New Prostaglandins from the Chemically Defended Soft Coral *Plexaura nina*

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Three new prostaglandins were isolated as minor constituents of the organic extract from the Caribbean soft coral *Plexaura nina*. The structures of the new natural products were established by means of spectral data analysis, including 2D NMR experiments. The unpalatability of the lipid extract of the coral and the defensive role of the major prostaglandin metabolites were determined by laboratory and field fish-feeding assays.

Introduction

Prostaglandins constitute an important group of biologically active substances with a wide-spectrum of pharmacological and ecological properties (Pawlik and Fenical, 1989; Coll, 1992; Evans, 1996). Structurally, all compounds are based on prostanoic acid and six series (A-F) arise by modification of the cyclopentane ring (Evans, 1996). Soft corals constitute the most significant marine source of prostaglandins. The first report dealt with the isolation of large quantities of two prostaglandin derivatives, 15-epi-PGA_2 and its ester, from the gorgonian coral *Plexaura homomalla* (Weinheimer and Spraggins, 1969). Since then a number of researchers isolated several prostaglandins from other soft corals, especially from members of the genus *Plexaura* (Schneider *et al.*, 1972; Light and Samuelsson, 1972; Schneider *et al.*, 1977a; Schneider *et al.*, 1977b; Ciereszko *et al.*, 1985). Additionally, total syntheses and structural modifications of these molecules have been the subject of considerable synthetic effort (Lincoln *et al.*, 1973; Grieco and Reap, 1973; Patterson and Fried, 1974; Luo and Negishi, 1985). The most frequently isolated prostaglandins are PGA_2 and PGE_2, as well as their acetyl derivatives and methyl esters. Especially, for the marine environment it has been suggested that only the acetylated methyl ester derivatives are true secondary metabolites and the hydroxy acids are decomposition by-products (Schneider *et al.*, 1977a). It was reported that marine prostaglandins possess *S* configuration at the C_{15} asymmetric center like the ones of mammalian origin (Schneider *et al.*, 1972; Light and Samuelsson, 1972; Schneider *et al.*, 1977a; Schneider *et al.*, 1977b; Ciereszko *et al.*, 1985).

In the course of our investigations on isolation of bioactive metabolites from marine organisms and the elucidation of their ecological roles (Harvel *et al.,* 1993; Kakonikos *et al.*, 1999; Mihopoulos *et al.*, 1999a) we recently examined some *Plexaura* species collected from the Caribbean Sea. In this report we describe the isolation and structure elucidation of three new prostaglandin derivatives, **4** - **6**, which were obtained from the medium polarity fractions of the organic extract of the gorgonian *P. nina*, along with the known compounds **1** - **3** that were proven to be defensive metabolites against fish predation.

In the past prostaglandins from other corals have been shown, to render chemical defence in the producing organisms (Pawlik and Fenical, 1989). Laboratory and field assays employing common soft coral predators were undertaken to determine the palatability of food pellets prepared with freshly-extracted lipid-soluble *P. nina* extract and pure metabolites. Crude extract and the combination of compounds **2** and **3** inhibited fish feeding at concentrations near or below the concentrations of metabolites in the gorgonian soft tissue. The experiments were performed according to protocols described (Pawlik *et al.*, 1987).

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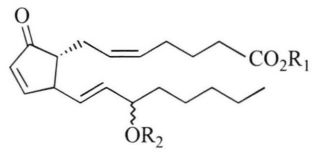
Results and Discussion

Structure determination

Metabolites **1** - **3** (Fig. 1) were isolated as the major constituents of the extract from the medium polarity fractions of the initial vacuum column chromatography. Comparison of the NMR data with literature data suggested that they are PGA_2 along with the acetoxy and the methyl ester derivatives. In the past these metabolites had been isolated from other *Plexaura* species as either 15*S* or 15*R* isomers (Schneider *et al.*, 1972; Schneider *et al.*, 1977a). In our fractions metabolites **1** - **3** were mixtures of both isomers as was indicated from the pair of doublets for H-10 and H-11. Upon further chromatographic separations the *S* (less polar) and the *R* (more polar) isomer (Schneider *et al.*, 1977a) were isolated in pure form but very soon both of them were racemized.

