THE NATURAL PRODUCTION OF ORGANOBROMINE COMPOUNDS

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Introduction

Of the 3,200 known naturally occurring organohalogen compounds, more than 1,600 contain bromine [1, 2]. These organobromines are produced by an array of biological and abiotic processes in our environment. Some of these compounds are identical to anthropogenic organobromines such as bromomethane, bromoform, bromophenols, brominated diphenyl ethers, and bromo dioxins, but many others are entirely new molecular entities, many of which possess extraordinary and important biological and medicinal properties, or are highly toxic natural pesticides. This presentation will summarize the status of this area of natural products, and will discuss the sources, structures, possible biological function, and implications of these natural pollutants for the environment.

Results and Discussion

Organobromines are produced for various purposes by marine plants and animals (sponges, corals, nudibranchs, gorgonians, tunicates, and bryozoans), fungi, lichen, bacteria, plants, and some higher animals. The mammalian immune system uses bromine to fight infection. Thus, cellular peroxidase enzymes in white blood cells convert natural blood bromide to active bromine or hypobromite, resulting in the death of the invading bacteria, yeast, fungi, or even tumor cells. A significant combustion product of biomass burning — natural or man-induced — is methyl bromide, the simplest organobromine.

In addition to producing large quantities of bromomethane (CH_3Br), the oceans have yielded CH_2Br_2, $CHBr_3$, CBr_4, CH_2ClBr, CH_2BrI, $CHCl_2Br$, $CHClBr_2$, $CHBr_2I$, $CHBrI_2$, $CHClBrI$, CH_3CH_2Br, $BrCH_2CH_2I$, $CH_3CH_2CH_2Br$, $CH_3CH_2CH_2CH_2CH_2Br$, $Br_2C{=}CHCHCl_2$, $Br_2C{=}CHCHClBr$, $Br_2C{=}CHCHBr_2$, and $BrIC{=}CHCHBr_2$, mainly from marine algae. The favorite edible seaweed of native Hawaiians is *Asparagopsis taxiformis*, and it contains at least 52 organobromine metabolites, several of which are potent lachrymators not unlike the tear gas "Mace."

The red alga *Bonnemaisonia hamifera* contains 21 organobromine compounds including several polybrominated 2-heptanones that account for the persistent sweet aroma and irritant properties of this seaweed. A haloform-like reaction of these bromoketones may be a source of marine bromoform.

Marine acorn worms are a rich source of bromine-containing metabolites, including benzoquinones, phenols, and related compounds. Thus, *Ptychodera flava laysanica* produces a tribromobenzoquinone and another species of *Ptychodera* from deep caves on Maui has yielded similar compounds. The Florida acorn worm *Ptychodera bahamensis* contains an impressive array of 11 bromophenols [3]. An octabromo metabolite is the major compound found in *Ptychodera flava*, and 4-bromophenol is the major metabolite in *Notomastus lobatus*. *Polyphysia crassa* contains 2,3,4-tribromopyrrole. *Ptychodera* and *Glossobalanus* spp. contain 3-bromoindole, 3,6-dibromoindole, 4,6-dibromoindole, 3,5,7-tribromoindole, and 3,4,6-tribromoindole. At least 22 bromophenols have been isolated from several species of red algae, one of which is the dibrominated lanosol, which has been found in 20 species of red algae.

Numerous brominated terpenes occur naturally, particularly from the marine realm, and more than 100 brominated marine monoterpenoid metabolites have been discovered, particularly in the genus *Laurencia* red algae. These compounds appear to act as feeding deterrents to reef fish. The causative agents of a red alga (*Gracilaria coronopifolia*) poisoning episode in Hawaii have been characterized as three bromine-containing manauealides, and the highly toxic aplysiatoxin, which is responsible for "swimmer's itch" in Hawaii, is produced by the blue-green algae *Lyngbya majuscula*, *Oscillatoria nigroviridis*, and *Schizothrix calcicola*.

Several natural sources have yielded brominated fatty acids. Two brominated stearic acids have been isolated from the seed oil of *Eremostachys molucelloides*, *Petrosia* sponges contain brominated hexacosadienoic acids, and the Okinawan sponge *Xestospongia* sp. has recently been found to produce 14 novel bromine-containing fatty acids [4]. A related fatty acid is produced by both an anemone (*Condylactis gigantea*) and a zoanthid (*Palythoa caribaeorum*). The Pohnpei sponge *Dysidea fragilis* contains the unusual bromine-containing antazirines [5], and the marine stolonifer *Clavularia viridis* produces the prostaglandin-like bromovulone I, which has antitumor activity.

The red algae *Delisea* and *Beckerella* spp. have yielded an array of nearly 35 brominated furanones, one of which contains six bromines. Other natural bromine-containing heterocycles are widely dispersed throughout the plant and animal kingdom. Numerous bromoindoles are known from natural sources. For example, One of the first naturally occurring organobromine compounds to be characterized was the ancient Egyptian dye Tyrian Purple which is found in several Mediterranean molluscs and was used by royalty to dye robes. The amino acid 6-bromotryptophan is incorporated into several peptides, and the bryozoan *Amathia convoluta* contains convolutamydine B as one of four related bromooxindoles that display differentiation of HL-60 cells [6], and the bryozoan *Chartella papyracea* has afforded the stunningly complex bromo alkaloids such as chartelline (A).

