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A diterpene, sandaracopimarinol, produced by Japanese cedar and found from the deep seawater pumped up from the Suruga Bay

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Abstract

Sandaracopimarinol, one of the diterpenes, has been isolated for the first time along with β-sitosterol from deep seawater pumped up from 687 m in the Suruga Bay. Sandaracopimarinol has been found in the Japanese cedar ("Sugi" *Cryptomeria japonica*), which is widely planted in the eastern Japan. We speculate that the above organic compounds have been discharged by rivers to the seawater from terrestrial environment.

Key Words: *deep seawater, sandaracopimarinol, β-sitosterol, Suruga Bay, diterpene*

1. Introduction

Deep seawater (below the depth of 200 m) has been characterized with low temperature, containing rich nutrients, cleanliness and relatively less polluted compared to the surface water (Takahashi, 2000). The utilization of deep seawater for aquaculture, foodstuffs, medicinal treatments and as natural renewable energy source is widely appreciated. Since 1976, basic research and various applications of the deep seawater have led to constructing deep seawater pumping stations in Kohchi, Toyama, Okinawa, Shizuoka, and several other locations in Japan. Suruga Bay in Shizuoka Prefecture is situated at a unique location having multi-layer water masses, resulting from the Kuroshio Current from the south and the Kurile Current from the north (Nakamura, 1982). Some studies showed that the organic components in seawater in the Suruga Bay are characterized with carbon-rich organic compounds with low molecular weight Dalton and its compositions consist of amino acids, carbohydrates, lipids and fulvic acid. Vertical profiles of nutrients in the Suruga Bay represent a kind of typical patterns in the ocean, that is, the concentrations of nutrients are low or near zero in surface water, and increase with depth (Iwata *et al.*, 2005). It was also observed that most particles produced biologically settle out from the surface to deeper water along with biological decomposition on its way. However, the basic research is still in preliminary phase and needs further elaboration. In this study, we have investigated the chemical changes in the quantity and quality of organic materials con-

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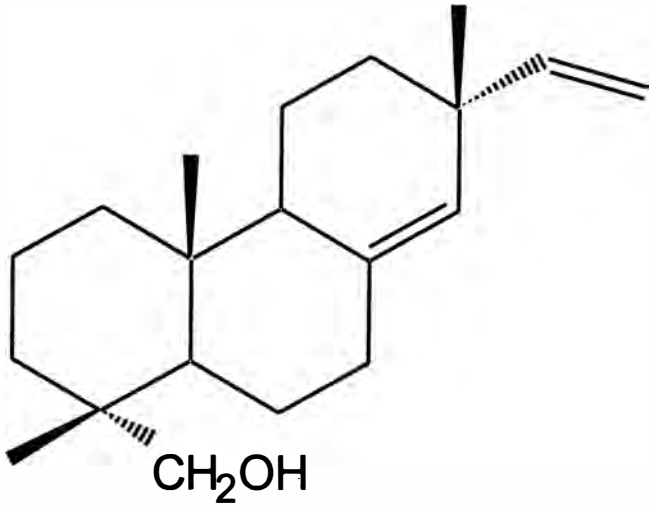

Fig. 1. Chemical structure of diterpene, sandaracopimarinol.

tained in seawater collected in different seasons from the Suruga Bay. The water samples used in this work were collected from the surface as well as from a depth of 687 meters in the Suruga Bay (City Yaizu) using the existing direct pumping system (through PVC pipes). The collected samples were filtered and directly extracted with ethyl acetate (EtOAc) prior to be separated on silica-gel column chromatography. The samples were then analyzed by ^{1}H- and ^{13}C-NMR spectroscopy.

In this paper, for the first time, we report the isolation of one of diterpenes, "sandaracopimarinol" found in Japanese cedar from deep seawater in Suruga Bay together with β-sitosterol. Furthermore, we expect a possible utilization of deep seawater resources as a functional food due to its significant bio-organic contents.

