

CHAPTER 3

FIELD EXPERIMENTS OF ANTIFOULING TEST PAINTS USING SYNTHESIZED COMPOUNDS

As described in chapter 2, some synthesized compounds showed promising activity. In order to examine whether these compounds prevent the settlement of fouling organisms in the field, promising compounds **131** (paint A) and **153** (paint B) were incorporated in paints and evaluated their antifouling activity in the fields. Field experiments were carried out at two field sites; Shizugawa bay, Miyagi and Tokyo bay, Tokyo for 3 and 2 months, respectively.

In both field experiments, test paints A and B showed promising performance as antifouling paint. These paint surfaces were settled by diatoms and hydrozoans, however macrofouling organisms, such as barnacles, ascidians, and bryozoans, were not observed on their surfaces during the test periods. These results suggest that isocyanides are very useful model compounds for the development of environmentally benign antifouling paints.

Results and Discussion

a) Experiment in Shizugawa bay

The applied concentrations of **131** and **153** were 20.5% (in 7.6g) and 24.7% (in 8.4g), respectively. Paint A was very excellent in dryness, on the other hand, the surface of paint B is little bit sticky. As shown in Figure 3-1, a test panel was suspended from a fishing boat at a depth of 0.5m for about three months starting from August 30, 2003.

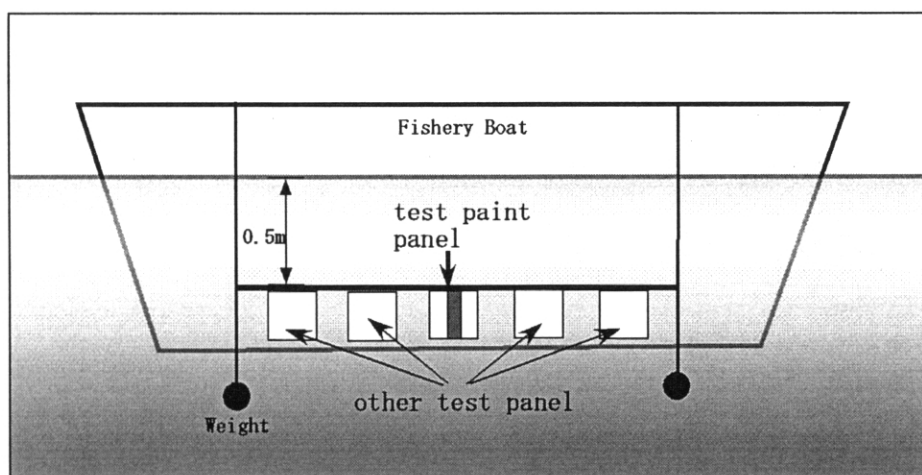


Figure 3-1. The installation method of test panel in Shizugawa bay.

Figure 3-2 shows the conditions of the painted surfaces. The variation of fouling organisms on submerged test paints is shown in Table 3-1. Copper-based paint, covered slightly with diatoms after 2 months, showed excellent antifouling activity during the experiment period. Both trial paints showed good activity against sessile organisms within a period of more than one month: then tubicolous amphipods and algae were observed on the surface of paint A, and attaching diatoms were found on the paint B, respectively. Macrofouling organisms, such as ascidians and bryozoans, started to settle on the blank's surface (backside) in 2 months, thereafter they grew up to cover all over the surface at the end of experiment (Figure 3-2). On the other hand, both trial paints prevented ascidians and bryozoans (a few settled on paint A) to settlement during the experiment period. Compared with the blank surface, paints A and B retained antifouling activity for 3 months. Barnacles and oysters, observed settlement of alongside the pier were not found on the test plate during the test period.

Table 3-1. Abundance and Timing of Appearance of Major Attaching Organisms Groups on Test Paints in Shizugawa bay

Experiment period	30, August to 27, November, 2003									
Observation date	30, September			24, October			27, November			
Test paint type ¹	A	B	C	A	B	C	A	B	C	D
Species and abundance²										
Attached diatoms			+		+++	+	++	+++	+++	++
Algae				++		+	±	+		
Hydorochoans							+	+		++
Bryozoans							±			++
Barnacles										
Amphipods	+			+			+			+
Solitary ascidians										++
Compound ascidians										++

1) A: paint A, using compound 131, B: paint B, using compound 153, C: copper-based paint, D: non-paint PVC (backside).

