The Presence of Volatile, Nonpolar Bromo Organic Compounds Synthesized by Marine Organisms

GULBRAND LUNDE, Central Institute for Industrial Research,
Forskningsv. 1, Blindern, Oslo 3, Norway

ABSTRACT

The presence of volatile, nonpolar bromine-containing compounds in marine organisms is demonstrated. These compounds represent, especially in tissue containing a high fat content, ca. 0.1-1.0% of the total amount of bromo organic compounds present in marine oils. In tissue with a low fat content, a higher concentration of bromo organic compounds is found. It is concluded that these compounds are probably synthesized in one or more stages in the marine food chain. These compounds may follow and disturb the analyses when isolating and determining chlorinated hydrocarbons originating from industrial and other sources of pollution.

INTRODUCTION

It has been shown that marine oils contain lipid soluble bromo organic compounds (1-3). The content of organic bound bromine varies between 3 and 50 ppm. When samples of marine oils are fractionated on a silica gel column using mixtures of chloroform and methanol as eluting agents, bromine is found in all the groups of different components fractionated. It does not seem that the bromine is localized to any particular group of compounds. It is concluded that marine organisms are able to synthesize lipid soluble bromo organic compounds (3).

In connection with contaminants that are released from industrial and other sources of pollution, extensive studies involving the analysis and characterization, especially of compounds consisting of chlorinated aliphatic and aromatic hydrocarbons, have been initiated. These compounds are in the main lipid soluble and when present in the marine environment they tend to be stored and also enriched in the lipid phase in marine organisms. Although the effect of many of these compounds is not known, one must assume that they will be harmful when present even in small quantities. This applies particularly to those organs such as fish liver that, in addition to being a storage place for fat, also have a high enzyme activity.

Most of these compounds may be enriched by steam distillation using a nonpolar solvent such as cyclohexane to collect the distilled compounds.

The presence of halogenated compounds has been demonstrated, mainly by gas chromatography (GC), where an EC-detector (electron capture) has been used. By connecting the GC with a mass spectrometer (MS) it is possible to identify the various components. In order to carry out such an identification ca. 10 ng or more of each compound is required. Using a gas chromatograph with an EC-detector only, it is not possible to distinguish between chlorinated, brominated, iodinated or other EC-sensitive organic compounds. Among the latter some esters, ketones, nitro compounds and thioles should be mentioned. An identification of the different compounds depends here on available standards.

Relating to the pollution aspect, it should be of interest to determine whether there exist volatile compounds among the lipid soluble bromo organic compounds, which marine organisms are able to synthesize themselves. They may then be detected under the same conditions applying to the detection of the chlorinated hydrocarbons from pollution sources.

Such a hypothesis could be confirmed if one were able to demonstrate the presence of bromo organic compounds among the volatile organic compounds that can be steam distilled from marine organisms. Following such a distillation the absolute quantity of bromine present can be determined by neutron activation of the cyclohexane phase.

TABLE I

The Bromine Content (ppb in the Lipid Phase) in the Volatile, Nonpolar Fraction of Marine Organisms

Organism	Sample		Locality	Yield of oil, %	Br μg/kg oil, ppb	
					1 Dist.	2 Dist.
Cod liver oil	*Gadus morhua*	Oil	Northern Norway, 1924	100	4	3
Cod liver oil	*Gadus morhua*	Oil	Northern Norway, 1940	100	5	2
Cod liver oil	*Gadus morhua*	Oil	Lofoten Norway, 1960	100	4	2
Cod liver oil	*Gadus morhua*	Oil	Lofoten Norway, 1969	100	5	2
Cod	*Gadus morhua*	Filet	Western Norway, 1971	0.4	63	68
Cod	*Gadus morhua*	Filet	Western Norway, 1972	0.4	50	50
Cod	*Gadus morhua*	Liver	Western Norway, 1972	55	3	1
Cod	*Gadus morhua*	Filet	Lofoten Norway, 1972	0.4	130	44
Cod	*Gadus morhua*	Liver	Lofoten Norway, 1972	61	2	1
Mackerel	*Scomber scomber*	Filet	Southern Norway, 1969	20	8	4
Halibut	*Hippoglossus hippoglossus*	Filet	Helgeland Norway, 1971	9.3	6	4
Halibut	*Hippoglossus hippoglossus*	Filet	East of Greenland, 1971	10.5	10	8
Capelin	*Mallotus villosus*	Whole fish	Northern Norway, 1969	9.2	25	7
Capelin	*Mallotus villosus*	Whole fish	Northern Norway, 1972	9.3	5	<0.2
Herring	*Clupea harengus*	Whole fish	Langesund Fiord, 1971	8.8	7	2
Shrimp	*Pandalus borealis*	Whole fish	Oslo Fiord, 1969	0.4	75	82
Mussel	*Mytilus edulis*	Whole animal	Trondheim Fiord, 1971	16[a]	0.9	<0.3
Seaweed	*Laminaria hyperborea*	Whole plant	Western Norway, 1971	3.2	557	358
Seaweed	*Ascophyllum nodosum*	Whole plant	Western Norway, 1971	3.1	592	227

[a]Dried material.

This analytical method is significant and has a high sensitivity for bromine and the other halogens. When the amount of each halogenated compound is too low to make an identification with a coupled GC-MS the method for determining if the compounds giving response on the EC-detector also contain halogens will be of special interest.

