

**ELEMENTAL STUDIES ON MARINE ALGAE BY
X-RAY ANALYTICAL MICROSCOPE
PART III. SEPARATION OF BROMO-COMPOUND IN SARGASSUM
FUSIFORMIS GUIDED BY X-RAY ANALYTICAL MICROSCOPE***

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INTRODUCTION

Halogens are known to be accumulated in all kinds of marine algae and through halogen peroxidases, chloride, bromine and iodine can be assimilated as organic compounds by the same mechanism (Vilter, 1984; Soedjak *et al.*, 1990). Although a number of bromine-containing organic compounds are found in various red algae (*e. g.* Blackman *et al.*, 1982; Weinstein, 1975; Chantraine *et al.*, 1973; Pedersen *et al.*, 1974; Craigie *et al.*, 1967), it has not still been well studied in brown algae, as shown by the very few reports on this subject (Koch *et al.*, 1982).

The metabolic role and physiological importance of the bromine-containing compounds in seaweeds is poorly understood, although there were some speculations on these compounds' antialgal, antibacterial and growth promotion activities (Pedersen *et al.*, 1974; Nisizawa *et al.*, 1979). Attention was recently paid on the role of volatile organic bromine compounds playing their potential effects on the upper atmosphere and environment (Muosmaa *et al.*, 1990). The bromo-compounds in marine algae should be under further investigated to elucidate the production pathway and ecological cycle of bromine in the excreta and huge biomass of marine algae. In this paper, describes a new method, guided by X-ray analytical microscope to fractionate and isolate the organic bromine compounds in *Sargassum fusiformis*, is described.

MATERIALS AND METHODS

Collection of samples Fresh samples of *Sargassum fusiformis* were purchased in early February, 1997, and immediately deeply frozen in medical refrigerator (- 30°C) (Sanyo, MDF-U536D) until further treatment.

Extraction and isolation of bromo-compounds The fresh-frozen algal material (*ca.* 1kg) was thawed, and cut into pellets by cooking cutter (Toshiba, CQM-62), and extracted with 2L methanol under

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nitrogen bubbling overnight at room temperature (15°C). The extract was concentrated *in vacuo* at 50°C. Two volumes of 1.5% caffeine solution was added to the aqueous phase, which, after nitrogen bubbling, was left overnight at 4°C. The turbid solution was centrifuged at 8000r/min for 10min (Hitachi 20PR-52), the precipitate was re-dissolved after treatment with chloroform and water (1:1, v/v); the water phase was washed thrice with equal volume of chloroform, and after removal of trace chloroform, was loaded on the insoluble polyvinylpyrrolidone (TCI Co., Japan) column (40cm in length, 2.8 cm i. d) connected to a peristaltic pump (Atta, AC-2110) set at 3ml/min. Then the column was washed by 5 volumes of pure water (Millipore water purification system) until no color appeared in the eluent, and eluted again with ammonia solution pH 8.8, 10.2, 11.4 respectively. The eluent was collected and concentrated *in vacuo*, and freeze dried (Eyela FDU-810).

Elemental fluorescence spectrum measurement by X-ray analytical microscope model XGT-2000V (Horiba Co. Ltd, Japan) The test sample was put on the sample plate, covered with polyethylene membrane, and moved into the center under the optical microscope by joystick. Other experimental conditions were: spot diameter of X-ray beam, 100 μ m; X-ray tube voltage, 30keV, tube current 1mA; pulse time, P1.

The solid sample could be scanned directly, while the liquid sample was first mixed with a small amount of microcrystalline cellulose powder, then oven dried. The dried cellulose powder was used for scanning in the same as that for solid sample.

MALDI-TOFMS (matrix-assisted-laser-desorption-ionization time-of-flight mass spectrometry) **measurment** MALDI-TOFMS mass spectrum was recorded by REFLEX II (Bruker-Franzen Analytik, Bremen, Germany). The sample was irradiated under high vacuum with a nitrogen laser (wavelength 337nm, 3ns pulse). Spectrum was obtained in linear mode using 20kV ion acceleration without postacceleration; detector voltage was set at 1.6kV.