2) +++: very common (covered over 50%), ++: common (covered 10~50%), +: present, ±: rare (a few individual observed).

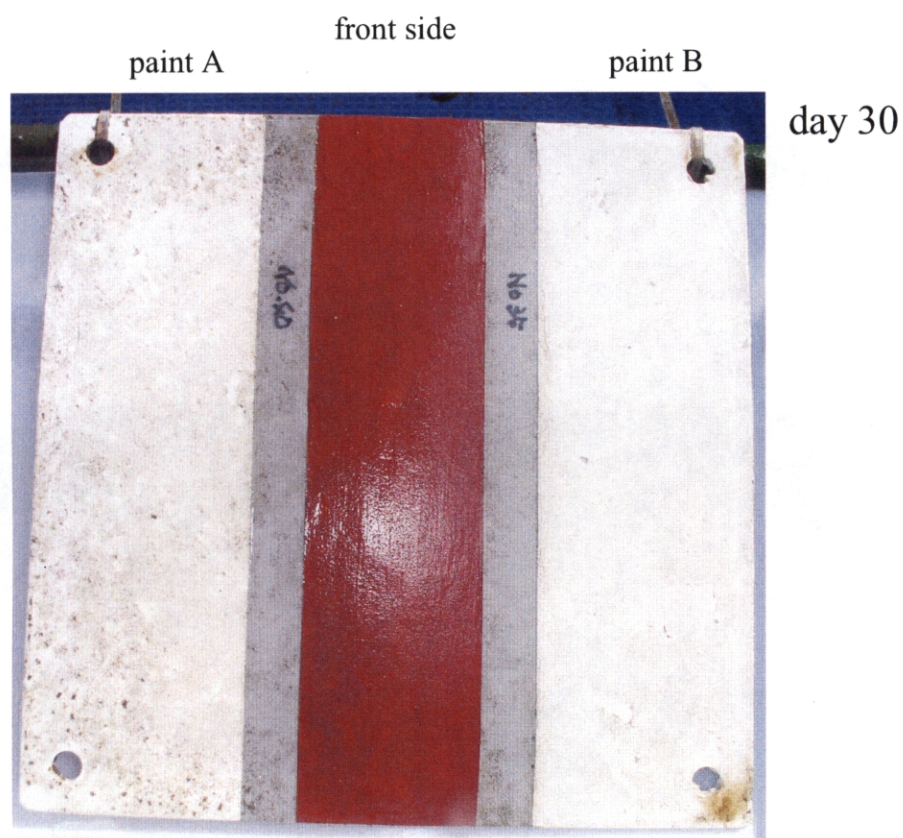


Figure 3-2. Surface conditions of test panel after 30 days and 60 days in Shizugawa bay (1).