Compounds **4** and **5** were purified by HPLC in minute quantities as yellowish oils. Both ^{13}C NMR data and HRMS measurements supported the molecular formula $C_{23}H_{34}O_5$. The EI mass spectra for both metabolites revealed a molecular ion $[M]^+$ at *m/z* 390. The IR spectra of **4** and **5** displayed absorptions for enone and ester carbonyls at 1710 and 1735 cm^{-1}. All spectral data of the two compounds were very similar with slight differences at the olefinic carbon and proton resonances. The ^{13}C NMR spectra showed the presence of 23 carbons corresponding to five quartenary, five methine, ten methylene and three methyl carbon atoms. With seven degrees of unsaturation the structure was suggested to contain one ring, three double bonds and three carbonyl groups. The presence of the enone moiety was also confirmed by the carbon signals at δ 208.7 and 208.4. The ^1H NMR spectra showed signals characteristic for a methyl ester group (at δ 3.6 ppm), an acetyl group (at δ 2.07 ppm) and for a terminal methyl (at δ 0.86 ppm).

Comparison of ^1H and ^{13}C NMR data of compounds **4** and **5** with literature values (Lincoln *et al.*, 1973; Patterson and Fried, 1974; Schneider *et al.*, 1977b; Luo and Negishi, 1985) showed that they possessed the prostaglandin skeleton with a high structural similarity to PGB_2. Based on spectral interpretation, the structure of the acetoxymethyl ester of PGB_2, that to the best of our knowledge has not yet been reported as a natural product, was proposed for metabolite **5**. The considerable shift of C-7 and C-4 (α- to Δ^5) methylene resonances to higher fields suggested **4** to be the 5*Z*-13*E* geometric isomer of **5** (Sims *et al.*, 1978; Mihopoulos *et al.*, 1999b). The assignments of all proton and carbon resonances in the molecules were supported by correlations provided by 2D homonuclear and heteronuclear NMR experiments.



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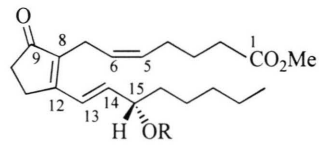
2: R_1=Me, R_2=H
3: R_1=H, R_2=H



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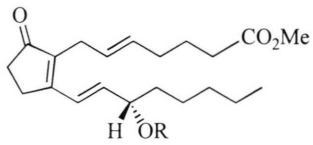
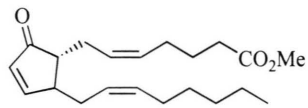
4a: R=H



5: R=Ac
5a: R=H



6

Fig. 1. Structures of the natural products **1**–**6**.

Table I. ^{13}C NMR spectral data of prostaglandins **4**, **5** and **6**.[a]

Position	Compound **4**	Compound **5**	Compound **6**
1	174.0 (s)	174.0 (s)	174.0 (s)
2	33.4 (t)	33.3 (t)	33.3 (t)
3	24.6 (t)	24.4 (t)	24.7 (t)
4	26.6 (t)	31.7 (t)	26.6 (t)*
5	129.7 (d)	130.4 (d)	126.7 (d)
6	126.7 (d)	127.0 (d)	131.1 (d)
7	21.4 (t)	26.0 (t)	39.7 (t)*
8	162.6 (s)	163.0 (s)	46.2 (d)
9	208.7 (s)	208.4 (s)	208.0 (s)
10	24.7 (t)	24.7 (t)	133.0 (d)
11	33.7 (t)	33.7 (t)	166.5 (d)
12	140.0 (s)	139.6 (s)	50.9 (d)
13	125.7 (d)	125.7 (d)	31.4 (t)
14	135.7 (d)	135.6 (d)	132.3 (d)
15	73.9 (d)	73.9 (d)	127.2 (d)
16	34.2 (t)	34.2 (t)	27.4 (t)
17	25.5 (t)	25.5 (t)	23.5 (t)
18	31.4 (t)	31.4 (t)	28.3 (t)
19	22.4 (t)	22.4 (t)	22.4 (t)
20	13.9 (q)	13.9 (q)	13.9 (q)
21 (OCH_3)	51.5 (q)	51.4 (q)	51.4 (q)
22 ($O\mathbf{C}OCH_3$)	171.0 (s)	171.0 (s)	
23 ($OCO\mathbf{C}H_3$)	21.1 (q)	21.1 (q)	

[a] All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in ppm.
* Resonances may be interchanged.