RESULTS AND DISCUSSIONS

X-ray elemental fluorescence spectra from *Sargassum fusiformis*

Although brown algae are known to be accumulators of iodine rather than bromine, this study showed that bromine, along with the potassium, calcium, arsenic and strontium (Fig. 1a), could form distinctive peaks in the X-ray elemental fluorescence spectrum of raw material of *Sargassum fusiformis*. Fig. 1b shows clearer peaks after the energy axis (x-axis) was expanded. This result indicated that some edible brown algae can assimilate bromine at relatively high level.

Organic bromine almost always appeared as phenol conjugate (Nisizawa *et al.*, 1979). The underlying mechanism was that bromide ion in seawater was oxidized into free bromine atom by peroxidase located on the algal cell surface, and then undergo nucleophile reaction to form a halogen-phenyl group. The fact that *Sargassum fusiformis* contains as much as 2.0% d. w. polyphenols (Yan, 1996, using Folin-Denis method) led us to do the further fractionation guided by X-ray analytical microscope.

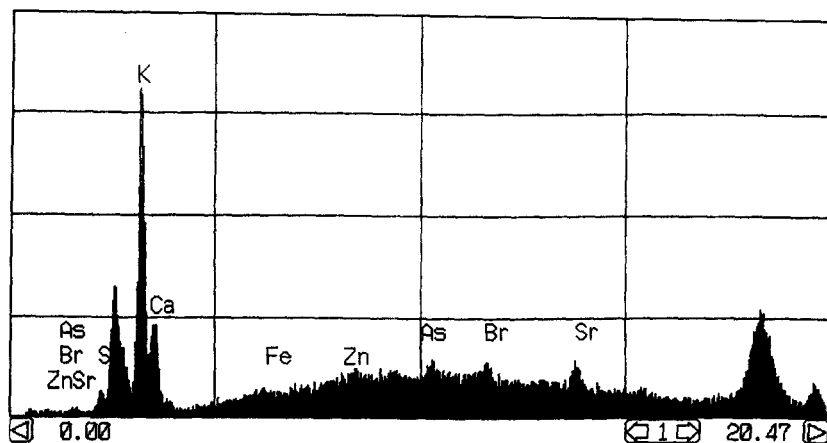


Fig. 1a The whole X-ray fluorescence spectrum

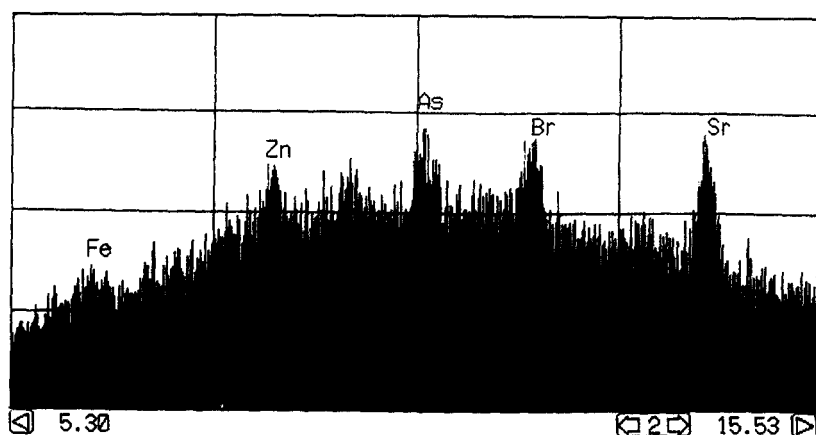


Fig. 1b Partial amplification of X-ray fluorescence spectrum

The aqueous phase (after the methanol extract was concentrated in vacuo to evaporate the methanol) was brown showing presence of tannin-like substances in it. The X-ray fluorescence spectrum (Fig. 2) of sediment precipitated by caffeine solution showed peaks attributed to potassium, arsenic and bromine. Thereafter, the sediment was dissolved by a mixture of chloroform and water; caffeine would easily dissolve in the chloroform while the polyphenol substances remained in the aqueous phase, which was somewhat labile, and would form precipitates during storage in a refrigerator, as shown in Fig. 3.

