Analysis of Arsenic and Bromine in Marine and Terrestrial Oils

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ABSTRACT

Samples of marine and terrestrial oils of both plant and animal origin have been analyzed for arsenic and bromine content. Two oil samples (cod liver oil and oil extracted from mackerel fillets) were fractionated on silica gel columns and bromine was determined in the different fractions. The results obtained indicate that lipid soluble bromine and arseno organic compounds are characteristic components of marine animal and marine plant oils (seaweed). The results also show that the bromine is not localized in any particular compound or type of compounds. The bromine-containing compounds seem to be relatively stable, but the arseno-containing compounds are not. When oils containing arsenic and bromine were saponified, some of the arsenic and bromine compounds were found in the fatty acid fraction while others appeared in the water soluble fraction.

INTRODUCTION

It has been shown in previous work (1-3) that lipids extracted from various marine organisms contain arsenic and bromine as organic compounds. In analyses made on lipids from whale and fish, the contents of these elements were found to vary between 3-25 ppm for arsenic and 1-50 ppm for bromine. It has also been shown that arsenic is present as two or more lipid soluble arseno organic compounds in herring (4). Bromine and arsenic are likewise found in the lipid phase of fresh water fish (3,5). Bromine is also present in lipid extracts from fresh and salt water algae, 20-150 ppm and 10-15000 ppm respectively (3). Arsenic was not determined in these samples.

The results obtained so far indicate that the presence of arseno and bromine organic compounds is characteristic of oils produced from marine and fresh water fish. The purpose of this work was to analyze oils from other species of both terrestrial and marine origin in order to see if this assumption has a more general validity. In addition to analyzing, these oils—some in which both arsenic and bromine were present—were saponified so that the fatty acids could be isolated and analyzed separately.

It was also of interest to ascertain whether bromine was present as one or more specific bromine organic compound. Such a study was undertaken by fractionating some of the oil samples and analyzing the different fractions. By calculating the bromine as parts per million (μg Br/g oil), it should be possible to obtain an indication as to whether this element was present as one or a few discrete compounds, i.e., present in one or a few fractions, or whether it is more distributed in several or all of the different compounds in the oil.

EXPERIMENTAL PROCEDURE

Methods

The production and saponification of the oils were carried out using conventional methods. In some samples where little material was available and where the yield of oil was low, the saponification was carried out directly on the raw material.

Analyses for bromine and arsenic were made using neutron activation. Such analyses may be made without chemical treatment of the sample (oil and fatty acid) after the activation, providing that after activation they do not contain trace elements that will interfere with the detection of the induced radioactivity of the arsenic and bromine. Among elements which do interfer, sodium should be emphasized. The content of sodium and other inorganic ions may, however, be reduced by washing with distilled water. When little material is available, a solution of the material in a suitable solvent may be washed. Some solvents appear to have an affinity to inorganic ions, and may carry the ions into the oil phase where they are left behind when the solvent is evaporated. Chloroform seems to be such a solvent, and should be avoided (unpublished results obtained in the author's laboratory). Among the solvents which may be used are diethylether and toluene. By this method it is also possible to analyze small amounts of material (milligram amounts) without taking any special precautions. Transfer of the samples before and after the neutron activation should be accomplished with the aid of a suitable solvent. Further details concerning activation analyses of oils are discussed elsewhere (3,4,6).

The fractionation of the two oil samples was made on silica gel columns according to the directions given by Barron and Hanahan (7).

Materials

The raw materials used for the production of the oil samples were obtained from the local fish and meat market and also through the kind assistance of other laboratories.