Numerous brominated pyrroles also occur naturally, including 2,3,4,5-tetrabromopyrrole, which is a metabolite of the marine bacterium *Pseudomonas bromoutilis*, and 2,3,4-tribromopyrrole is produced by the acorn worm *Polyphysia crassa*. Several brominated bis-pyrroles are secreted as fish antifeedants by the bryozoan *Sessibugula translucens*. A brominated bis-pyrrole was recently discovered in sea bird eggs and eagle liver samples, a compound that is believed to be diet-derived [7].

Several brominated diphenyl ethers and diphenylmethanes are known, usually being found in red algae, sponges, and acorn worms. Even more remarkable is the report of two polybromodibenzo-*p*-dioxins from the sponge *Tedania ignis*. Since brominated diphenylethers can be easily imagined to undergo oxidative cyclization, additional natural bromine-containing dibenzo-*p*-dioxins may await discovery.

Many studies have shown that these naturally occurring organobromine metabolites arise from the action of a peroxidase enzyme, such as bromoperoxidase (BPO), on a suitable substrate in the presence of bromide ion and hydrogen peroxide. The BPO enzyme(s) responsible for the oxidation of bromide to bromine (hypobromite) or to an enzyme-bound bromine complex have been isolated from nearly 100 species of marine algae and phytoplankton, terrestrial lichen, bacteria, an acorn worm, and a marine annelid [8]. Chloroperoxidase and other peroxidases also have the ability to oxidize bromide. For example, chloroperoxidase is the principal enzyme involved in the production of bromophenols in the acorn worm *Notomastus lobatus*. Two BPO genes from *Streptomyces aureofaciens* have been cloned and sequenced, and one of these BPO enzymes has been characterized by X-ray crystallography. A BPO has also been recently isolated for the first time from marine phytoplankton, and this enzyme is presumed responsible for the production of $CHBr_3$, CH_2Br_2, and CH_2BrI [9].

Many of these organobromine metabolites function as chemical defensive agents (e.g., antifeedants). An interesting example is a bromine-containing amide, which is secreted by the Thai plant *Arundo donax* to repel weevils. Some organobromines promote larval settlement in the life cycle of marine invertebrates and others prevent the growth of fungi, bacteria, and barnacles. For example, the brominated compounds in the sponge *Verongia aerophoba* seem to prevent overgrowth by fouling organisms such as barnacles. When the cells of the sponge are perturbed, an inactive compound is enzymatically transformed into two active repellent bromine compounds. A bromoester, which has been found in mammals, including human cerebrospinal fluid, may play a role in the sleep phenomenon since it is a potent inducer of rapid-eye-movement sleep.

The large quantities of natural bromomethane and bromoform in the environment may serve to recycle bromine/bromide between the oceans, the atmosphere, and land. For example, the emission of bromoform from marine sources is estimated to be 1-2 million tons per year. The current best estimate of the atmospheric budget of bromomethane puts the marine emissions at 56,000 tons/per year [10], and biomass burning, as shown by direct measurement, may produce 20,000-50,000 tons/year. The marine production of bromoform is much larger than that of bromomethane, and is estimated at 1-2 million tons/year. Macroalgae may contribute an estimated 200,000 tons/year, and Arctic ice microalgae may contribute an estimated maximum of 70,000 tons/year.

Another major source of organobromine compounds — yet to be explored in detail — is the high molecular weight matter in marine sediments as mentioned earlier. These studies indicate that there is large-scale bromination of high molecular weight organic matter in the marine environment.

A detailed study of the acorn worm *Ptychodera flava* has established that the annual fecal secretion of the 64 million worms living in a one-square kilometer habitat on Okinawa contains four tons of organic matter (primarily bromophenols and bromoindoles, *vide supra*). If one extrapolates this quantity to the world's acorn worm population, then an enormous quantity of organobromine compounds is biosynthesized and excreted by these acorn worms. A similar study of the Floridian *Ptychodera bahamensis* acorn worm estimates an annual output of 0.5-1.3 tons per kilometer of coastline. A study of the brown alga *Ascophyllum nodosum* has determined that two tons of HOBr is produced annually by this seaweed along a 30-kilometer stretch of dike in the Netherlands [11].

A surprising recent discovery is the production of bromomethane in significant quantities by several terrestrial plants and vegetables (rapeseed, mustard, cabbage, Chinese cabbage, broccoli, pak-choi, alyssum, wild mustard, turnip, radish) from natural bromide in the soil. The amounts of bromomethane range from 18-36 ng/g plant material per day. The authors conclude that this source of natural bromomethane contributes significantly to the overall atmospheric concentration of bromomethane [12].

The number of reported natural organobromine compounds from living organisms continues to increase. Since only a small percentage of living organisms have been examined for their chemical content, especially marine organisms, it is indisputable that a large number of new organobromines is awaiting discovery. For example, of the 4,000 species of bryozoans, fewer than 1% have been studied in this regard and all of these produce organobromine compounds. Moreover, since mammals, including humans, utilize *in vivo* bromination in white blood cells as part of the immune process, it is inevitable that additional organobromine compounds will be found to occur naturally in humans.

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