After removing caffeine and trace chloroform in it, the aqueous phase was immediately loaded on an insoluble polyvinylpyrrolidone (also named as polyvinylpolypyrrolidone, PVPP) column. It was found that the ammonia solution (pH 8.8) contained most of the bromine compounds. In Fig. 4, the X-ray fluorescence spectrum only showed one big bromine peak, not any signal of arsenic. The height of the peak showed that it was relatively pure. This is the first time that organic bromine compound

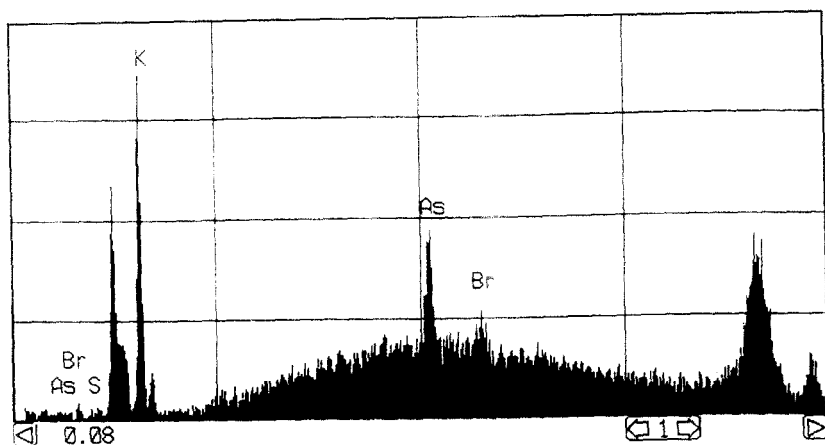


Fig. 2 X-ray fluorescence spectrum of caffeine precipitated polyphenol

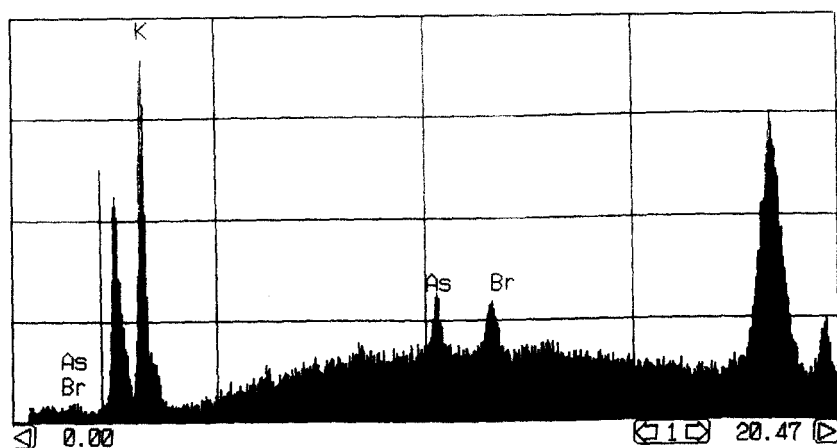


Fig. 3 X-ray fluorescence spectrum of bromine containing water phase was isolated by a non-destructive "element monitor".

During the fractionation, the bromine compounds showed some chemical characteristics (Table 1). It may be interesting for some researchers thinking about the role of halogens in marine algae on the geochemical and ecological cycle of halogens.

