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NOTE

A re-evaluation of the ichthyodeterrent role of prostaglandins in the Caribbean gorgonian coral *Plexaura homomalla*

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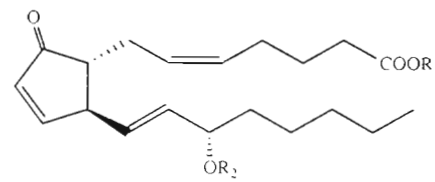
ABSTRACT: Previous investigations have indicated that the Caribbean gorgonian coral *Plexaura homomalla* derives a chemical defense from prostaglandins present at high concentrations within the coral's soft tissues. These conclusions were based on laboratory and field experiments with the hydroxy acids of prostaglandin A_2 (PGA_2), and not the acetoxy methyl esters of PGA_2 that are present in the living gorgonian. In the present study, the consumption of food strips containing the acetoxy methyl ester of 15(R) PGA_2 from *P. homomalla* by a natural assemblage of Caribbean coral reef fishes did not differ from consumption of control strips. In support of previous findings, treatment with the acetoxy acid, hydroxy methyl ester and hydroxy acid of 15(R) PGA_2 inhibited consumption of food strips by reef fishes. Although the acetoxy methyl esters of PGA_2 undergo hydrolysis when the soft tissues of *P. homomalla* are damaged, the process appears to be too slow to provide the gorgonian with an effective inducible defense mechanism.



A. HYDROXY ACID: R_1=H, R_2=H
B. HYDROXY METHYL ESTER: R_1=CH_3, R_2=H
C. ACETOXY ACID: R_1=H, R_2=$COCH_3$
D. ACETOXY METHYL ESTER: R_1=CH_3, R_2=$COCH_3$

Fig. 1. Structure of 15(R) prostaglandin A_2 (PGA_2) from the gorgonian coral, *Plexaura homomalla* (A). Structures of the 3 derivatives assayed in this study are also indicated (B, C, D)

Some species of Caribbean gorgonian corals of the genus *Plexaura* (in particular, *P. homomalla*) contain high concentrations of prostaglandins within their soft tissues; estimates range from 1 to 8% of wet tissue weight (Schneider et al. 1977, Domingez et al. 1980) and 1 to 2% of dry tissue weight (Weinheimer & Spraggins 1969). Non-esterified prostaglandins mediate a variety of biological responses, particularly in mammals, and are generally found in only trace concentrations in animal tissues (Nomura & Ogata 1976). The biological function of such high concentrations of these compounds in the tissues of *P. homomalla* is unknown, particularly because co-occurring species of gorgonians do not contain prostaglandins in appreciable amounts. Equally noteworthy, fully-esterified prostaglandin A_2 (PGA_2; Fig. 1D), the most abundant prostaglandin in the tissues of *P. homomalla*, occurs predominantly in the 15(R) isomer rather than the 15(S) form found in all other animal tissues. The 15(R) isomer is much less active than the 15(S) in representative pharmacological assays (Spraggins 1972).

Gerhart (1984) tested the hypothesis that the prostaglandins in *Plexaura homomalla* function as a chemical defense against predatory reef fishes. He found that both the 15(S) and 15(R) isomers of unesterified PGA_2 induced regurgitation in the yellowhead wrasse *Halichoeres garnoti* in feeding experiments conducted on coral reefs off Curaçao, and obtained similar results in laboratory assays with the killifish *Fundulus heteroclitis* (Gerhart 1984). As Gerhart pointed out, however, the compounds used in his assays were the hydroxy acids of 15(S) and 15(R) PGA_2 (Fig. 1A) (Gerhart 1984, p. 183), while the acetoxy methyl esters (Fig. 1D) of these compounds are the sole derivatives stored in the living tissues of the gorgonian (Schneider et al. 1977). Gerhart jus-

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tified his use of the hydroxy acids because they are the ultimate products of enzymatic hydrolysis of the acetoxy methyl esters after the coral tissue is damaged; however, completion of this process is reported to take ~24 h (Schneider et al. 1977).

Gerhart (1986) also tested the hypotheses that prostaglandins function as allelopathic agents or as inhibitors of biofouling of *Plexaura homomalla*. He found little or no PGA_2 in the seawater surrounding colonies of *P. homomalla*, and neither isomer of PGA_2 inhibited the fouling of gorgonian axial skeletons. It is unclear whether the hydroxy acids or acetoxy methyl esters of PGA_2 were employed in the fouling assays.

In a survey of the chemical defenses of Caribbean gorgonian corals, we found that extracts of most of the gorgonian species of the genus *Plexaura* were palatable to the bluehead wrasse *Thalassoma bifasciatum* in laboratory assays (Pawlik et al. 1987). One sample, *Plexaura* sp. voucher No. 29, yielded an extract containing esterified prostaglandins. This extract was unpalatable in preliminary assays, but did not significantly deter fish feeding in more rigorous assays conducted at diminishing concentrations (Pawlik et al. 1987, Table II). The purpose of the present study was to ascertain whether naturally-occurring prostaglandins contained in the tissues of *P. homomalla* (i.e., the acetoxy methyl esters) provide an effective chemical defense against a natural assemblage of predatory reef fishes in field assays.