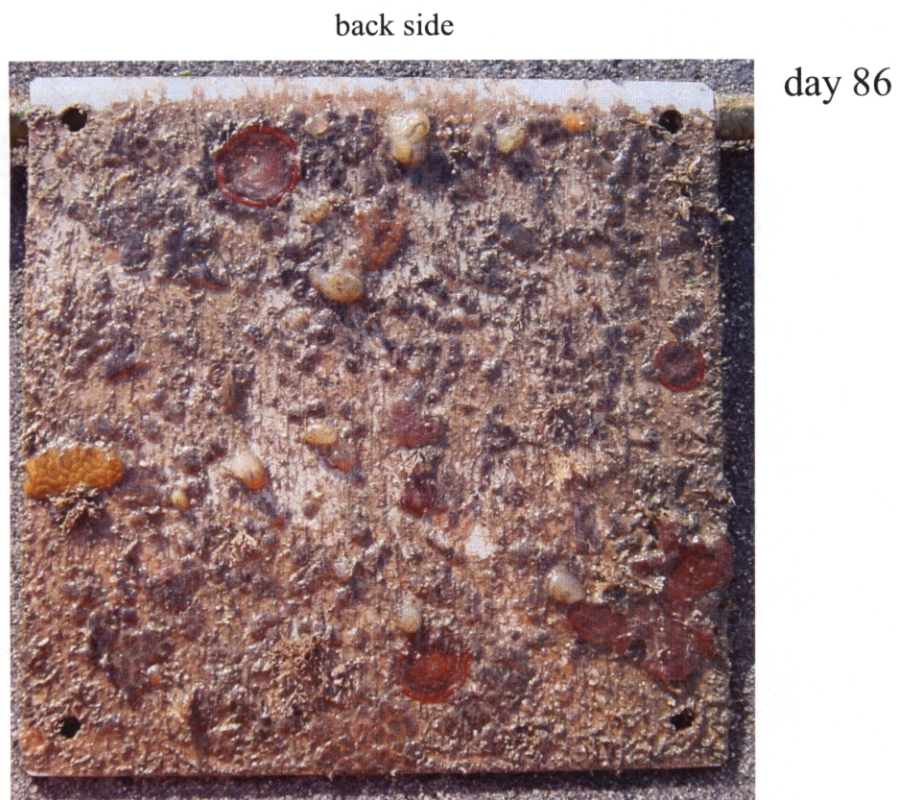
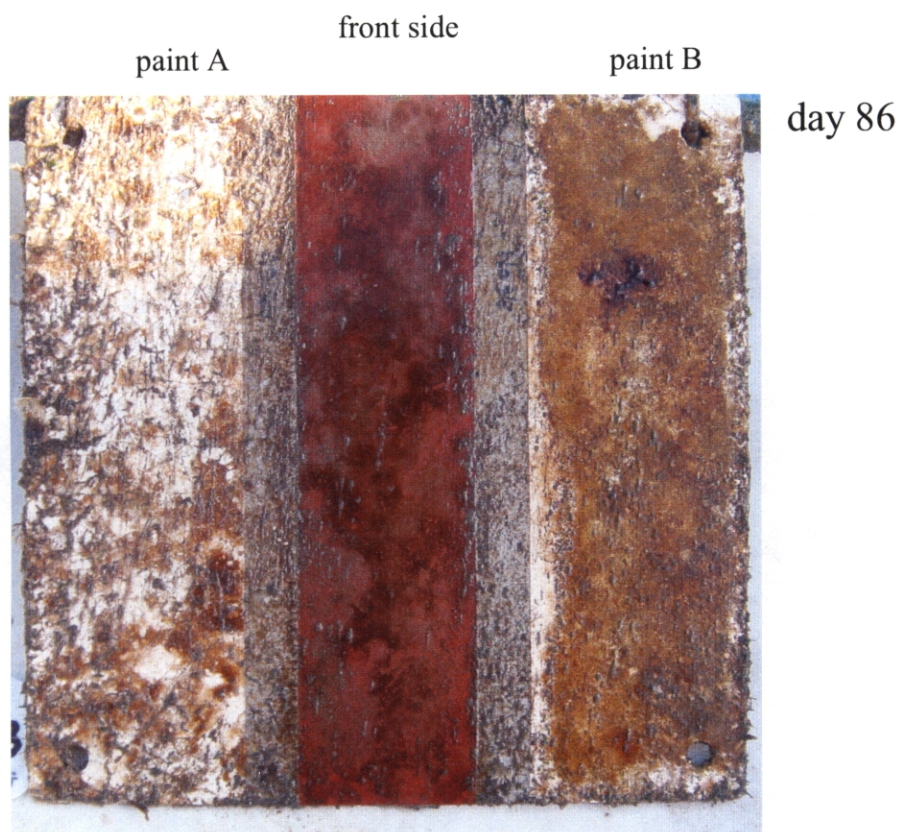


Figure 3-2. Surface conditions of test panel after 86 days in Shizugawa bay (2).

b) Experiment in Tokyo bay

The applied concentrations of **131** and **153** were 20.5% (in 7.2g) and 24.7% (in 9.1g), respectively. A test panel, fixed on the stainless frame was submerged with ropes from the pier at a depth of 1.0 m below the water surface for about two months from October 16, 2003 (Figure 3-3). Figure 3-4 shows the conditions of the painted surfaces. The variation of fouling organisms submerged test paints is shown in Table 3-2.