Table 1 Some chemical characteristics of organic bromine compounds

● Organic bromine compounds in Sargassum can be water soluble
● They can be precipitated by caffeine solution
● They can be absorbed on PVPP column, and eluted by slightly alkaline solution
● They may have some interaction with arsenic compounds
● They are not so stable in water phase, and tend to form sediments, and may be involved in the geochemical and ecological cycle of halogens
● Their UV-vis spectra are similar to polyphenol compounds (Fig. 5)

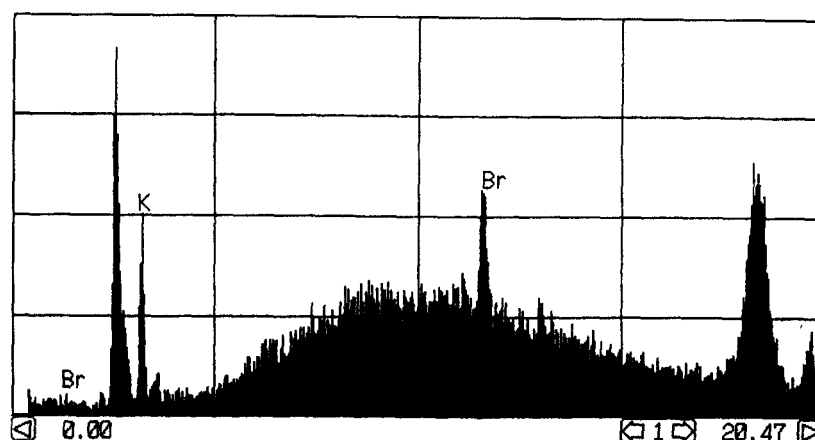


Fig. 4 X-ray elemental fluorescence spectrum of PVPP eluent at pH 8.8

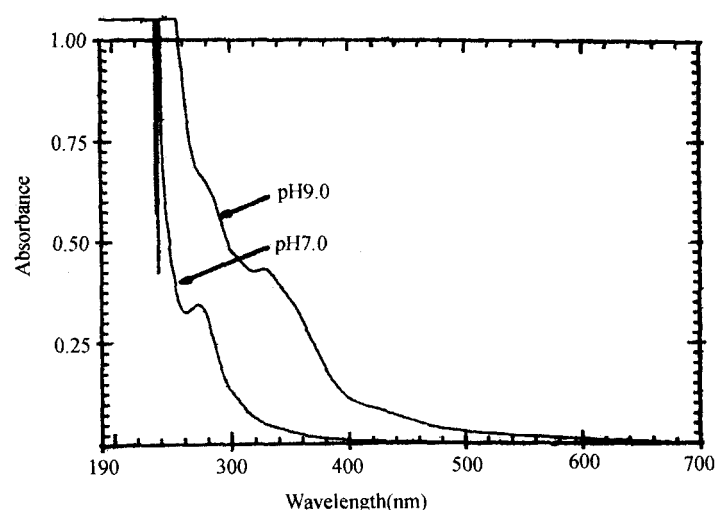


Fig. 5 UV-visible spectrum of organic bromine compounds

MALDI-TOFMS spectrum

MALDI-TOFMS is the abbreviation of “matrix-assisted laser desorption ionization time-of-flight mass spectrometry”, which is the only method to determine the mass of molecule in a mixture state. Although its major application is on the macromolecules such as proteins and DNA fragments (e. g. Juhasz, *et al.*, 1996), it also can be used for small molecules. After comparing and subtracting the background mass peaks, foreign peaks can be found among the matrix peaks. In this case, it was fortunate that the tested bromine compounds could show very clear mass peaks (Fig. 6). The spectrum indicated the existence of two kinds of organic bromine compounds, with molecular weights of 330 and 554, respectively. The isotopic molecular weights of these compounds were 332 and 554. It can

