Journal of Chemical Ecology, Vol. 26, No. 6, 2000

STRUCTURE–ACTIVITY RELATIONSHIP OF INHIBITION OF FISH FEEDING BY SPONGE-DERIVED AND SYNTHETIC PYRROLE–IMIDAZOLE ALKALOIDS

THOMAS LINDEL,[1,*] HOLGER HOFFMANN,[1] MATTHIAS HOCHGÜRTEL,[1] and JOSEPH R. PAWLIK[2]

[1] *Universität Heidelberg, Pharmazeutisch-chemisches Institut*
Im Neuenheimer Feld 364
D-69120 Heidelberg, Germany

[2] *Biological Sciences and Center for Marine Science*
University of North Carolina at Wilmington
Wilmington, North Carolina 28403-3297

(Received August 31, 1999; accepted February 9, 2000)

Abstract—We investigated the relationship between the structures of pyrrole-containing alkaloids from marine sponges of the genus *Agelas* and their capacity to deter feeding by the omnivorous Caribbean reef fish, *Thalassoma bifasciatum*. Seven natural products were assayed at volumetric concentrations of 1, 5, and 10 mg/ml: dispacamide A, keramadine, oroidin, midpacamide, 4,5-dibromopyrrole-2-carboxylic acid, 4,5-dibromopyrrole-2-carboxamide, and racemic longamide A. We also assayed 14 structural analogs obtained mostly by chemical synthesis. Of the seven natural products, only *rac*-longamide A was not significantly deterrent at any of the assay concentrations. The pyrrole moiety was required for feeding inhibition activity, while the addition of the imidazole group enhanced this activity. Variously functionalized imidazoles lacking the pyrrole moiety were not deterrent. Combinations of the natural products appeared to have an additive effect on feeding inhibition; there was no evidence of synergy. Given their high concentrations in sponge tissue, dispacamide A and oroidin most probably serve as the primary chemical defenses of many *Agelas* sp., while minor compounds such as keramadine are not present in high enough concentrations to contribute much to chemical defense.

Key Words—Chemical defense, sponges, predation, *Agelas*, pyrrole–imidazole alkaloids, structure–activity relationship, synthesis.

*To whom correspondence should be addressed.

0098-0331/00/0600-1477$18.00/0

INTRODUCTION

Sponges are the source of the greatest diversity of marine natural products (Braekman et al., 1989; reviewed in Faulkner, 1998, and previous reviews cited therein). Despite decades of natural products isolation, structure elucidation, and pharmacological assays, little is known about the functions of sponge secondary metabolites in ecological interactions (Pawlik, 1993). Because natural products are often structurally complex or present at high concentrations in sponge tissues, they are likely to be metabolically expensive for sponges to make and store, and should have some adaptive purpose.

Sponges are soft-bodied and sessile and thus appear to be physically vulnerable to predation. Caribbean demosponges, for example, represent a rich protein source (mean of 20.7 mg soluble protein per milliliter of tissue; Chanas and Pawlik, 1995) in an environment noted for intense grazing activity by fishes (Hixon, 1983). Nevertheless, very few fish species are known to feed on Caribbean sponges (Randall and Hartman, 1968). Only recently have ecologically relevant methods been used to test whether sponge secondary metabolites deter consumption by predatory fishes (e.g., Duffy and Paul, 1992; Pennings et al., 1994; Chanas et al., 1996; Wilson et al., 1999).

In a survey of the chemical antipredatory defenses of 73 species of Caribbean sponges (Pawlik et al., 1995), we discovered that all of the species within the genus *Agelas* yielded crude organic extracts that strongly deterred the feeding of a predatory reef fish in aquarium assays. Subsequently, oroidin and 4,5-dibromopyrrole-2-carboxylic acid were identified as the principal fish antifeedant metabolites of *Agelas clathrodes*, present at concentrations of 1–5 mg/ml of sponge tissue in *A. clathrodes* and in five other species of *Agelas* common to the Caribbean (Chanas et al., 1996). Similar compounds have been identified from species of *Agelas* collected worldwide and from other sponges in the orders Agelasida, Axinellida, and Halichondrida (e.g., Forenza et al., 1971; Walker et al. 1981; Nakamura et al., 1984; Gunasekera et al., 1989; Cafieri et al., 1996, 1997, 1998).