Along with the acetylated metabolites **4** and **5** were obtained in small quantities the hydroxy analogues **4a** and **5a**. A portion of these metabolites was acetylated and the structures of the products were confirmed by comparison with **4** and **5**. Another part of **4a** and **5a** was treated with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride in pyridine (Dale *et al.*, 1969) and after workup of the resulting diastereomers it was evident the shift of H-13 from 6.83 ppm to 6.77 ppm. That shift proves the 15*S* stereochemistry (Schneider *et al.*, 1977a) for metabolites **4** and **5**.

Compound **6** was purified by HPLC as yellowish oil. Both ^{13}C NMR data and HRMS measurements supported the molecular formula $C_{21}H_{32}O_3$. The EI mass spectrum revealed a molecular ion $[M]^+$ at *m/z* 332. The IR spectrum of **6** displayed absorptions for enone and ester groups at 1710 and 1736 cm^{-1} respectively. The ^{13}C NMR spectrum showed the presence of 21 carbons corresponding to two quartenary, eight methine, nine methylene and two methyl carbon atoms. With an unsaturation degree of six, the structure was suggested to contain one ring, three double bonds and two carbonyl groups. Metabolite **6** shared many structural features with **4** but it was also clear the absence of the hydroxyl group from C-15 (no oxygenated carbons) and the displacement of the double bond to Δ^{14} as that was suggested from the shift of H-12 to higher fields (supported also by the 2D correlations). The *Z* geometry of the Δ^{14} double bond was proposed based on literature comparisons for similar carbon chains (Barrow and Capon, 1991). The stereochemistry on the cyclopentane ring is proposed to be the same with metabolites **1** - **3** by comparison of the H-8 chemical shifts, coupling constants and stereochemical correlations. This structure has in the past been proposed to be a biosynthetic precursor of the marine natural product clavulone (Corey *et al.*, 1988).

Biological assays

The octocorals comprising the order Gorgonacea (sea whips and fans) occur at their greatest diversity and abundance in the tropical northwestern Atlantic. Despite their relative abundance apparently gorgonian corals have few predators. A definite explanation for the low predation rates on gorgonians has not been provided, but may include both physical defences and chemical methods of predator deterrence (Hay, 1984; Fenical and Pawlik, 1991; Pawlik, 1993; Van Alstyne *et al.*, 1994). Studies of the natural products chemistry of gorgonian corals have yielded a wealth of novel metabolites, which occur in numerous Caribbean species at astonishingly high concentrations (Coll, 1992). Among them *P. homomalla* has been found to contain esterified prostaglandins at concentrations frequently as high as 2% of the dry tissue (Weinheimer and Spraggins, 1969).

In this study the preliminary coral palatability evaluation was performed on the ship using the common predatory reef fish *Thalassoma bifasciatum*. The crude lipid extract of *P. nina* as well as the chromatographically separated fractions and pure compounds were assayed in serial dilutions as low as their natural concentration in the coral. Tank assays were repeated with *Thalassoma pavo* in order to establish a significant number of experiments and perform a paired samples t- test statistics against control assay (Zar, 1999) that is shown in Table II.

The same set of chemicals in strip preparations was tested in field assays in Saronicos gulf and the results of these assays were found to follow the pattern of the tank assays. The extract of the gor-

Table II. Palatability assessment (paired samples t-test statistics against control assay) of the gorgonian extract and major metabolites.

	Tank assays					Field assays
Sample	Eaten pellets	N	T	df	P	Eaten strips (%)
Control	10 out of 10	5		4		100
Extract	0 out of 10	5		4		0
Metabolite **1**	9 out of 10	5	2,138	4	>0.05	95
Metabolite **2**	7 out of 10	5	8,552	4	0.01	50
Metabolite **3**	3 out of 10	5	15,652	4	0.00	15
Metabolite **2** + **3**	0 out of 10	5		4		0

gonian was found highly unpalatable, whereas the non-polar fractions were palatable in magnitudes comparable to the control levels.