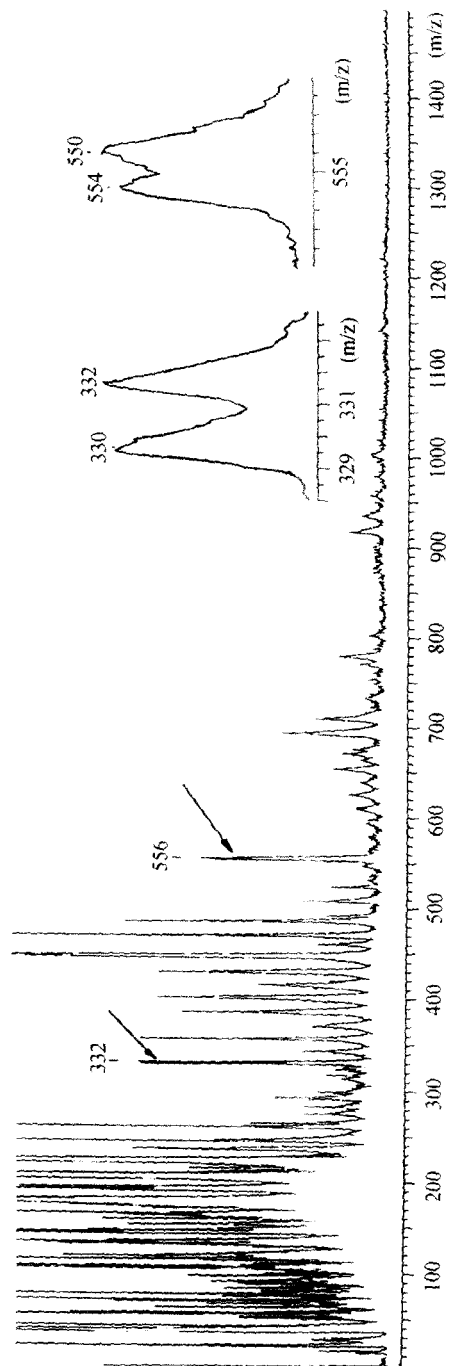


Fig. 6 MALDI-TOFMS spectrum of PVPP eluent

be deduced from the principles of isotope in mass spectrometry that there is only one bromine in one molecule. It was noteworthy that the molecular weight of 554 was the largest among the bromine compounds already found in marine algae. The full structure of these two compounds is still under study.

The efficiency of this new method

X-ray analytical microscopy is a rapid and nondestructive method for measurement of elemental composition (Hosokawa, 1995). Using such a technique as the non-destructive bromine monitor, organic bromine compounds were separated and fractionated successfully. Although elucidation of the total structure of the bromine compound has not been achieved until now, this new isolation strategy, *i. e.* fractionation and isolation guided by X-ray analytical microscope was proved in this study to be useful and efficient for studying special element containing compounds such as halogen containing, arsenic-containing, and metal ion containing compounds, to some extent, it is analogous to bioactivity guided separation strategy.

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用 X 射线分析显微镜对海藻元素的研究Ⅲ. X 射线分析显微镜引导下羊栖菜中含溴化合物的分离*

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摘 要

本研究采用了一种新的元素检测方法,即用 X 射线分析显微镜来引导分离羊栖菜(*Sargassum fusiformis*)中有机溴化合物。新鲜羊栖菜经提取、浓缩、洗脱等处理,所得洗脱液经冷冻干燥后,用 X 射线分析显微镜分析其元素组成。X 射线荧光光谱得到的结果表明,这种检测方法十分有效,能够证实有机溴化合物在 PVPP 柱色谱中得到了有效的浓缩与分离。MALDI-TOFMS 质谱显示存在两个有机溴化合物,分子量分别为 330 和 554,质谱的同位素特征表明,每个有机溴化合物分子中分别含有一个溴原子。该有机溴化物具有以下化学特征:水溶性较强,能够被 PVPP 柱吸附,而在弱碱条件下溶出;在水溶液中不太稳定,容易形成沉淀。该有机溴化物的紫外可见光谱特征具有多酚化合物的特点,并与海藻中的砷化物具有某种程度的相互作用。

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