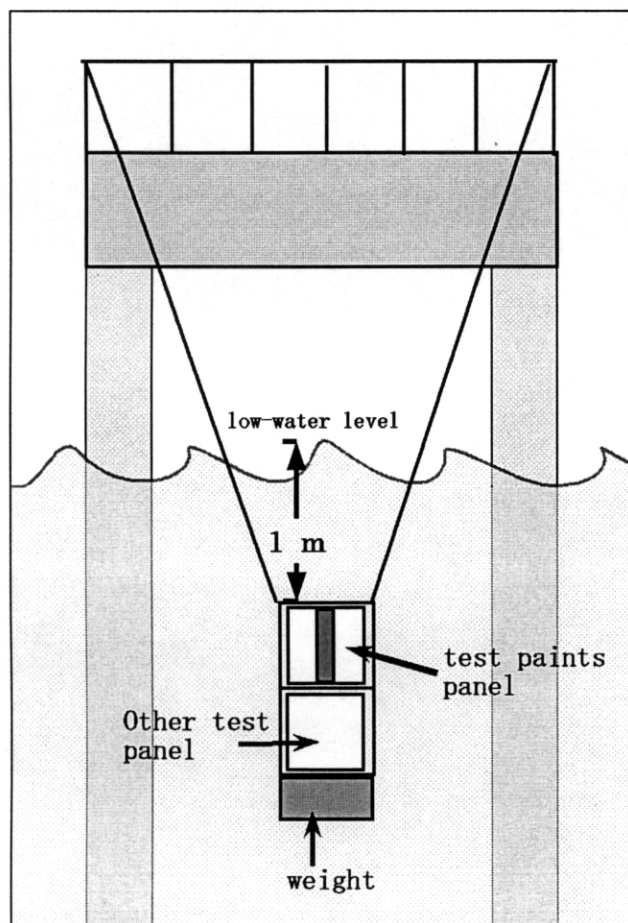


Figure 3-3. The installation method of test panel in Tokyo bay.

Copper-based paint completely prevented sessile organisms from attachment on the surface for the test period end. On the surface of blank (backside), the barnacle *B. eburneus* were observed in one month, in addition, ascidians were found in 2 months. Both trial paints prevented ascidians from settlement completely, and reduced barnacle settlement comparison with blank surface. In 2 months, the surface of paint A was settled by hydrozoans, while 14 individuals of barnacles settled (7.47 indiv./100cm²). Though paint B was covered with diatoms and hydrozoans, only 6 individuals of

barnacles (3.2 indiv./100cm²) were found on its surface in 2 months. On the other hand, barnacles settled 202 individuals on blank surface (32.3 indiv./100cm²). It seems that both trial paints kept promising anti-barnacle activity for a few months in the field.

In this study, two trial paints were prepared and evaluated for their antifouling activity in the field. From the results, these paints showed promising antifouling activity in the field. These paint surfaces were settled by diatoms and hydrozoans, however the macrofouling organisms, such as barnacles, ascidians, and bryozoans, were not observed on their surfaces during the test periods. It appears that these trial paints prevent settlement of barnacles, ascidians, and bryozoans. But the antifouling effect was not retained for so long. Especially, paint B was covered with diatoms comparatively easily, it seems to be due to the sticky surface of paint B. These results suggest that isocyanides are very useful model compounds for the development of environmentally benign antifouling paints.

Table 3-2. Abundance and Timing of Appearance of Major Attaching Organisms Groups on Test Paints in Tokyo bay

Experiment period	16, October to 9, December, 2003							
Obsevation date	12, November			9, December				
Test paint type ¹	A	B	C	A	B	C	D	
Species and abundance²								
Attached diatoms		+++			+++		+++	
Algae								
Hydrozoans				+	+		++	
Bryozoans								
Barnacles	±	±		+	+		+	
(numbers)	(4)	(3)		(14)	(6)		(202)	
Amphipods								
Solitary ascidians							+	
Compound ascidians								

1) A: paint A, using compound 131, B: paint B, using compound 153, C: copper-based paint, D: non-paint PVC (backside).

2) +++: very common (covered over 40%), ++: common (covered 10~40%), +: present, ±: rare (a few individual observed).