The conservation of brominated pyrrole–imidazole alkaloids as natural products in the tissues of sponges within the genus *Agelas* suggests that these compounds have been evolutionarily elaborated and retained as chemical defenses. The purpose of the study reported herein was to determine the relative activity of the naturally occurring compounds as deterrents of fish feeding, and to determine the chemical functionalities necessary and sufficient for this activity. Our continuing program on the synthesis of pyrrole–imidazole alkaloids from marine sponges (Lindel and Hoffmann, 1997a,b; Lindel and Hochgürtel, 1998) provided sufficient quantities of seven natural products and 14 derivatives for fish-feeding assays employing the bluehead wrasse *Thalassoma bifasciatum*, a generalist predator on Caribbean reefs.

METHODS AND MATERIALS

The syntheses of dispacamide A (**1**) (Lindel and Hoffmann, 1997a), keramadine (**2**) (Lindel and Hochgürtel, 1998), midpacamide (**4**), and the alkylidene hydantoin **8** and its dimethylated derivative **9** (Lindel and Hoffmann, 1997b) have been described earlier (Figure 1). Oroidin (**3**), first isolated by Forenza et al. (1971), was obtained by isolation from *A. clathrodes* [for details regarding the procedure see Lindel et al. (1999)]. Compounds **14** and **18–21** were commercially available from Aldrich. *rac*-Longamide A (**7**) was prepared in two steps starting from the pyrrolyl trichloromethylketone **22** (Bailey and Johnson, 1973), which was first reacted with 2-aminoacetaldehyde dimethyl acetal (**23**) in DMF to **24**, followed by hydrolysis and spontaneous regioselective cyclization under acidic conditions (79% yield, Figure 2a). Treatment of **7** with tosyl chloride in the presence of triethylamine led to the formation of the elimination product **16** in 66% yield. The β-alanine derivative **12** was obtained via ester **25** by treatment of **22** with ethyl 3-aminopropionate and subsequent hydrolysis (Figure 2a). The natural products 4,5-dibromopyrrole-2-carboxylic acid (**5**) and its amide **6** were obtained via hydrolysis and ammonolysis, respectively, of **22**. The histamine derivative **10**, the 2-aminoethanol derivative **13**, and the allylic amide **14** were prepared by reaction of **22** with histamine, with 2-aminoethanol, and with allylic amine, respectively. The aminopropylidenehydantoin hydrochloride **17** was synthesized in 50% overall yield by reaction of 3-phthalimidopropionic aldehyde (**23**) (Moe and Warner, 1949) with the hydantoin-5-phosphonate **24** (Meanwell et al., 1991), followed by hydrazinolysis at 0°C in ethanol (Figure 2b).

Characterization and Purification. Melting points of compounds used in this study were determined with a Reichert hot stage and are uncorrected. NMR spectra were taken with a Bruker WM-250 (250 MHz for ^{1}H and 62.9 MHz for ^{13}C) and a Bruker WM-360 spectrometer (360 MHz for ^{1}H and 90.6 MHz for ^{13}C). Chemical shifts refer to those of residual solvent signals based on $\delta_{TMS} = 0$. All measurements were carried out at 300°K. Mass spectra were obtained with a Varian MAT-311 A mass spectrometer (EI). IR spectra were recorded with a Perkin-Elmer PE 1600 FT-IR spectrometer. UV/Vis spectra were measured with a Hewlett-Packard HP-8452A diode array spectrophotometer. Elemental analyses were performed with a Foss-Heraeus Vario EL. Solvents were purified and dried according to standard procedures. Silica gel 60 (60–200 mesh, Merck) was used for column chromatography. Reaction products were purified by column chromatography (silica gel), trituration in an ultrasonic bath in the presence of a precipitating solvent, or by crystallization, until analytical purity was achieved. The purity of all tested compounds was secured by TLC analysis and by ^{1}H NMR spectroscopy.

