学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Antifouling compounds isolated from two Red Sea organisms: a *Hyrtios* sp. sponge and an *Okeania* sp.cyanobacterium

(紅海由来の海綿 *Hyrtios* sp.およびラン藻 *Okeania* sp.から得られた付着阻害物質)

Biofouling is defined as the accumulation of organisms on submerged structures such as ships' hulls, underwater pipelines, oil rigs, piers, buoys etc. It causes large economic loss and serious ecological problems worldwide. For instance, in naval industry, not removing biofouling leads to corrosion, resistance in the water and increase of the weight of ships, which contribute to an increase of fuel consumption. This increase results in a raise of contaminant gases, harmful particles and so of global warming. To avoid all these troubles and keep our planet livable, antifouling strategies are needed. One of them is to copy Nature by mimic sessile organisms which produce chemical defenses to be free of fouling and avoid predation. Several marine natural compounds have already been isolated from marine organisms, mainly from cnidaria, sponges and algae.

For this study, about eighty extracts of different marine organisms from the Red Sea were tested on barnacle larvae which are hard-to-remove macrofoulers and found worldwide. The Red Sea is a special ecosystem because it is partially isolated from the open ocean and, because of its location between deserts, evaporation occurs, making this sea the warmest and most saline one in the world. So, because of these hard conditions, organisms should produce compounds to adapt, including antifouling ones. About 18 % of the tested extracts were very active (active at 1 µg/mL) and 31 % were moderately active (active at 10 µg/mL). Among the active extracts, two were selected because of their large amount of material: the sponge *Hyrtios* sp. and the cyanobacterium *Okeania* sp. Sponge are prolific producers of antifouling compounds while cyanobacteria are not yet well studied for such compounds although they can be cultured in large scale to afford large amounts of active compounds for the industries.

Three known compounds were isolated from *Hyrtios* sp.: *N*-phenethylacetamide and the two fatty acid methyl esters (FAMEs), methyl-(5*Z*,9*Z*)-hexacosa-5,9-dienoate and methyl-(*Z*)-octadec-11-enoate. The position of the double bonds of these FAMEs was determined by study of GC-MS-MS fragments of their dimethyl disulfide adducts while their configuration was ascertained by NMR (coupling constants, carbon shifts of the allylic methylenes). *N*-Phenethylacetamide was previously isolated from a fungus while methyl-(5*Z*,9*Z*)-hexacosa-5,9-dienoate was isolated from different sponges. Both FAMEs have already been synthesized but, to our knowledge, it is the first time that methyl-(*Z*)-octadec-11-enoate was isolated from a natural source.

Okeania sp. afforded two fatty acid amides, serinolamides C and D, the known antifoulant dolastatin 16, and several lyngbyabellins: the known 27-deoxylyngbyabellin A, lyngbyabellins F, G, H and the new lyngbyabellins O and P. The absolute configuration of the two serinolamides was established by partial synthesis and Marfey's analysis of the synthetic (*R*)- and (*S*)-*O*-methyl serinol and the hydrolysate of both fatty acid amides. They were named serinolamides in reference to serinolamides A and B, isolated from *Moorea producens*, a cyanobacterium genetically close to *Okeania* sp. The planar structures of the two new

lyngbyabellins, O and P, were elucidated by MS and NMR techniques. The absolute configuration at C-14 and C-20 of lyngbyabellin O was determined to be *R* and *S*, respectively, by chiral-phase chromatography of its degradation products: the glyceric acid and the 2,3-dihydroxyisovaleric acid methyl ester residues. Its configuration at C-2 and C-3 was assessed by methanolysis of lyngbyabellin G. Methanolysis of lyngbyabellin G opens its structure at C-16, giving lyngbyabellin O. Both lyngbyabellins have as a result a 2*S* and 3*S* configuration. Lyngbyabellin F was deacetylated to give lyngbyabellin P, which therefore shares the same absolute configuration 2*S*, 3*S*, 14*R*, 20*S*, 26*R* and 27*S*. Methanolysis of lyngbyabellins F, G and P led to a regioselective ester cleavage at C-14 and C-16, respectively, giving lyngbyabellin O. Therefore, we can wonder if acyclic lyngbyabellins and lyngbyabellins without a side chain are artifacts. But, as previously reported with the biosynthetic pathways of lyngbyabellin A and hectoclorin, macrocyclization occurs, meaning that lyngbyabellins with cyclic structure are natural compounds. Clusters for incorporation of side chain were not observed in both pathways so lyngbyabellins without a side chain are probably precursors, and other clusters are used for addition of a side chain. Only acyclic structures are probably artifacts.

Antifouling study was conducted on *Amphibalanus amphitrite* barnacle larvae. *N*-Phenethylacetamide was the least active compounds from *Hyrtios* sp. (EC_{50} 41.7 µM), while both FAMEs were potent (EC_{50} 0.91 and 1.86 µM, respectively). Structure of *N*-phenethylacetamide is close to dopamine, which is a reported antifoulant. Dopamine induces metamorphosis without prior settlement in *A. amphitrite* and inhibits the ciliary activity in mussel larvae. Mussel larvae stop swimming and therefore cannot reach the substratum. As some FAMEs were reported as antioxidants, they could act as antifoulants by inhibiting the oxidative chemistries barnacle larvae use to settle and metamorphose. *Okeania* sp. gave interesting compounds such as the potent serinolamides C and D (EC_{50} 2.45 and 0.1 µM, respectively), the extra acetyl of serinolamide D apparently increasing the activity. However, after 120 hour-exposure, larvae started to settle a little and the recovery test with serinolamide D showed that almost all the exposed larvae could attach after seven days, which means that the activity of serinolamides is reversible. As fatty acid amides, they could have biosurfactant properties which reduce the surface tension and so avoid settlement from larvae. Some fatty acid amides such as oleamide and erucamide are already used in antifouling paints because of these properties. Lyngbyabellins are known antineoplastic compounds and their structure seems to play an important role in their cytotoxicity toward cancer cells, with the cyclic structure with a side chain being the most potent. Their structural features are apparently also important to prevent larval settlement for barnacle. Indeed, the acyclic structure (lyngbyabellin O) seems more active while cyclic structure (lyngbyabellin G) and addition of a side chain (lyngbyabellin P) decrease the activity. 27-Deoxylyngbyabellin A, which is different from the other lyngbyabellins by additional lactams, exhibits almost total toxicity at 0.7 µM, making it potent but also toxic, which is not the strategy of ecologically friendly antifouling compounds.

This study showed that Red Sea organisms such as *Hyrtios* sp. and *Okeania* sp. produce antifouling compounds. Up to now, only one paper reported such compounds and three papers active extracts. The structure elucidation of compounds from *Hyrtios* sp. and *Okeania* sp. was possible thanks to MS and NMR techniques, together with partial synthesis, study of degradation products, derivatization and chiral chromatography. A relation between structure and activity of the lyngbyabellins was observed, leading to a possible future further research to understand their mode of action on barnacle larvae.