2. Methods

1.5 L deep seawater sample (from a depth of 687 meters) was extracted with 300 mL of EtOAc for 10 min. The water layer was separated and repeatedly extracted with 200 mL portion of EtOAc. All EtOAc extracts were dried by anhydrous Na_2SO_4 and concentrated by evaporation. By repeating the above process for several times, we obtained 175 mg (0.362%) of concentrated EtOAc extract from 483 L of deep seawater. Then the above concentrated EtOAc extract was successively further extracted with hexane and $CHCl_3$ in order to separate the compounds more precisely. The weight of concentrated $CHCl_3$ extract was measured to be 63 mg. On the other hand the hexane extract was concentrated to get a residue of 97 mg. The hexane extract was chromatographed over a silica gel column (ϕ 1.3 × 45 cm) and eluted with hexane, hexane-$CHCl_3$ solvent system (20:80) to get fractions 1 to 8. The fraction 7 (8.9 mg) was again chromatographed on silica gel column with hexane-$CHCl_3$ (85:15) to give four sub fractions (namely fractions a, b, c and d). Isolation of fraction 7-c gave sandaracopimarinol (1.1 mg), which was identified by comparison of its spectral data with those of an authentic sample (Morisawa *et al.*, 2002). The fraction 7-d was again isolated on PLC (chloroform-100%) to get further four fractions (7-d-i to 7-d-iv). Spectral analysis of fraction 7-d-ii gave β-sitosterol (0.7mg) as a second compound, which was identified by comparison of

its spectral data with those of an authentic sample (Arigoni *et al.*, 1997; Lee *et al.*, 2003; Robert, 1977).

3. Results and Discussion

We successfully isolated the sandaracopimarinol twice out of three observations carried out in our studies from August to November in 2003 (dates of water collections were August 6 and 21, September 2 and 26, and November 25) and again from June to September in 2004 (dates of water collections were June 16, August 2 and 5, September 6, 12 and 18) from 687 meters depth of seawater. However, no presence of sandaracopimarinol was observed from the surface seawater. Earlier, sandaracopimarinol has been isolated from a tree species called Japanese cedar (*Cryptomeria japonica*) and exhibited repellent activities (Morisawa *et al.*, 2002; Ashitani *et al.*, 2001). The tea made from the leaves of this tree is commonly known as "*Sugi*" tea in Japan. The isolated sandaracopimarinol scents the same odor as that of Japanese cedar and is anticipated to have some antibacterial properties.

Near the Suruga Bay, Shizuoka Prefecture (Japan) has rich mountain regions covered by the Japanese cedar. We speculate that the above organic materials may have been supplied to the seawater through river water from these terrestrial areas (Opsahl and Benner, 1997). It was observed that organic matter is degraded in the upper layer of seawater by biological processes and is consumed and/or transported downwards by deep mixing (Shinomura *et al.*, 2005). However, some terrigenous refractory materials are difficult to degrade even by biological processes and they get transported towards the depth by gravitational settling without having much destruction to their structure (Suzuki, 2005). In deep waters, the bacterial activities are weak as the water temperature is low. Thus, such materials might be remained in deep sea water for prolonged period. This is the first isolation of sandaracopimarinol from deep seawater indicating relationship between the forests, rivers and oceans. The isolated compounds may also provide information on the original sources of taxonomic or useful organic components in the sea water, which are currently unknown.

Considering probable antibacterial characteristics of sandaracopimarinol, the present study suggests a possible potential use of deep seawater as drinking water on account of its contents, provided that the contents are retained during processing. It is possible that it may contain some other useful components that need to be studied further. In Shizuoka Prefecture there are few paper manufacturing factories that elute waste material of used Japanese cedar pulp in the river water. Therefore, the wastes of the paper factories may also be one of probable sources of sandaracopimarinol in deep seawater. Keeping this possibility in mind, we are currently working on seawater collected from different locations of Kohchi, Toyama, Okinawa, Shizuoka and other prefectures in Japan to broaden this research and to study biogeochemical and physical processes occurring in ocean water.

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駿河湾の深層水に含まれている杉由来成分のジテルペンの一種，サンダラコピマリノールについて

要　旨

駿河湾海洋深層水中からジテルペンの一種である sandaracopimarinol および β-sitosterol を単離同定した．Sandaracopimarinol は，東日本の多くの森に大規模に植林されている杉から単離された化合物で，今回，駿河湾から取水されている焼津の海洋深層水から初めて検出された．この物質は，山から川を通して海水まで流れていったものと考えられる．

キーワード：海洋深層水，サンダラコピマリノール，β-シトステロール，駿河湾；ジテルペン