*Synthesis of 4,5-Dibromo-1*H*-pyrrole-2-carboxylic Acid (**5**).* The ketone **22** (5 g, 13 mmol) was dissolved in aqueous NaOH (10%, 30 ml) and stirred at

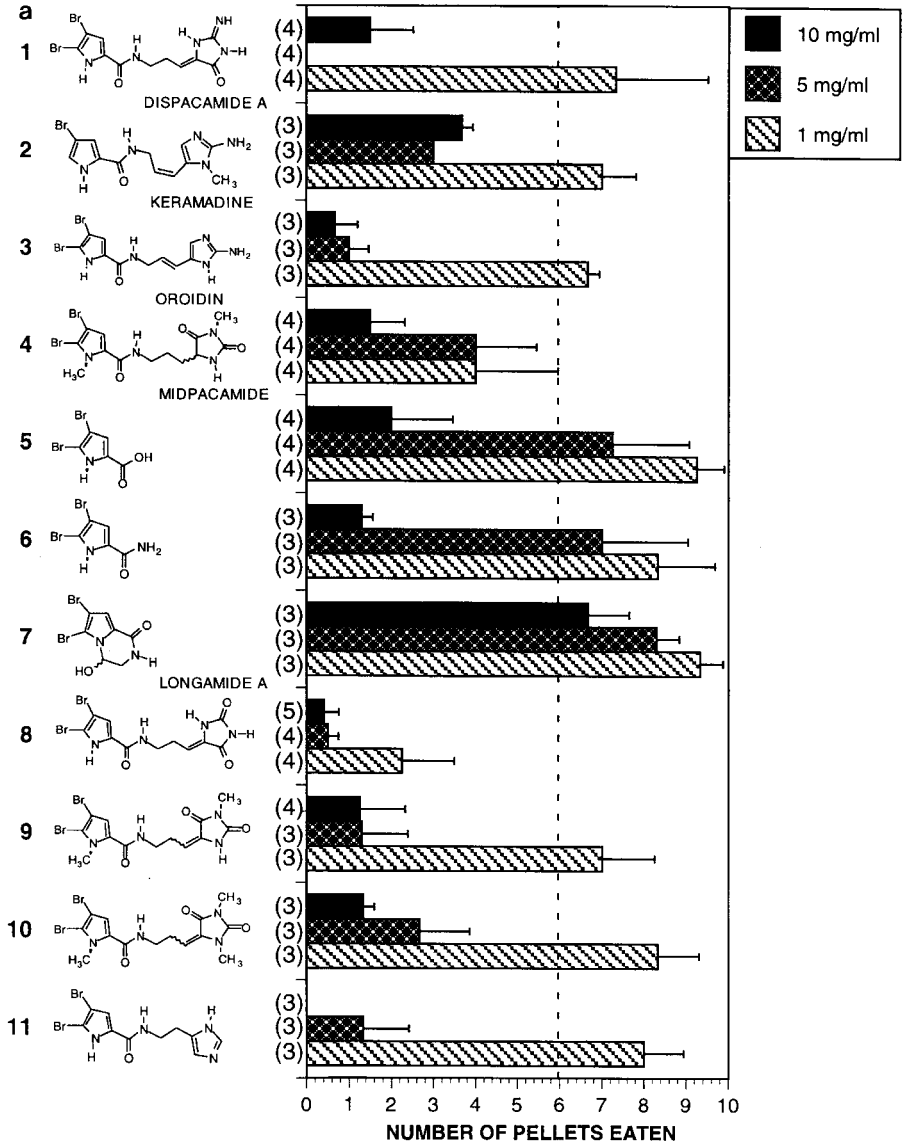

FIG. 1. (a and b): Consumption by *Thalassoma bifasciatum* of food pellets (mean + SE) containing naturally occurring and synthetic pyrrole-imidazole alkaloids at 10, 5, and 1 mg of compound per milliliter of food paste. Fish consumed all 10 control pellets in all cases. The number of replicate assays performed at each concentration is shown in parentheses.