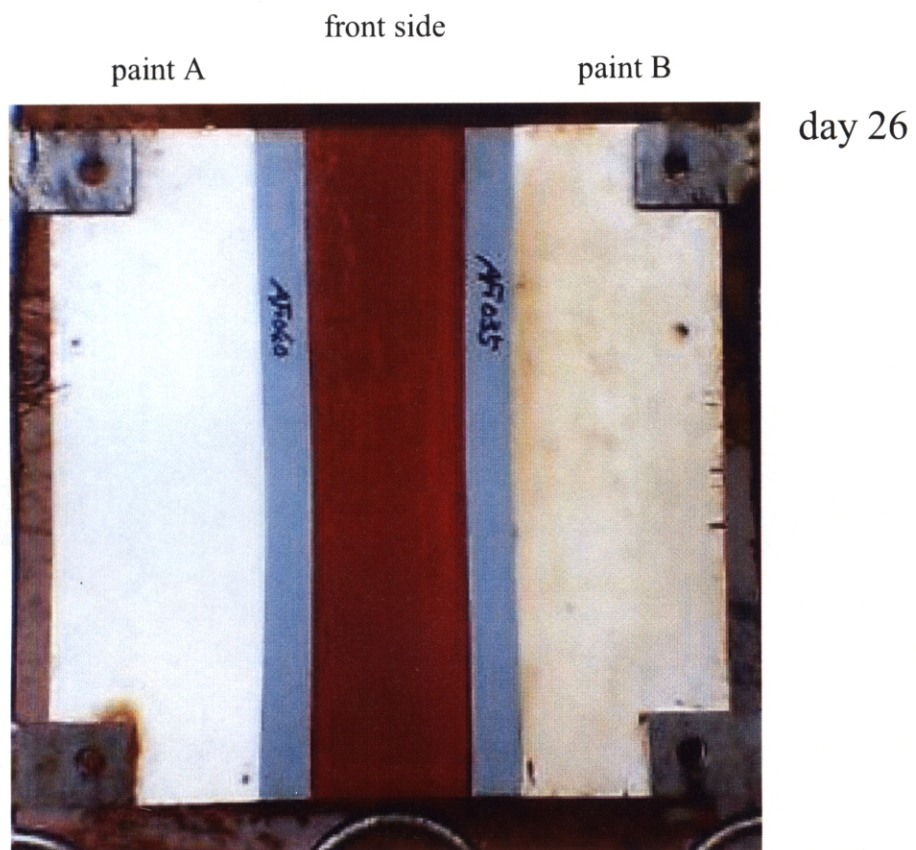


Figure 3-4. Surface conditions of test panel after 26 days in Tokyo bay (1).

