher trophic level in that environment and may be expected to be an integrating organism to measure the accumulation of persistent, naturally occurring or man-made compounds. With the exception of the concentration of bromine observed in the liver lipid of the California sea lion, bromine levels in the tissue lipids of these animals were not particularly high. The sea lion also contains high levels of chlorinated hydrocarbons, both polychlorinated biphenyls and DDE, a metabolite of DDT (7). These high residues in the sea lion, which live and feed along the Pacific coast of the U.S., are associated with the probability of exposure to man-made contaminants. Whether man-made components are contributing to the bromine levels in marine lipids will be resolved once the identity of these compounds is established.

TABLE III

Distribution of Bromine in Marine Lipid

	Recovery following methylation		
	mg	µg Br/g Lipid	Total Br-µg
Crude lipid	100	10.8	1.08
Fatty acid methyl ester	90.2	12.2	1.10

TABLE IV

Distribution of Bromine in Fatty Acid Ester Fraction Derived from 100 mg Crude Lipid

Column fraction	Mass of lipid mg	Total bromine μg	μg Br/g Lipid
5% Acetone/hexane	82.9	.907	10.9
	.19	.0048	25.2
Acetone TLC[a]	.18	.0028	15.8
	.21	.0048	22.8
	3.04	.104	34.1
$CHCl_3$/MeOH	3.76	.0838	22.3
	90.2	1.03	

[a]15% ether in hexane.

TABLE V

Distribution of Bromine between Fatty Acid and Nonsaponifiable Fractions of Marine Oils

	Mass - mg	Bromine ppm	Bromine μg
1. Ling Cod			
Lipid extract	102.4	322 ± 14	32.9 ± 1.4
Fatty acid ester	40.9 ± 2.7	479 ± 45	19.6 ± 2.2
Nonsaponifiable	37.5 ± 8.8	202 ± 55	7.3 ± 1.4
2. Flounder			
Lipid extract	105.1	96.1 ± 1.4	10.1 ± 0.20
Fatty acid ester	59.1 ± 3.2	114.7 ± 1.6	6.78 ± 0.36
Nonsaponifiable	32.3 ± 1.2	115.6 ± 3.7	3.73 ± 0.11

Distribution of Bromine in Lipid Fractions

When a crude lipid extract from a sample of hake was transesterified with methanolic HCl, especially all the bromine was recovered in the methyl ester fraction (Table III). This fraction was further partitioned on a silicic acid column and on a thin layer plate (Table IV). The major proportion (81%) of the bromine was recovered in the least polar fraction, which would be comprised primarily of fatty acid methyl esters. This evidence is suggestive but not conclusive for the presence of brominated fatty acids since other nonpolar components could be included in this fraction. The highest bromine concentration was observed in one of the more polar fractions. The bromine in marine oils is thus associated with a number of compounds.

When crude lipids from either cod or flounder were hydrolyzed and partitioned against base to extract the fatty acids, 60% and 67%, respectively, of the initial bromine was recovered in the fatty acid esters (Table V). The recovery of the bromine was good – 82% in the case of the cod and 100% with the flounder. One can conclude from these data that these oils contained brominated acids. Further studies are in progress to establish the identity of these compounds.

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