Some preliminary GC and GC-MS analyses were carried out on cyclohexanedistillate from seaweed *(Laminaria hyperborea)*, shrimp and cod liver oil (1940). More than 50 compounds were detected with EC-detector, but no one present in sufficient amount for MS analyses contained bromine, supporting the hypothesis that the bromine is distributed on many volatile compounds.

EXPERIMENTAL PROCEDURES

Various samples of fish, seaweed, shrimp and shellfish as well as a series of cod liver oils were included in the experiments. One of the oil samples was produced in 1924, the other in 1940, 1960 and 1969, respectively. Table I should be consulted for further details on the samples.

The cod liver oil, 100-200 ml, was mixed with distilled water in the ratio of 1:5 in a spherical 2 liter flask, the latter being connected to a conventional distillation apparatus. Five milliliters cyclohexane was added and the solution was then heated to boiling. The cyclohexane together with ca. 50 ml of water was distilled over. The solution was cooled to below 50 C, a further 5 ml of cyclohexane was added and the distillation was repeated a second time, now collecting 5-10 ml of water. The function of the last added cyclohexane is essentially that of flushing out remnants of the volatile nonpolar compounds in the apparatus into the receiving flask. Each sample was distilled once more following the same procedure, and the two distillates were kept separate in the subsequent work.

The extraction of volatile organic compounds from the fish samples and the other raw materials was performed by the same distillation procedure as that used for cod liver oil. The samples were homogenized, and distilled water was added to give a ratio of one part of raw material to two parts of water.

After distillation the cyclohexane phase was collected, and 25 ml distilled water was added and redistilled. This is to remove any remaining inorganic ions, in particular bromide ions, which could have contaminated the distillate and disturbed the subsequent analysis. The total amount of lipids in these samples was determined by chloroform-methanol extraction.

The bromine content of the cyclohexane solution was finally determined by neutron activation analysis. One milliliter of the cyclohexane solution was transferred to a quartz ampoule previously treated with concentrated nitric acid and washed in distilled water. The ampoule was sealed, and together with a bromine standard irradiated with a neutron flux of ca. 5×10^{12} n/cm^2 sec in a nuclear reactor for 2 hr. Following irradiation the samples were transferred to nonactive glass vials and their activity measured using a multichannel γ-spectrometer without any prior chemical treatment.

RESULTS AND DISCUSSION

All the neutron-activated cyclohexane solutions gave nearly pure bromine spectra when their activity was measured ca. 1 day after irradiation. If these solutions also contain corresponding chloro- and iodine-organic compounds, then the chlorine isotope Cl-35 with a half life of 37.3 min and the iodine isotope I-128 with a half life of 25 min will be formed when they are subjected to neutron irradiation. These radioactive isotopes can be detected using a γ-spectrometer, if the measurements are carried out immediately after the irradiation, i.e., within the time of a few half lives. In a few samples only traces of the radioactive sodium isotope Na-24 were detected. Contamination due to the presence of inorganic ions can therefore be regarded as negligible.

The results of the bromine determinations in the two distillations together with the content of lipids extractable with chloroform-methanol are given in Table I. The amount of volatile bromo organic compounds is given as μg Br/kg extractable lipids (ppb). The amount of bromine found in the distillate from fish raw material represent ca. 0.1-1% of the total bromine content of the marine oils. This is especially the case for cod liver oil and the fat fish. In cod fillet there seems to be a certain accumulation of the bromine-containing compounds compared with the liver. Also the other samples with a low content of lipids have a higher concentration of these compounds. When evaluating the considerable higher bromine content in the two distillates produced from brown algae, it should be borne in mind that the content of bromine found in the lipids extracted from seaweed is higher here than in marine fish (3).

The results obtained from the analysis of cod liver oil should be regarded as minimum values, as some of the volatile compounds may have evaporated during the production of the oil.

The presence of bromo organic volatile compounds in all the samples analyzed, especially in the samples from 1924 and 1940, indicate that the marine organisms are able to synthesize this type of compound. The presence of bromo organic compounds originating from pollution sources together with chlorinated hydrocarbons cannot be disregarded, but the contribution of such compounds is probably negligible compared with compounds originating from marine organisms, especially in relatively uncontaminated areas. Based on earlier results where it has been demonstrated that no one or no group of soluble bromo organic compounds dominate in the lipid phase in marine organisms, it is also probable that several volatile bromo organic compounds are present. This is also demonstrated by comparing the content of the bromo organic compounds found in the first distillate with that in the second (Table I). In most of the samples analyzed there is a certain reduction of the bromo organic compounds from the first to the second distillate, indicating the presence of both volatile and less volatile compounds.

One of the conclusions that may be drawn from these findings is that most of these bromine-containing compounds will probably pass the usual methods of sample clean-up employed in analysis for chlorinated hydrocarbons of man-made origin in the environment, and they will be detected by electron capture gas chromatography. The possibility of their being mistakenly identified as pollutants with long biological half lives therefore exists. Furthermore the ubiquitous occurrence of these brominated hydrocarbons among all classes of organic compounds indicates that their mechanism(s) of formation is not very specific, and that chlorine may be incorporated as well.

REFERENCES

1. Lunde, G., Int. Rev. Gesamten Hydrobiol. 52:265 (1967).
2. Lunde, G., JAOCS 48:517 (1971).
3. Lunde, G., Ibid. 49:44 (1972).

[Received September 12, 1972]