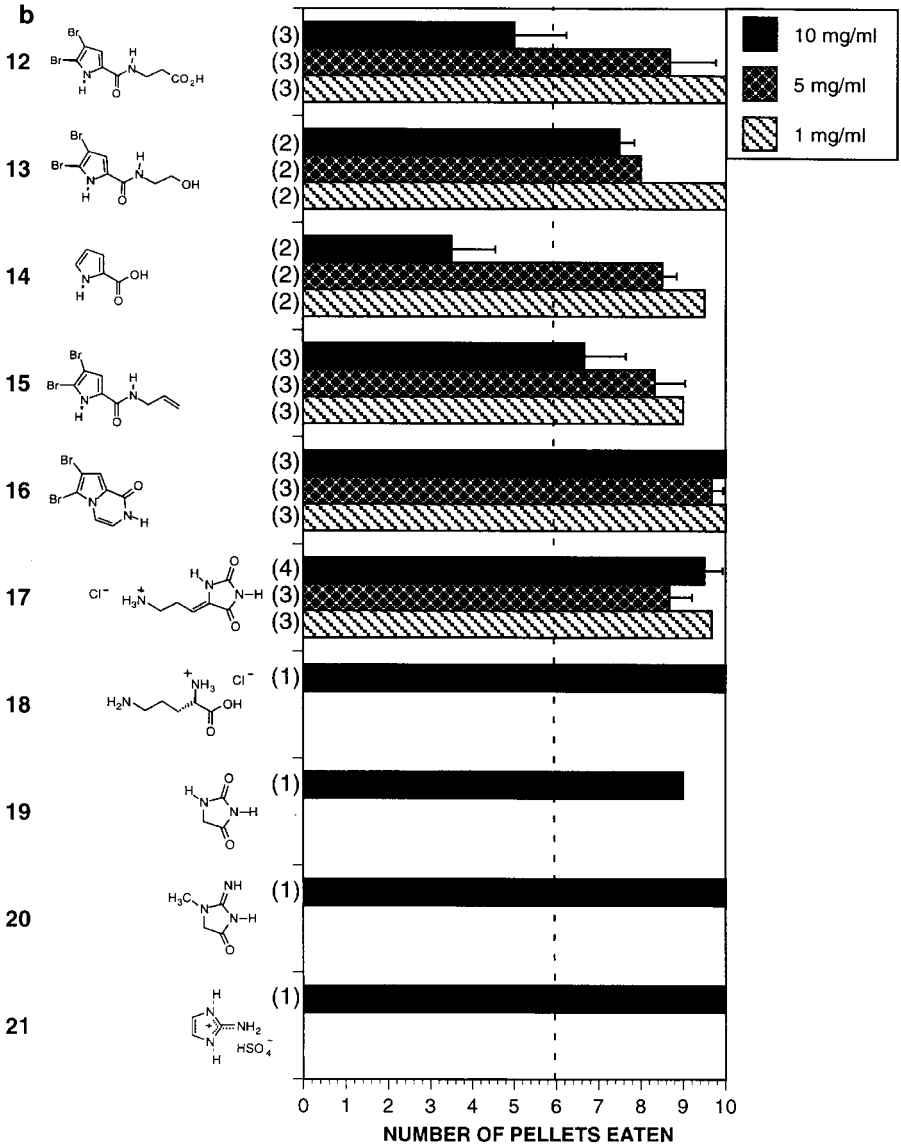

FIG. 1. Continued. For compounds **18–21**, assays were not performed at concentrations <10 mg/ml. For any individual assay, compounds were considered deterrent if the number of pellets eaten was ≤6 (Fisher exact test, one-tailed) as indicated by the dotted line on the graphs.