TABLE I

Eluting Agent and Characterization of the Main Fractions From Chromatography of Fish Lipids on Silicic Acid

Fraction	Eluting agent	Amount, ml	Characterization of main components
A	Hexane (H)	150	Hydrocarbons
B	15% Benzene in H	400	Sterol esters
C	5% Diethylether in H	1330	Triglycerides and free fatty acids
D	15% Diethylether in H	950	Free sterol
E	30% Diethylether in H	600	Diglycerides
F	50% Diethylether in H	600	Diglycerides–monoglycerides
G	Diethylether (D)	600	Monoglycerides
H	3% Methanol in D	250	Phospholipids ?
I	5% Methanol in D	150	Phospholipids
J	80% Methanol in D	250	Phospholipids
K	Methanol	100	Phospholipids

TABLE II

Bromine and Arsenic in Oil and Fatty Acids Extracted From Marine Fishes and Invertebrates

Sample			Locality	Br, ppm		As, ppm	
				Oil	Fatty acid	Oil	Fatty acid
Capelin	*Mallotus villosus*	whole fish	Northern Norway	9.2	8.1	12.1	6.3
Herring	*Clupea harengus*	whole fish mature	Western Norway	5.8	2.2	13.8	9.2
Herring	*Clupea harengus*	whole fish	Skagerak	2.6	2.7	19.3	12.1
Mackerele	*Scomber scomber*	fillet	Southern Norway	2.8	3.1	8.2	4.1
Mackerel	*Scomber scomber*	liver	Southern Norway	16.5	8.5	13	6.2
Cod[a]	*Gadus morrhua*	liver	Western Norway	34	12	8.4	6.1
Cod[a]	*Gadus morrhua*	liver	Western Norway	36	9.2	9.9	7.2
Cod	*Gadus morrhua*	liver	Western Norway	26	6.8	10	6.0
Plaice	*Pleuronectes platessa*	fillet	Southern Norway	17.3	7.6	6.1	5.2
Clam	*Pecten maximum*	whole animal	Northern Norway	15	7.8	4.8	1.9
Squid	*Ommatostrephes sagittatus*	whole animal	Northern Norway	---	3.9	---	0.7
Starfish	*Asterias rubens*	whole animal	Oslo Fiord Norway	14.6	6.4	9.1	7.5
Shrimp	*Pandalus borealis*	whole animal	Oslo Fiord Norway	17	13.0	10.1	4.8
Lobster	*Homarus vulgaris*	whole animal	Southern Norway	50	≈3	4.7	≈3
Mussel	*Mytilus edulis*	whole animal	Western Norway	137	35	18	22
Snail	*Littorina littorea*	whole animal	Western Norway	294	82	84	32

[a]From the same locality.

Some of the vegetable raw oils were factory produced.

Treatment of the Samples

All samples were homogenized in a mechanical blender, and extracted with two volumes of chloroform-methanol 2:1. The extractions were carried out for 2-4 hr at 50 C on a waterbath, under reflux. The extract was then filtered, and the extraction was continued with a similar amount of solvent for another 2-4 hr. The two extracts were combined, the chloroform phase was removed in a separatory funnel, and finally removed under reduced pressure at 35-40 C. If a sufficient amount of oil was available, the oil phase was washed twice with distilled water. Usually it was necessary to add magnesium sulphate as a deemulsifying agent. The oil was then centrifuged. Where the oil yield was small, the oil was dissolved in toluene or diethylether and washed with water. After two washing processes the organic phase was separated by centrifuging and the solvent evaporated.

The saponification of the oils was performed in an alcoholic potassium hydroxide solution (2 N KOH in C_2H_5OH). The solution was subsequently made acidic with sulphuric acid and extracted with diethylether. The ether solution was then washed with water and the ether evaporated. Some seaweed samples with a low oil content were saponified directly. The fatty acids were separated as described for the other samples.

Fractionation of Oil

Oil produced from mackerel fillets and from cod liver were used for the fractionation experiments. The silica gel (Merck AG, Darmstadt, 0.2-0.5 mesh) used as solid phase was prepared in the following way: The gel was first dried at 110 C and then treated with the following solvents: diethylether, 15% benzene in *n*-hexane, and *n*-hexane. About 90 g of gel was added to a column with diameter 2.5 cm. Approximately 20 mg oil per gram of gel was used. The different eluting agents used and the amounts of each are given in Table I. The absorption of the eluate at 254 mμ was obtained with an Uvicord-Unit (LKB, Sweden). From the elution curves obtained, and information given by Barron and Hanahan (7), the eluate was devided into different fractions and the solvents were removed by evaporation.