Materials and methods. Prostaglandins employed in the assays were purified from a frozen, crude dichloromethane extract of *Plexaura homomalla* collected in August 1983 at Acklins Island, Bahamas. The gorgonians had been air-dried prior to extraction. Extracts contained a mixture of the acetoxy methyl ester, hydroxy methyl ester and hydroxy acid (approximate ratio of 2:1:1, respectively) of 15(R) PGA_2 (Fig. 1), as identified by NMR spectroscopy The 15(S) isomer of PGA_2 was not detected. The prostaglandins in the extract were purified by flash chromatography on silica gel employing a 0 to 100 % ethyl acetate in iso-octane gradient, followed by silica gel HPLC on a preparative column with 2:1 hexanes:ethyl acetate as the eluant. A portion of the purified hydroxy acid was acetylated with acetic anhydride in pyridine to yield the acetoxy acid (Fig. 1C). Purified samples were kept frozen for the 1 to 2 wk prior to use in field assays. Extracted, esterified prostaglandins were stable to further hydrolysis.

Field assays were conducted near Looe Key Reef, south of Big Pine Key, Florida, USA, in September 1987. Prostaglandins were incorporated into a matrix of 4 % carageenan in the form of 1.0 × 0.5 × 5.0 cm strips (Harvell & Fenical 1988). Each strip enclosed a length of cotton string that protruded from one end of the strip. Strips were made by adding water to 2.5 g carageenan (Gelcarin, FF961L; FMC Corp., Philadelphia) and 0.6 g freeze-dried brine shrimp (*Artemia* sp.) to a total volume of 60 ml and heating the mixture to boiling. To this mixture, 1.5 g of prostaglandin were added in a minimal amount of ethanol. The mixture was stirred and then poured into plastic molds crossed by lengths of cotton string. After the matrix cooled and gelled, the strips were sliced to size with a scalpel and removed from the mold. Control strips contained an equivalent volume of ethanol, but no prostaglandin.

The assay concentration of 1.5 g prostaglandin in a 60 ml carageenan mixture (~2.5 % by volume) is a high value relative to the levels of prostaglandins found in *Plexaura homomalla*. We calculated ~0.86 g of dry *P homomalla* tissue per ml of wet tissue volume (excluding gorgonin axial skeleton). Assuming that 2 % of the dry weight of the gorgonin is composed of prostaglandins (Weinheimer & Spraggins 1969), then 60 ml of this tissue would contain ~1.0 g of prostaglandins (~1.7 % by volume). Prostaglandin concentrations of ~2.5 % by volume (~3 % dry weight) would be at the upper limit of their natural concentration in *P. homomalla*. Therefore, any conclusions drawn from assays conducted at this concentration on the ineffectiveness of the compounds to deter predation would be conservative.

One treatment and one control strip each were affixed to a 50 cm length of 3-strand nylon rope (at a distance of ~4 and 14 cm from one end) by tying the cotton string protruding from each strip through the strands of the rope. Twenty such ropes were deployed on the reef for each experiment, with the end of each rope opposite the food strips unwound and clamped onto a piece of coral or rock at 10 to 15 m water depth. After 1 to 4 h, the ropes were retrieved from the reef and the amount of each strip eaten was recorded as a percentage decrease in the strip eaten was recorded as a percentage decrease in the strip length (to the nearest 5 %). Strip length was adjusted for bites taken out of the edges of the strip (see Hay 1984, for a detailed discussion of these assay methods). The Wilcoxon paired-sample test (one-tailed, Zar 1984) was employed to analyze the assay results after first excluding pairs for which both control and treatment slices had been either completely eaten, or not eaten at all.

Results and discussion. In support of Gerhart's (1984) results, the hydroxy acid of 15(R) PGA_2 from *Plexaura homomalla* (Fig. 1A) inhibited the feeding of a natural assemblage of reef fishes (Fig. 2A). The principal reef fishes observed feeding on assay strips were wrasses, primarily *Thalassoma bifasciatum*, and 2 or 3 species of *Halichoeres*. Damselfishes, *Abudefduf* sp. in particular, were occasionally observed biting at the strips.