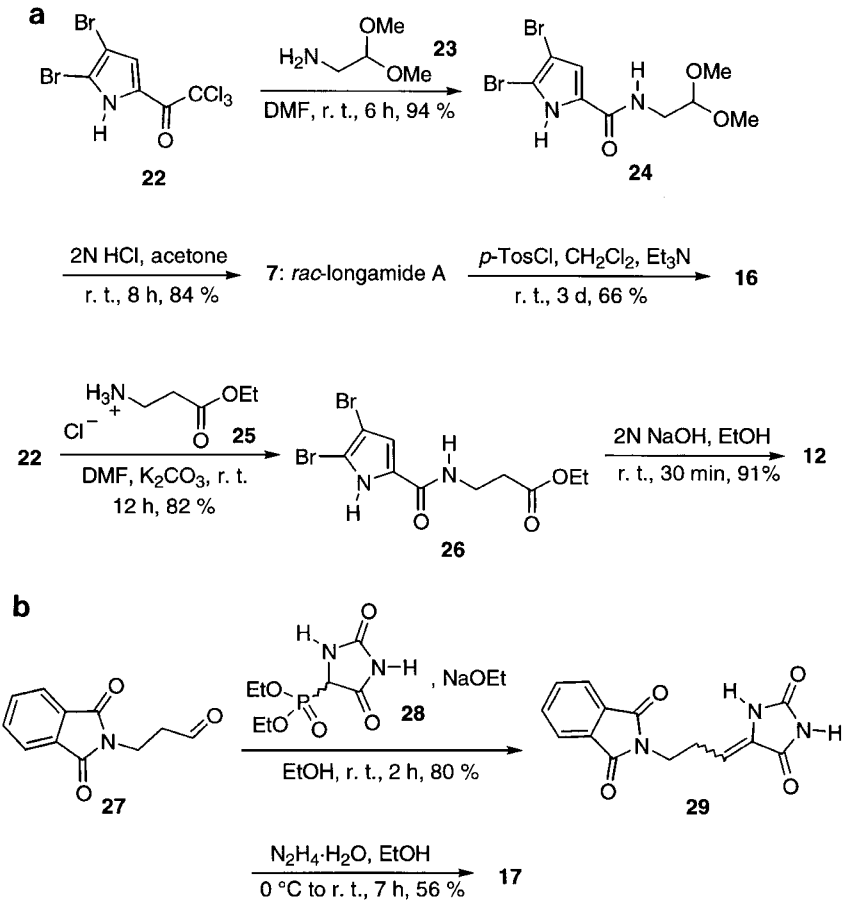

FIG. 2. (a and b): Previously undescribed syntheses of compounds used in feeding assays.

room temperature. After 35 min, pH 2 was adjusted by adding aqueous HCl (2 N). The precipitate was collected by filtration to yield **5** as a pale brown solid (2.6 g, 72%): mp 179–183°C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 12.90 (s, 1H, N*H*), 6.84 (s, 1H, CBrC*H*C); ^{13}C NMR (90.6 MHz, DMSO-d_6) δ 160.22 (*C*O), 124.99 (CH*C*C), 116.80 (*C*H), 106.67 (*C*H), 106.67 (*C*Br), 98.70 (*C*Br), IR (KBr) 3444, 3191, 1645, 1556 cm^{-1}; UVλ_{max} (MeOH) 272, 204 nm; EI-MS *m/z* (rel intensity) = 267/269/271 (22/43/22) [M$^+$], 249/251/253 (59/100/50); HR-EI-MS calcd for $C_5H_3N_2O^{79}Br^{81}Br$ 268.8592, found 268.8510.

*Synthesis of 4,5-Dibromo-1*H*-pyrrole-2-carboxylic Acid Amide (**6**).* The ketone **22** (1 g, 2.7 mmol) was dissolved in aqueous NH_3 (25%, 10 ml) and

stirred at room temperature. After 45 min, pH 7 was adjusted by adding aqueous HCl (2 N). The precipitate was collected by filtration, to yield **6** as a pale brown solid (563 mg, 78%): mp 87–90°C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 12.17 (s, 1H, N*H*), 7.58 (s, 1H, N*H*H), 7.16 (s, 1H, NH*H*), 6.33 (s, 1H, CBrC*H*C); ^{13}C NMR (90.6 MHz, DMSO-d_6) δ 160.47 (*C*O), 128.23 (CH*C*C), 113.16 (*C*H), 104.65 (*C*Br), 97.71 (*C*Br); IR (KBr) 3632, 3195, 1658, 1592, 1452, 1426 cm^{-1}; UV λ_{max} (MeOH) 274, 204 nm; EI-MS *m/z* (rel intensity) 266/268/270 (39/93/38) [M$^+$], 249/251/253 (55/100/59) [M$^+$-OH]; HR-EI-MS calcd for $C_5H_4N_2O^{79}Br^{81}Br$ 267.8690, found 267.8671.