Neutron Activation

The oil and fatty acid samples were neutron activated in quartz ampoules. The quartz ampoules were first washed with warm nitric acid and rinsed with distilled water. Standards (PA Chemicals: Merck AG, Darmstadt) of ammonium bromide and arsenic dissolved in hydrochloric acid (0.1 N) were neutron activated under the same conditions as the samples. The activation took place in the nuclear reactor JEEP 2, Kjeller, Norway, with a neutron flux of approximately 5×10^{12} n/cm^2 sec for 2 hr. The quartz ampoules were cooled to -196 C before opening in order to reduce the pressure from volatile compounds formed during the irradiation. The samples were then transferred to inactive glass vials. The standards were treated in the same way.

TABLE III

Bromine and Arsenic in Oil and Fatty Acids Extracted From Seaweed

Sample[a]	Br, ppm		As, ppm	
	Oil	Fatty acid	Oil	Fatty acid
Laminaria digitata	566	640	221	36
Laminaria saccharina	496	477	155	7.5
Laminaria hyperborea	368	385	197	16
Ascophyllum nodosum (1968)	61	27	7.8	5.2
Ascophyllum nodosum (1969)	56	25	49	21
Fucus vesiculosus	43	44	35	5.1
Fucus Serratus	40	39	27	6.1
Fucus spiralis	12	19	5.7	5.0
Pelvetia canaliculata	34	41	10.8	7.3

[a]The samples were collected at the west coast of Norway.

TABLE IV

Bromine in Oil Extracted From Birds, Terrestrial Mammals and Vegetables

Sample		Locality	Bromine, ppm
Hare	*Lepus timidus*	Inland Norway	<0.1
Reindeer	*Rangifer torandus*		0.12
Elk	*Alces alces*		<0.01
Cow	*Bos taurus*		<0.05
Wood-grouse	*Tetrao urogallus*		0.01
White-grouse	*Lagopus lagopus*		0.03
Lesser black-backed gull	*Larus fuscus*	West coast Norway	1.0
Common gull	*Larus canus*		0.6
Herring gull	*Larus argentatus*[a]		11.0
Herring gull	*Larus argentatus*[a]		13.2
Soybean oil			0.06
Olive oil[b]			0.03
Peanut oil			0.09
Linseed oil[b]			0.12
Coco fat			0.18

[a]Traces of arsenic present.

[b]Commercially produced raw oil.

Registration of the Samples

After neutron activation, measurement of the induced activity in the samples was made with the aid of a 2 x 2 in. sodium iodide detector coupled to a multichannel γ-spectrometer. The samples were not treated chemically before these measurements. Some of the samples contained traces of sodium and the registration of their activity was therefore postponed for 4-5 days to ensure that the activity from sodium -24 would not disturb the registration of the induced bromine and arsenic activity. The sensitivity for the analysis of bromine and arsenic when using this method is approximately 0.005-0.01 μg and 0.002-0.005 respectively. The sensitivity is reduced when the registration after activation is postponed, or if activities from other radioactive isotopes increase the "background" activity.

