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Feeding attractants for the muricid gastropod *Drupella cornus*, a coral predator

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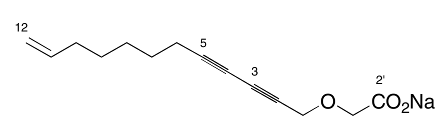
Abstract—The muricid gastropods genus *Drupella* are known to be voracious coral predator. Outbreaks of them have accelerated significant destruction on coral reefs, but its precise mechanism is poorly understood. Here, we describe the identification of montiporic acids C (**1**) and A (**2**) isolated from sea water extracts of the coral *Montipora* sp., which showed potent feeding-attractant activity toward *D. cornus*.

Coral reefs are important sources of revenue for the fishing and tourism industries, and have aesthetic and cultural values, but they are in serious decline worldwide, primarily due to over-harvesting, pollution, disease and climate change.[1] With deterioration of the marine environment, coral bleaching and outbreaks of coral predators have accelerated conspicuous and significant coral mortality. Among several coral-feeding marine creatures, the crown-of-thorns starfish *Acanthaster planci* and the muricid gastropods genus *Drupella* are known to be voracious coral predators.[2–5] The muricid *Drupella* sp. are widely distributed throughout the Indo-Pacific region.[6] Population explosions of *Drupella* have been noted worldwide from 1976; that is, at the island of Miyake-jima, Japan, at Mactan Island, Philippines, and at Enewetak, Marshall Islands. Although muricid gastropods are rather small (10–40 mm) compared to *A. planci* (20–60 cm), aggregates of these creatures are capable of inflicting significant destruction on coral reefs. In one case, more than 100 individuals of *D. rugosa* were counted on a single colony of corals in an area measuring 18×17 cm in the Marshall Islands.[7] Although several reasons for the periodic outbreak of *Drupella* sp. have been proposed,[5] that is, overabundance of nutrients, climatic aberration such as heavy rainfall or typhoon, and elevated water temperature, the precise mechanism is poorly understood. Control efforts are hampered by the fact that there are no effective methods for capturing these coral predators other than those which are massively labor intensive.



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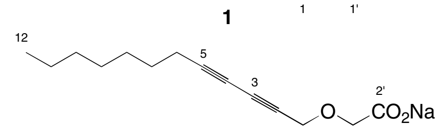

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We have focused on 'feeding attractants' for these voracious coral predators, and recently reported that both arachidonic acid and alpha-linolenic acid were effective attractants for the starfish *A. planci*.[8] Here, we describe the identification of montiporic acids C (**1**) and A (**2**) isolated from sea water extracts of *Montipora* sp., which

Keywords: Feeding attractant; Montiporic acid; *Drupella cornus*; Isolation and structure determination; Synthesis.

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Figure 1. The muricid gastropod *Drupella cornus*.

showed potent feeding-attractant activity toward the muricid gastropod *D. cornus*.

Field observations were carried out on the reef around the village of Nakijin, Okinawa, Japan. In this area, the representative gastropod species was *D. cornus* (Fig. 1), and it preferentially fed on colonies of *Montipora* sp. We collected both species, and conducted a feeding attractant assay in an aquarium. We found that *D. cornus* was indeed attracted and fed not only on coral branches but also on agar containing sea water in which coral was bled ('sea water extract'). Attractant activity was observed with a 1/200 dilution of sea water extract (4 L, before concentration) of coral (35 g). Interestingly, extension of the proboscis, which is a characteristic feeding behavior of *D. cornus*, was observed during agar feeding (Fig. 2). Thus, it was expected that the coral *Montipora* sp. contained some feeding attractant(s) for this gastropod. Distilled water extracts of corals also showed feeding attractant activity. However, to minimize damage to living coral, sea water extracts were used in this experiment.

Guided by this simple assay, separation of the feeding attractants for *D. cornus* was carried out. Concentrated

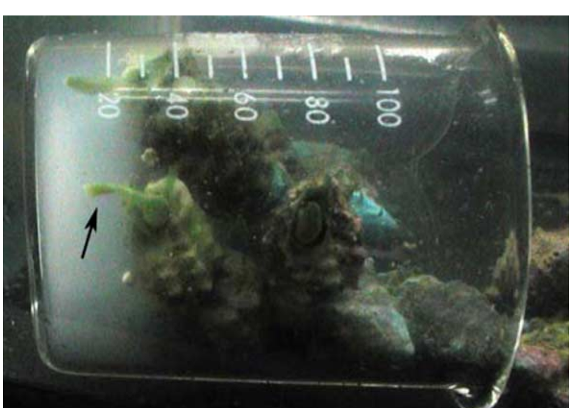
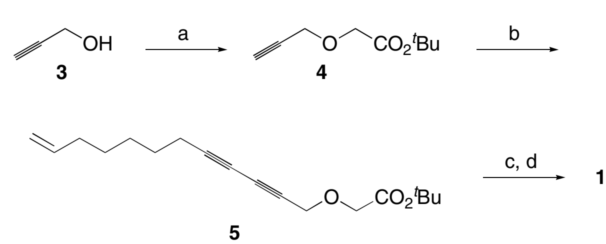


Figure 2. Bioassay in an aquarium. *D. cornus* (20–30 mm body length) were attracted to and fed on agar (20 mL) that contained sea water extracts of the coral *Montipora* sp. in a 100-mL beaker. The arrow indicates the proboscis of *D. cornus*.