*Synthesis of 4,5-Dibromo-1*H*-pyrrole-2-carboxylic Acid (2,2-Dimethoxyethyl) Amide (**24**).* To a solution of the ketone **22** (10.00 g, 27.00 mmol) in 50 ml dry DMF was slowly added 1-amino-2,2-dimethoxyethane (**23**, 3.50 ml, 32.40 mmol). After 6 hr the solvent was removed under reduced pressure. The brown residue was triturated with 100 ml water in the ultrasound bath. Filtration gave a beige solid. Recrystallization from dichloromethane/acetone gave colorless crystals (9.10 g, 94%): mp 128°C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 3.29 (s, 6H, OCH_3), 3.30–3.33 (m, 2H, CH_2), 4.44 (t, J = 5.4 Hz, 1H, OC*H*O), 6.97 (s, 1H, ═C*H*), 8.18 (t, J = 6.2 Hz, 1H, NH, amide), 12.67 (s, 1H, NH, pyrrole); ^{13}C NMR (90.6 MHz, DMSO-d_6) δ 40.41 (1 C, *C*H$_2$), 53.21 (2 C, O*C*H$_3$), 97.78 (1 C, *C*Br), 101.97 (1 C, O*C*HO), 104.56 (1 C, *C*Br), 112.81 (1 C, ═*C*H), 127.84 (1C, ═*C*NHC), 158.87 (1 C, *C*═O); IR (KBr) 3430, 3148, 1644, 1563, 1520, 1436, 1399, 1363, 1210, 1193, 1137, 1104 cm^{-1}; UV λ_{max} (EtOH) 274,204 nm; EIMS *m/z* (rel intensity) 354/356/358 (1/1.7/1) [M$^+$], 322/324/326 (0.8/1.5/0.8), 323/325/327 (0.4/1.2/0.5) [M$^+$-OMe], 250/252/254 (2.4/3.6/2.1), 75 (100); HR-EI-MS calcd for $C_9H_{12}N_2O_3{}^{79}Br^{81}Br$ 355.9194, found 355.9196. Anal. Calcd for $C_9H_{12}N_2O_3Br_2$: C, 30.36; H, 3.40; N, 7.87. Found: C, 30.82; H, 3.58; N, 7.95.

Synthesis of rac*-Longamide A(**7**).* To a solution of **24** (10.00 g, 27.03 mmol) in 250 ml acetone was added 6 ml aqueous HCl (2 *N*). After 8 hr of stirring at room temperature, the solvent was removed, the residue was diluted with 100 ml diethyl ether, and the solution filtered to yield *rac*-longamide A (**7**) as a beige-colored solid (7.01 g, 84%). Analytically pure material was obtained by crystallization from methanol: mp 217°C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 3.44 (dd, J = 1.4, 5.1 Hz, 1H, C*H*H), 3.74 (dd, J = 2.8, 13.7 Hz, 1H, CH*H*), 5.64 (br d, J = 6.0 Hz, 1H, NC*H*OH), 6.85 (s, 1H, ═C*H*), 7.01 (d, J = 6.7 Hz, 1H, OH), 7.94 (br d, J = 4.8 Hz, 1H, NH); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 46.41 (1 C, *C*H$_2$), 73.64 (1 C, N*C*HOH), 100.02 (1 C, *C*Br), 105.85 (1 C, *C*Br), 113.85 (1 C, ═*C*H), 125.96 (1C, ═*C*NHCBr), 157.29 (1 C, C═O); IR (KBr) = 3194, 1665, 1559, 1471, 1428, 1245, 1165 cm^{-1}; UV λ_{max} (EtOH) 280, 236 and 204 nm; EI-MS *m/z* (rel intensity) 308/310/312 (19/40/19) [M$^+$], 280/282/284 (16/28/14) [M$^+$-CO], 279/281/283 (14/27/15) [M$^+$-CHO], 250/252/254 (55/100/55); HR-

EI-MS calcd for $C_7H_4N_2O^{79}Br^{81}Br$ 309.8776, found 309.8776. Anal. calcd for $C_7H_4N_2OBr_2$: C, 27.13; H, 1.95; N, 9.04. Found: C, 27.40; H, 2.32; N, 9.09.