Metabolite **1** was not inhibiting feeding at all, whereas metabolites **2** and **3** were respectively, moderately and highly unpalatable. Among the tested compounds, in natural abundance, metabolite **3** was proven to be the most active even though less active than the crude extract. It is noteworthy that when metabolites **2** and **3** were combined showed a profound synergistic effect. The new metabolites **4** - **6** when assayed at their natural concentrations did not significantly deter fish feeding.

Experimental

General details

Optical rotations were measured using a Perkin-Elmer model 341 polarimeter and a 10cm cell. IR spectra were obtained using a FT IR PERKIN ELMER model PARAGON 500 infrared spectrophotometer. UV spectra were recorded on a SHIMADZU UV model 160A. 1D and 2D NMR spectra were recorded using a BRUKER AC 200 and a BRUKER DRX 400 spectrometers. Chemical shifts are given on a δ (ppm) scale using TMS as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). HRMS data were provided by the Mass Consortium Corporation, San Diego. EIMS data were recorded on a HEWLETT PACKARD 5973 Mass Selective Detector. Column chromatography was performed with Kieselgel 60 (Merck), HPLC was conducted using a PHARMACIA LKB 2248 model with Spherisorb S10W (25 cm × 10 mm) column and a UV detector. TLC was performed with Kieselgel 60 F_{254} (Merck aluminum support plates).

Plant material

Gorgonians were collected by SCUBA from medium depths (25–30 m) on reefs of Acklins Island (Caribbean Sea), as part of an expedition on board the research vessel Seward Johnson, July 1995. Colonies were labelled, frozen, and small voucher samples set aside for identification. A voucher specimen is deposited at the Herbarium of the Pharmacognosy Laboratory, University of Athens (ATPH-MO-36).

Extraction and isolation

The freshly collected soft coral (wet vol. 1.2 l) was exhaustively extracted at room temperature with mixtures of CH_2Cl_2/MeOH (3/1, v/v). The organic extract after evaporation of the solvents afforded a brown oily residue (8.75 g). The residue was subjected to vacuum column chromatography using silica gel and a step-wised gradient solvent system (increments of 10% EtOAc) ranging from 100% cyclohexane to 100% ethyl acetate. The V (40% EtOAc in cyclohexane) fraction was further subjected to normal phase HPLC chromatography, using as mobile phase cyclohexane/EtOAc (80/20), to yield compounds **1** (137.8 mg), **2** (92.6 mg), **4** (2.0 mg), **5** (5.0 mg). The same process was repeated for the fraction VII (60% EtOAc in cyclohexane) to yield compound **6** (2.8 mg) and also for the fraction XI (90% EtOAc in cyclohexane) to yeild compound **3** (49.9 mg).

Spectral data

5Z-Acetyl-(15S) Prostaglandin B_2 methyl ester **(4)**: yellowish oil; $[\alpha]_D^{20} = -25.5°$ ($CHCl_3$); IR (thin film): ν_{max} = 2955, 2860, 1735, 1710, 1641, 1458, 1438, 1369, 1235, 1020, 965 cm^{-1}; UV (*n*-Hexane): λ_{max} (ε) = 252.6 (1628) nm; FAB HRMS $[MNa]^+$

found: 413.2318, $C_{23}H_{34}O_5$ requires 413.2304; EIMS: *m/z* (% rel. int.) = 390 ([M]$^+$, 8), 359 (11), 330 (92), 317 (5), 273 (30), 229 (39), 215 (36), 203 (100), 161 (52), 145 (32), 133 (54), 117 (27), 105 (33), 91 (35), 55 (27), 43 (77); ^{1}H NMR ($CDCl_3$, 400MHz) δ = 6.76 (1H, d, *J*=15.7 Hz, H-13), 6.11 (1H, dd, *J*=15.7, 6.6 Hz, H-14), 5.41 (1H, m, H-5), 5.32 (1H, dd, *J*=11.5, 5.2, H_2, H-6), 5.24 (1H, m, H-15), 3.64 (3H, s, OCH_3), 3.0 (2H, d, *J*=5.2 Hz, H-7), 2.61 (2H, dd, *J*=8.7, 4.7 Hz, H-10), 2.42 (2H, m, H-11), 2.07 (3H, s, OAc), 2.13–2.33 (4H, m), 1.62–1.77 (4H, m), 1.23–1.40 (6H, m), 0.86 (3H, t, *J*=6.4 Hz, H-20); ^{13}C NMR ($CDCl_3$, 200MHz): see Table I.