Both partially-esterified prostaglandins, the hydroxy methyl ester (Fig. 1B) and the acetoxy acid (Fig. 1C),

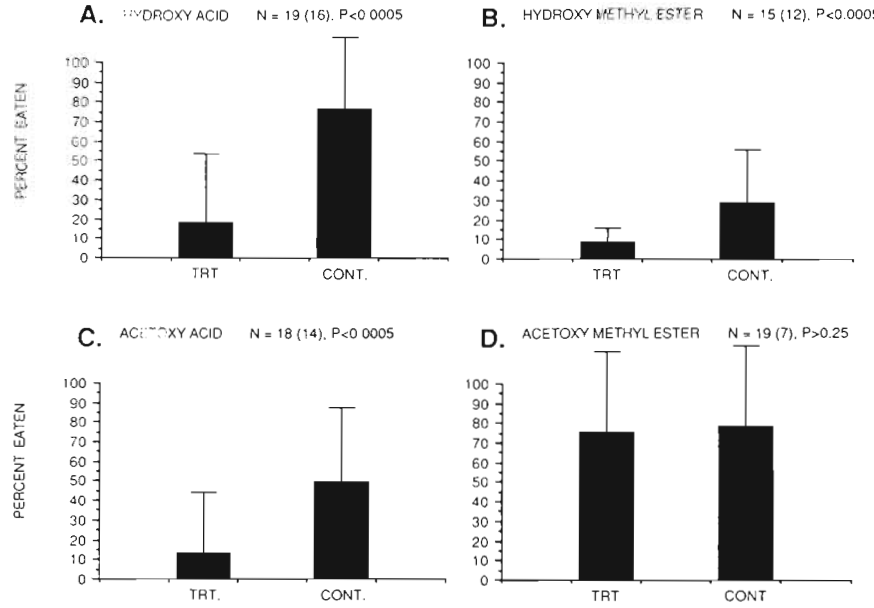

Fig. 2. Percentage consumption of assay strips treated with prostaglandins (TRT.) and untreated (CONT.) by a natural assemblage of coral reef fishes in field experiments. Compounds A, B, C are hydrolysis products of the fully-esterified 15(R) PGA_2 found in the undamaged tissues of *Plexaura homomalla* (D). Vertical lines through each histogram indicate one standard deviation above the mean. N: no. of pairs retrieved of 20 deployed (no. of pairs used in statistical analysis). Probability values were calculated from the Wilcoxon paired-sample test

exhibited equally effective deterrent properties (Figs. 2B, C). The hydroxy methyl ester is slowly generated by the enzymatic hydrolysis of the acetoxy methyl ester, and begins accumulating after damage to the gorgonian soft tissue (Schneider et al. 1977). It is unclear whether the acetoxy acid is produced under normal hydrolysis conditions; our intent was to test the relationship between molecular structure and biological activity, and this compound proved to be equally deterrent (Fig. 2C).

The fully-esterified, acetoxy methyl ester of 15(R) PGA_2 isolated from *Plexaura homomalla* (Fig. 1D) did not significantly deter consumption of treated assay strips by reef fishes as compared with untreated strips (Fig. 2D). Of the 19 pairs of strips deployed for this experiment, 12 were entirely eaten (both treatment and control) before the ropes could be retrieved; this left only 7 pairs for statistical analysis. The 15(S) isomer of PGA_2 was not present in our collections of *P. homomalla*, although it has been detected in specimens collected from other localities (Schneider et al. 1977). The effectiveness of the esterified 15(S) isomer as an inhibitor of fish predation is unknown, but it seems unlikely that 15(R) PGA_2, the exclusive prostaglandin in the tissues of *P. homomalla* from many parts of the Caribbean, serves a defensive function in its fully-esterified form.

An alternative hypothesis is that esterified prostaglandins function as an inducible defense of *Plexaura homomalla:* damage to the gorgonian tissue results in the enzymatic hydrolysis of otherwise innocuous acetoxy methyl esters, resulting in the formation of noxious hydroxy methyl esters and hydroxy acids. While an attractive hypothesis, no evidence indicates that hydrolysis occurs at a sufficiently rapid rate. Schneider et al. (1977) reported that 24 h were required for the conversion of the acetoxy methyl esters to the hydroxy acids. We observed that only about one-half of the prostaglandins underwent hydrolysis when *P. homomalla* was air-dried over several hours.

For a chemical defense to be effective, the defensive compounds should cause the predator to reject the prey almost immediately after the prey is sampled; the predator then learns to avoid the prey on subsequent forays. The conversion of the acetoxy methyl esters of PGA_2 to the hydroxy methyl esters and hydroxy acids in the guts of predatory reef fishes several hours after the ingestion of gorgonian tissues might result in subsequent regurgitation, or possibly the death of the fish, but these responses would be unlikely to result in prey avoidance by the predators.

Plexaura homomalla is a common constituent of the Caribbean gorgonian fauna, and apparently defends itself through some means, perhaps by virtue of the silicious spicules that pervade its soft tissues. Studies by Harvell and colleagues (Harvell & Fenical 1988, Harvell et al. 1988) indicate that the physical defense afforded by spicules may be important in defending gorgonians of the genus *Pseudopterogorgia* from fish predators. Although the results of the present study

suggest that the esterified prostaglandins present in *Plexaura homomalla* do not deter fish predators, these compounds may have very different effects on other potential predators of gorgonians, particularly invertebrates.

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