*Synthesis of 6,7-Dibromo-2*H*-pyrrole [1,2-*a*]pyrazin-1-one (**16**).* To a suspension of *rac*-longamide A (**7,** 1.50 g, 4.84 mmol) in 40 ml dry dichloromethane was added *p*-toluene sulfonylchloride (1.86 g, 9.75 mmol) and triethyl amine (3.30 ml, 26.40 mmol). After three days at room temperature the solvent was removed under reduced pressure. Purification of the residue by column chromatography (chloroform–methanol 10 : 1) gave (**16**) as colorless solid (920 mg, 66%): mp 140–142°C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 6.78 (dd, J = 5.7, 5.7 Hz, 1H, CH═C*H*NH), 7.15 (s, 1H, pyrrole-C*H*), 7.16 (d, J = 5.6 Hz, 1H, C*H*═CHNH), 10.88 (br. s, 1H, NH); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 101.60 (1 C), 102.79 (1 C), 105.62 (1 C), 111.00 (1 C), 116.52 (1 C), 125.55 (1 C, ═*C*NHCBr), 154.12 (1 C, C-6, C═O); IR (KBr) 1656, 1376 cm^{-1}; UV λ_{max} (EtOH) 278, 234 and 204 nm; EI-MS m/z (rel intensity) 290/292/294 (52/100/52) [M$^+$]; HR-EI-MS calcd for $C_7H_4N_2O^{79}Br^{81}Br$ 291.8670, found 291.8669.

*Synthesis of (*E/Z*)-4,5-Dibromo-1-methyl-pyrrole-2-carboxylic Acid [3-(1,3-Dimethyl-2,5-dioxo-imidazolidin-4-yliden)-propyl] Amide (**10**).* To a solution of the alkylidene hydantoin **8** (2.00 g, 4.93 mmol) in dry DMF (10 ml) was added K_2CO_3 (2.20 g, 15.91 mmol). After 15 min of stirring at room temperature, iodomethane (1.00 ml, 2.27 g, 16.00 mmol) was slowly added. The mixture was stirred for 4 hr and was then diluted with water (100 ml). The resulting suspension was extracted with ethylacetate (5 × 50 ml) and the combined organic layers were dried ($MgSO_4$). Concentration to dryness gave a yellow oil. the product precipitated on addition of methanol as a colorless solid (1.28 g, 58%): mp 154°C; ^{1}H NMR (250 MHz, DMSO-d_6, E/Z isomer ~ 2 : 3) δ 2.67/2.87 (dt, J = 6.8, 7.0/6.8, 7.0, 2H, C*H*$_2$CH═), 2.90/2.92 (s, 3H, hydantoin-NC*H*$_3$), 2.99/3.26 (s, 3H, hydantoin-NC*H*$_3$, 3.25–3.40 (m, 2H, NHC*H*$_2$), 3.87/3.88 (s, 3H, pyrrole-NC*H*$_3$), 5.57/5.72 (t, J = 7.9/8.1 Hz, 1H, CH$_2$C*H*═), 6.96/6.95 (s, 1H, pyrrole-C*H*), 8.38/8.28 (t, J = 5.6/5.6 Hz, 1H, NH); ^{13}C NMR (90.6 MHz, DMSO-d_6) E/Z isomer δ 24.04/24.53 (1 C, CH$_3$), 25.46/26.03 (1 C, *C*H$_2$CH═), 25.74/28.48 (1 C, CH$_3$), 35.23/35.26 (1 C, CH$_3$), 38.41/38.78 (1 C, NH*C*H$_2$), 96.75/96.80 (1 C, CBr), 110.32/110.55 (1 C, CBr), 110.86 (1 C), 113.82 (1 C), 113.84 (1 C), 114.46 (1 C), 127.74/127.79 (1 C), 129.97/130.23 (1 C, CH═*C*), 153.07/154.57 (1 C, C═O), 159.71/159.78 (1 C, C═O), 162.31/162.49 (1 C, C═O); IR (KBr) 3383, 1760, 1712, 1666, 1652, 1544, 1466, 1436, 1391, 1257, 1128 cm^{-1}; UV λ_{max} (EtOH) 276, 254 nm; EIMS m/z (rel intensity) 446/448/450 (3/8/2) [M$^+$], 280/282/284 (6/11/6), 264/266/268 (38/78/41), 166 (100); HR-EI-MS calcd for $C_{14}H_{16}N_4O_3{}^{79}Br^{81}Br$ 447.9569, found 447.9568. Anal. calcd for $C_{14}H_{16}N_4O_3Br_2$: C, 37.52; H, 3.60; N, 12.50. Found: C, 37.90; H, 3.75; N, 12.61.