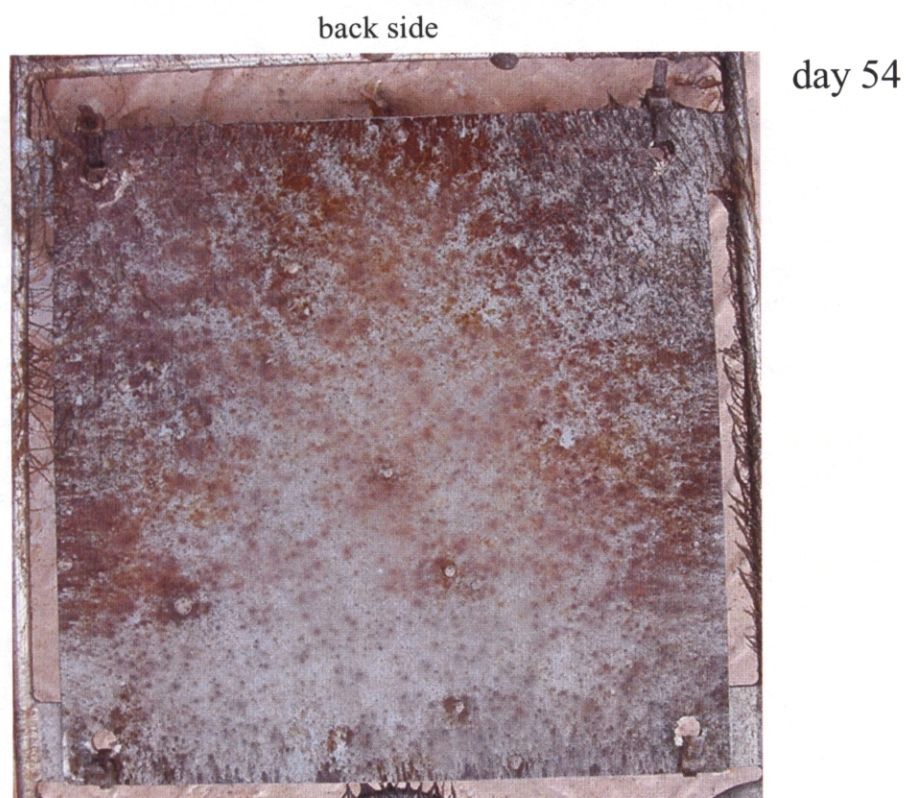
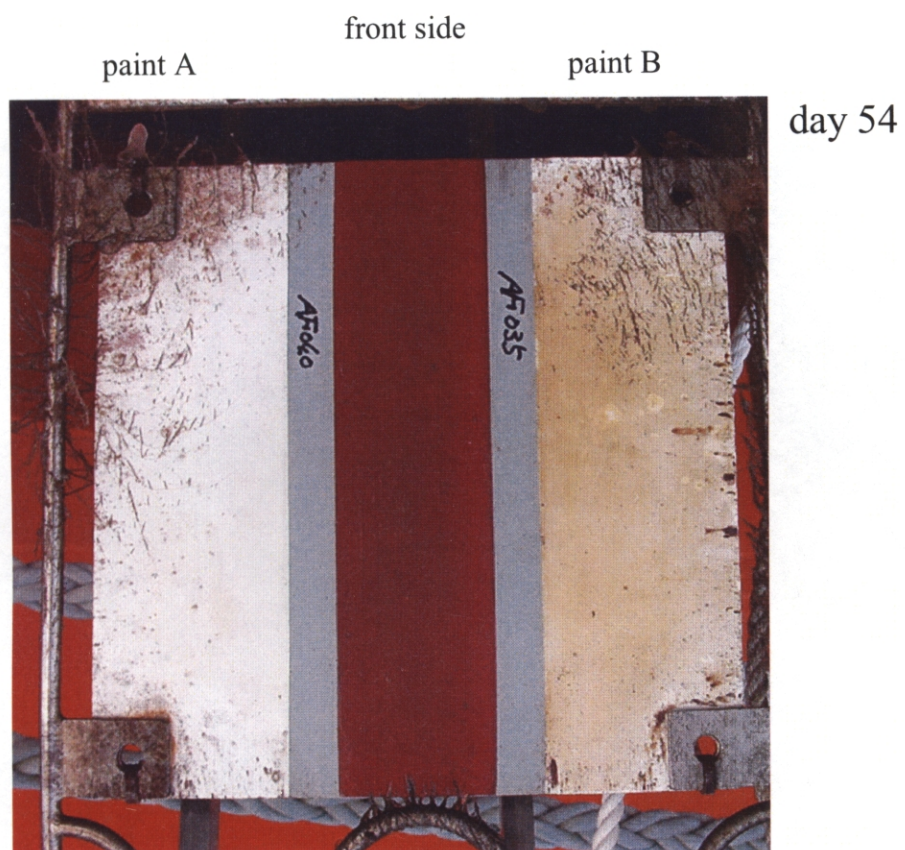


Figure 3-4. Surface conditions of test panel after 54 days in Tokyo bay (2).

Experimental Section

Preparation of test paints

Paint A (using compound 131)

The test paint A (100g) was prepared by combining **131** (15g), acrylic copolymer including carboxylic acid (10.8g), rosin (5.5g), tricresyl phosphate (2.0g), and titanium oxide (40g) in a solution of 2-acetoxy-1-methoxypropane (26.7g), and the mixture was stirred for 4h. After stirred, paint A was immediately applied onto PVC plates with brush.

Paint B (using compound 153)

The test paint B (100g) was prepared by combining **153** (15g), acrylic copolymer including carboxylic acid (10.8g), rosin (5.5g), tricresyl phosphate (2.0g), titanium oxide (27.5g) in a solution of 2-acetoxy-1-methoxy propane (39.2g), and the mixture was stirred for 4h. After stirred, paint B was immediately applied onto PVC plates with brush.

Copper-based paint

The commercial copper-based antifouling paint was used as a positive control.