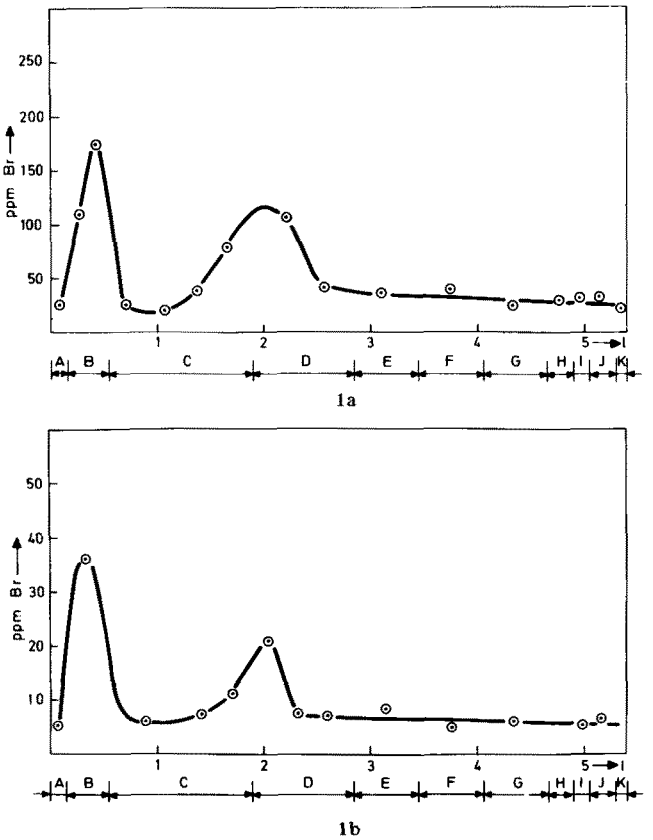

FIG. 1. The Bromine content in different fractions of cod liver oil (a) and oil extracted from mackerel fillet (b) fractionated in a silica gel column (see Table I for characterization of fractions).

RESULTS AND DISCUSSION

The results of the bromine and the arsenic analysis in the different oils and in the corresponding fatty acids are shown in the Tables II-IV. They confirm the earlier findings that lipid soluble arseno and bromine organic compounds are characteristic of oils of marine origin. This conclusion applies to lipids extracted from fish, different invertebrates, seaweed and algae. The amount of bromine in vegetable oils and in some oils extracted from terrestrial animals is significantly lower than in marine oils, and it is probably present as inorganic bromine which has not been removed from the oils by the washing process.

The only oil from organisms of terrestrial origin in which bromine was detected came from seabirds, and the bromine-organic components were here partly present in somewhat reduced amounts. Fish constitutes the main part of the diet of these birds and is most likely the source of the bromine-containing compounds found in them. The analytical results indicate both a gradual reduction in the content of bromine organic compounds through the feed chain from algae to fish and to seabirds, and that at least some of these components are quite stable. No arsenic was found in any of the terrestrial oils, except for some traces in the oil from seabirds.

When the arsenic content in marine oils is compared with the arsenic content in the corresponding fatty acids, the results show that at least two groups of arseno organic compounds are present in the marine oils. One type consists of an arsenic-containing acid which follows the fatty acids during the saponification process while the other type is converted to a water soluble compound during this process.

It is interesting to note that the lipid phase in seaweed also contains arseno organic compounds as do the lipids from the marine animals studied. The amount following the fatty acids in a saponification is, however, relatively lower than for the other marine oils studied. These compounds are probably formed from inorganic arsenic absorbed from the sea water, synthesizing first a water soluble arseno organic compound. Compared with the total amount of arsenic present in seaweed (30-110 ppm) (8,9), the amount of arsenic found in the oil represents a relatively small amount of the total arsenic present in the seaweed. The yield of extractable lipids here is usually from 3-6%. However it should be noted that the contents of arsenic particularly in oil extracted from some *laminaria* species exceeds that in any other marine raw material analyzed.

The analysis of bromine in the marine oils and in the

corresponding fatty acids shows that this element also is localized in the fatty acid fraction, but that the content is somewhat lower here than in the oils. This would indicate that the bromine organic compounds are not formed solely by an addition of bromine to double bonds in the fatty acids.

The results of the bromine analysis in the fractions from the two oils fractionated on a silica gel column (see Figs. 1a and b) give nearly the same picture of the bromine distribution in the different fractions, although the absolute amount of bromine is considerably higher in the cod liver oil than in the mackerel oil. Both diagrams show that the highest bromine concentration is located in fractions B and C-D. Fraction B (15% benzene in hexane) contains mainly sterolesters while the fractions C-D (5-15% diethylether in hexane) contains triglycerides, free fatty acids and sterols. It is also possible that the bromine may be present in other compounds which are eluted under these conditions, but which are present in smaller amounts and not found in this study. Although the results indicate that the bromine is randomly added to the main components of the oil, there may be one or more specific bromine organic compound located in fractions B and C-D.

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