5E-Acetyl-(15S) Prostaglandin B_2 methyl ester **(5)**: yellowish oil; $[\alpha]_D^{20}$ = −21.8° ($CHCl_3$); IR (thin film): ν_{max} = 2956, 2860, 1735, 1710, 1638, 1459, 1438, 1370, 1236, 1019, 966 cm^{-1}; UV (*n*-Hexane): λ_{max} (ε) = 252.6 (1616) nm; FAB HRMS [MNa]$^+$ found: 413.2319, $C_{23}H_{34}O_5$ requires 413.2304; EIMS: *m/z* (% rel. int.) = 390 ([M]$^+$, 8), 359 (14), 330 (74), 317 (7), 273 (30), 229 (41), 215 (36), 203 (100), 161 (52), 145 (32), 133 (54), 117 (27), 105 (33), 91 (37), 55 (27), 43 (77); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 6.73 (1H, d, *J*=15.7 Hz, H-13), 6.11 (1H, dd, *J*=15.7, 6.6 Hz, H-14), 5.40 (1H, m, H-6), 5.35 (1H, m, H-5), 5.28 (1H, m, H-15), 3.62 (3H, s, OCH_3), 2.95 (2H, d, *J*=2.9 Hz, H-7), 2.62 (2H, dd, *J*=8.8, 4.7 Hz, H-10), 2.42 (2H, dd, *J*=9.1, 4.8 Hz, H-11), 2.07 (3H, s, OAc), 1.70–2.33 (8H, m), 1.23–1.34 (6H, m), 0.86 (3H, t, *J*=6.6 Hz, H-20); ^{13}C NMR ($CDCl_3$, 200 MHz): see Table I.

9-oxo-prosta-(5Z,10,13Z)-trienoic acid methyl ester **(6)**: yellowish oil; $[\alpha]_D^{20}$ = +103° ($CHCl_3$); IR (thin film): ν_{max} = 2954, 2860, 1736, 1702, 1638, 1459, 1437, 1370, 1242, 1022, 967 cm^{-1}; UV (*n*-Hexane): λ_{max} (ε) = 316.5 (1267) nm; FAB HRMS [MK]$^+$ found: 371.1989, $C_{21}H_{32}O_3$ requires 371.1981; EIMS: *m/z* (% rel. int.) = 332 ([M]$^+$, 5), 317 (7), 299 (5), 249 (10), 234 (8), 217 (16), 208 (100), 190 (30), 174 (10), 147 (12), 133 (15), 119 (11), 109 (76), 94 (43), 81 (19), 71 (12), 55 (14), 43 (20); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 7.53 (1H, dd, *J*=5.8, 2.5 Hz, H-11), 6.12 (1H, dd, *J*=5.8, 1.8 Hz, H-10), 5.55 (1H, m, H-5), 5.54 (2H, m, H-14, H-15), 5.53 (1H, m, H-6), 3.64 (3H, s, OCH_3), 2.59 (1H, ddd, *J*=6.6, 4.3, 1.8 Hz, H-8), 2.45–2.38 (4H, m, H-7, H-4), 2.3 (2H, m, H-13), 2.28 (2H, m, H-2), 2.25 (2H, m, H-16), 2.0 (1H, m, H-12), 1.25–1.78 (8H, m), 0.87 (3H, t, *J*=6.7 Hz, H-20); ^{13}C NMR ($CDCl_3$, 200 MHz): see Table I.

Biological assays

Gorgonian extract and metabolites incorporated in food preparations (Pawlik *et al.*, 1987) along with control pellets were given one at a time to 10 different fish, kept separately, from the top of the aquaria. A food pellet was considered rejected if the fish accepted the pellet into the mouth cavity and then spat it out; the pellet was considered eaten if swallowed by the fish. Control and treated food strips for field assays were prepared according to the same protocol and were hung, in pairs, on plastic ropes that were fastened on rocks at a depth of approximately 25 meters. The ropes were let in the sea until at least 50% of the control strips were consumed. The eaten percentage of the strips was later determined volumetrically.

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