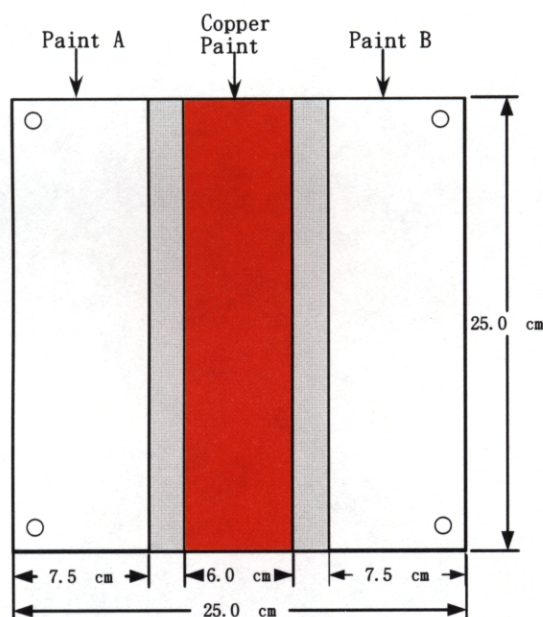


Figure 3-5. Test panels of coating with test paints, paint A, paint B, and copper based paint (front side).

Test panels

Gray PVC plates (25cm × 25cm × 3mm) were sandblasted and painted before the exposure. The test paints A and B (7.5cm × 25cm) were applied onto the both sides of panel, and the copper-based paint (6.0cm × 25cm) was applied onto center of the panel

as shown in Figure 3-5. The coating of these paints was done with a thin brush and repeated 3 times after painting dry. The coatings were applied to only front side of the panels, and their back sides were not treated. The panels were left to dry in dark, in order to avoid photooxidation of the paint. In Shizugawa bay experiment, the application quantities of test paint A, B and copper-based paints were 7.6g, 8.4g, and 12.0g, respectively. In Tokyo bay experiment, The application quantities of test paint A, B and copper-based paint were 7.2g, 9.1g, and 11.1g, respectively.

Field experiments

a) Experiment in Shizugawa bay

Field experiments were conducted at Shizugawa bay (Miyagi, Japan) between 30 August and 27 November 2003. The test panel (1 plate) was suspended vertically with ropes from a fishing boat at a depth of 0.5m. The test plate was evaluated monthly and photographed weekly during the test period.

b) Experiment in Tokyo bay

Field experiments were carried out at Tokyo bay between 16 October and 9 December 2003. The test panel (1 plate), suspended vertically from the pier at a depth of 1.0m below the low-water level as shown in Figure 3-3, The test plate was observed and photographed on 11 November and 9 December.

CONCLUSION

In order to apply not only effective but also “environmentally benign” antifouling agent, attempts were made to search novel antifouling substances from marine invertebrates, and to synthesize a variety of isocyano and related compounds based on 3-isocyanotheonellin, reported as a potent antifouling compound.

A total of 118 marine organisms, constituted of 96 sponges, 9 coelenterates, 5 bryozoans, and 8 tunicates collected from various places in Japan were extracted to prepare the lipophilic fractions, which were examined their antifouling activity against the barnacle *B. amphitrite* cypris larvae. Thirteen extracts showed promising activity, since they showed potent antifouling activity without significant toxicity. Among promising samples, the sponge *Acanthella cavernosa* collected off Atami, Shizuoka was chosen for further investigation of antifouling substances. Two terpenoids: a new compound 10-formamido-4-cadinene (**105**) and a known compound T-cadinol (**106**) were isolated from *A. cavernosa*. They inhibited larval settlement with EC₅₀ values of 0.50 and 0.53 µg/mL, respectively. Alcohol **106** did not show toxicity to cyprids at 30 µg/mL, while formamido **105** killed all cypris larvae at 10 µg/mL. T-cadinol **106** is a well-known compound contained in wood oil such as the genus *Chamaecyparis* (Hinoki), this is the first report of its antifouling activity. Since T-cadinol is included in the essential oil of the genus *Chamaecyparis* at 10%¹⁰⁶ and can be isolated comparatively easily,¹⁰⁷ further investigation into the use of T-cadinol is being allowed.