sea water extracts of the coral *Montipora* sp. (35 g) were subjected to TSK G-3000S gel permeation column chromatography. The column was first washed with water to remove most of the mineral salts, and then eluted with EtOH. Although most of the attractant activity was found in the aqueous layer (~90%), the specific activity of the EtOH layer was approximately 100 times higher than the aqueous layer. Thus, we investigated the purification of EtOH layer. The EtOH layer was ultrafiltered (cut-off M_r, 10,000), and the filtrate was then separated by reversed-phase column chromatography and thin-layer liquid chromatography to give two fatty acids, montiporic acids C (**1**, 1 mg) and A (**2**, 10 mg). The minimal amounts of **1** and **2** to show feeding attractant activity were 0.1 and 1 mg/20 mL agar, respectively. Thus, montiporic acids C and A may account for approximately 10% of the activity of crude coral extracts.[9] In general, mucus is produced in considerable quantities by branching hard corals and covers a large proportion of the reef area, which is suggested to carry nutrients.[10] Thus, montiporic acids may be released from corals with mucus in the field. Spectroscopic data for these compounds, including NMR and MS, were found to be identical to those reported in the literature.[11] Montiporic acids have been reported to be cytotoxic and antibacterial metabolites contained in the eggs of *Montipora* sp. However, this is the first report that the genus *Drupella* was attracted to these polyacetylenic fatty acids. Interestingly, none of the known feeding attractants for *A. planci*, that is, arachidonic acid, alpha-linolenic acid, and betaine,[12] were effective for *Drupella* sp.

To confirm the feeding attractant activity of montiporic acids, we investigated the synthesis of **1** (Scheme 1).[13] Propargyl alcohol (**3**) was coupled with 2-bromoacetate to give **4**. Acetylene **4** was converted to the diyne alcohol **5** by coupling it with 1-nonene-8-yne using Cu(OH)Cl·TMEDA catalyst.[14] Deprotection of the *t*-Bu group in **5** under acidic conditions followed by neutralization with $NaHCO_3$ gave the montiporic acid C sodium salt (**1**). The total yield of this sequence was 48%. The ^{1}H and ^{13}C NMR data of montiporic acid C sodium salt were completely consistent with those of the natural compound.[15]

Synthetic montiporic acid C sodium salt (**1**) showed feeding attractant activity for *D. cornus* at a concentration of 1.5 mg (or more)/20 mL agar. In the bioassay, we



Scheme 1. Synthesis of montiporic acid C (**1**). Reagents and conditions: (a) $BrCH_2CO_2{}^tBu$, NaH, THF–DMF (5:1), rt, 93%; (b) 1-nonene-8-yne, Cu(OH)Cl·TMEDA (10 mol %), O_2, CH_2Cl_2, rt, 53%; (c) HCOOH (neat), rt; (d) $NaHCO_3$ aq rt, 100% in two steps.

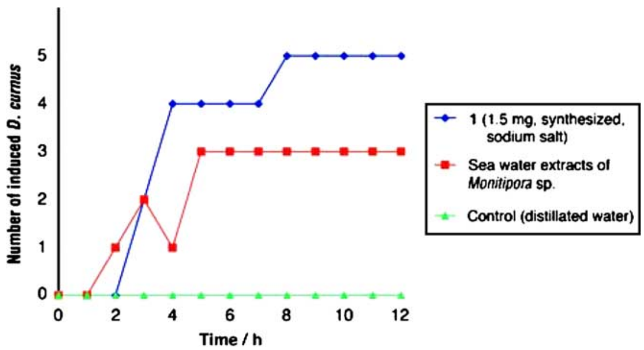


Figure 3. Feeding attractant assay for *D. cornus* by the hour. Twelve individuals of *D. cornus* were used in each assay. In the case of sea water coral extracts (indicated in red), 20 mL of the sea water, in which coral (35 g) was bled, was used.

counted the number of attracted *D. cornus* each hour, and observed that both synthesized **1** and sea water *Montipora* extracts showed persistent attractant activity (Fig. 3). Although the minimal effective amount of synthesized **1** was somewhat higher than that of natural **1**, we confirmed that montiporic acid C sodium salt (**1**) is indeed a feeding attractant for *D. cornus*.

In a previous study, we showed that arachidonic acid and alpha-linolenic acid were effective feeding attractants for *A. planci* in a natural setting.[8] Although a bioassay has not yet been conducted in for *D. cornus* or other *Drupella* species in a natural setting, it is expected that our method, that is, trapping of coral predators using feeding attractants, could contribute to an effective monitoring system for the prediction and prevention of coral damage caused by outbreaks of harmful marine coral predators.

Acknowledgements

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15. ^{1}H NMR data (800 MHz, CD_3OD) for **1** (synthetic, sodium salt): δ 5.80 (H-11, 1H, ddt, $J = 17.0$, 10.2, 7.0 Hz), 4.99 (H-12a, 1H, dd, $J = 17.0$, 1.5 Hz), 4.92 (H-12b, 1H, dd, $J = 10.2$, 1.5 Hz), 4.28 (H-1, 2H, s), 3.92 (H-1′, 2H, s), 2.29 (H-6, 2H, t, $J = 7.0$ Hz), 2.06 (H-10, 2H, q, $J = 7.0$ Hz), 1.52 (H-7, 2H, m), 1.41 (H-8 and H-9, 4H, m); ^{13}C NMR data (201 MHz, CD_3OD) for **1** (synthetic, sodium salt): δ 177.2 (C-2′, s), 139.8 (C-11, d), 114.9 (C-12, t), 81.7 (C-5, s), 72.6 (C-3, s), 72.1 (C-2, s), 69.7 (C-1′, t), 65.4 (C-4, s), 58.9 (C-1, t), 34.7 (C-10, t), 29.5, 29.3, 29.2 (C-7–C-9, 3C, t), 19.7 (C-6, t); MS (FAB) *m/z* 279 $(C_{14}H_{17}NaO_3{+}Na)^+$.