From the sponge *A. cavernosa* and the nudibranchs of family Phyllidiidae, many antifouling activity compounds were reported^{50,51,60} and most of which were terpenoids including isocyano, isothiocyantes, and formamide functional groups.^{50,51,60} Some kalihinene-type compounds such as kalihinol A (**51**), 15-formamidokalihinene (**49**), and 10-formamidokalihinene (**50**) isolated from the sponge *A. cavernosa* showed potent antifouling activity (EC₅₀ < 0.1 µg/mL) and their toxicity was quite low (LD₅₀ > 100µg/mL). However, only α-murolene (**54**) was reported as antifouling compounds in cadinene class sesquiterpenes, and it showed moderately active and toxic against cypris larvae.⁶¹ The present studies gave further information of antifouling compounds isolated from the sponge *A. cavernosa*. On the other hand, from nudibranchs of the family Phyllidiidae, 5 cadinene type sesquiterpenes, 10-isocyano-4-cadinene (**27**), 1,7-epidioxy-5-cadinene (**29**), (-)-10-isothiocyano-4-amorphene (**31**), 10-isocyano-4-amorphene (**32**), and 10-isocyano-5-cadinene-4-ol (**36**), were obtained as antifouling compounds, and showed activity against cyprids with EC₅₀ values of 0.14, >50, 7.2, 0.70, and 0.13 µg/mL, respectively.⁵¹ Considering the results of antifouling

activity of 8 cadinene derivatives (compounds **105**, **106**, **54**, **27**, **29**, **31**, **32**, and **36**), compounds **105** and **106** were also highly antifouling. For potent antifouling activity and significant toxicity, it seems that functional group of C-10 position and stereochemistry of C-7 and C-10 are important.

3-Isocyantheonellin (**35**), which was isolated as a promising antifoulant from the nudibranch *P. pustulosa* is a sesquiterpene of the bisabolone class with an isocyano functional group.^{51,52} In order to obtain the structure-activity relationships of a model compound, 3-isocyantheonellin, a total of 59 derivatives were synthesized and evaluated their antifouling activity and toxicity against cyprids larvae of the barnacle *B. amphitrite*. Among 15 compounds showed potent antifouling activity comparable to 3-isocyantheonellin ($EC_{50} < 0.20 \mu\text{g/mL}$), 14 compounds were isocyanides, whereas only *N*-(4-hexylphenyl) acetamide (**131**) showed promising antifouling activity without isocyano functional moiety.

Among 20 isocyanocyclohexanes synthesized, 8 compounds exhibited potent antifouling activity, in particular, 6 isocyanocyclohexanes possessing oxygenic functional group, showed excellent antifouling activity without significant toxicity. It should be noted that *trans*-4-acetoxy-1-methylcyclohexyl isocyanide (**116**) exhibited the most potent activity among the synthesized isocyano compounds. On the other hand, *cis*- and *trans*-4-methylcyclohexyl pivaloate (**124** and **125**) lacking isocyano functional moiety, did not show significant antifouling activity and toxicity. These results suggest that the isocyano functional group would play an important role of potent antifouling activity with non-toxic. In addition, the ester groups appear to be important functional group for the exhibition of a promising antifouling activity. However, the stereoisomers did not show clear difference in antifouling activity.

A total of 27 isocyanobenzenes and related compounds was synthesized, among which 4-[(*E,E*)-1,5-dimethyl-1-hexa-1,3-dienyl-phenyl] isocyanide (**126**) and *N*-(4-hexylphenyl)acetamide (**131**) showed promising antifouling activity. 4-Benzyloxyphenylisocyanide (**138**) exhibited not only potent antifouling activity but also highly toxic against cypris larvae. Considering the results of antifouling activity of other benzyloxy compounds, isocyano group seems to inhibit larval settlement owing to their toxicity. But, the results of this study did not make clear that some synthesized benzenes showed high toxicity. On the other hand, the functional groups of carboxy acid, amido, and aldehyde did not affect larval settlement.

Twelve simple linear isocyanides have been synthesized and evaluated as antifouling agents. Their antifouling activity indicated the importance of the isocyano group. All synthetic compounds showed antifouling activity, but much less toxicity.

However, the relationships of alkyl chain length and activity have not been completely clarified; compounds of different length of alkyl chains may provide information on non-toxic potent antifoulants.

Among promising compounds as described above, two promising compounds **131** and **153** were chosen and evaluated as paints in field experiments. Field experiments were carried out at two field sites; Shizugawa bay, Miyagi and Tokyo bay, Tokyo for 3 and 2 months, respectively. In both field experiments, test paints A and B showed promising performance as antifouling paint. Although these paint surfaces were settled by diatoms and hydrozoas, macrofouling organisms, such as barnacles, ascidians, and bryozoans, were not observed on their surfaces during the test periods.

Obviously more studies are required. Nevertheless, the results suggest that isocyanides are very useful model compounds for the development of environmentally benign antifouling paints.

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