*Synthesis of 4,5-Dibromo-1*H*-pyrrole-2-carboxylic Acid [2-(1*H*-Imidazole-*

*4-yl)ethyl] Amide (**11**).* To a solution of histamine dihydrochloride (300 mg, 1.63 mmol) and Na_2CO_3 [191 mg, 1.8 mmol) in 3 ml of dry DMF was added the ketone **22** (603 mg, 1.63 mmol). After 6 hr of stirring at room temperature, the solution was concentrated in vacuo and diluted with water (100 ml). the precipitate was collected by filtration to yield **11** as a colorless solid (497 mg, 78%): mp 218°C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 12.66 (s, 1H, CBrN*H*), 8.18 (t, J = 5.50 Hz, 1H, CON*H*), 7.53 (d, J = 1.22 Hz, 1H, NHC*H*N), 6.88 (s, 1H, CBrC*H*C), 6.80 (s, 1H, CC*H*N), 3.42 (dt, J = 7.32, 5.80 Hz, 2H, NHCH_2CH$_2$), 2.75 (t, J = 7.32 Hz, 2H, CH$_2$CH_2C); ^{13}C NMR [90.6 MHz, DMSO-d_6 + DCl/D_2O (25%)] δ 159.42 (*C*O), 133.65 (NH*C*HN), 131.36 (CH$_2$*C*CH), 128.13 (CH*C*CO)), 116.42 (C*C*HN), 113.71 (*C*H), 104.95 (*C*Br), 98.53 (*C*Br), 37.82 (NCH$_2$), 24.78 (NCH$_2$*C*H$_2$); IR (KBr) 1628, 1528, 1418 cm^{-1}; UV λ_{max} (MeOH) 274 and 209; EI-MS m/z (rel intensity) 360/362/364 (5/12/5) [M$^+$], 250/252/254 (6/14/5) [M$^+$-$C_5H_8N_3$], 95 (68), 82 (100; HR-EI-MS calcd for $C_{10}H_{10}N_4O^{79}Br^{81}Br$ 361.9201, found 361.9200. Anal. calcd for $C_{10}H_{10}N_4OBr_2$: C, 33.18; H, 2.78; N, 15.48. Found C, 32.92; H, 3.01; N, 15.27.

*Synthesis of 3-{[1-(4,5-Dibromo-1*H*-pyrrol-2-yl)-methanoyl]-amino}-propionic Acid Ethyl Ester (**26**).* To a solution of ethyl 3-aminopropionic acid hydrochloride (**25,** 3.00 g, 19.50 mmol) in dry DMF (40 ml) is added K_2CO_3 (2.70 g, 19.50 mmol). After 15 min of stirring at room temperature, the ketone **22** (7.00 g, 17.50 mmol) is added and the mixture is kept at room temperature for 12 hr. Water is added until precipitation of the colorless product is complete (5.30 g, 82%): mp 206–209°C; ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.18 (t, J = 7.1 Hz, 3H, CH_3), 2.53 (t, J = 6.8 Hz, 2H, NHCH$_2$CH_2), 3.43 (dt, J = 6.7, 5.9 Hz, 2H, NHCH_2), 4.07 (q, J = 7.1 Hz, 2H, CH$_2$CH$_3$), 6.90 (s, 1H, pyrrole-C*H*), 8.18 (br. t, 1H, amide-NH), 12.66 (br s. 1H, pyrrole-NH); ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 13.97 (1 C, *C*H$_3$), 24.83 (1 C, NHCH$_2$CH_2), 33.79 (1 C, NH*C*H$_2$), 59.85 (1 C, *C*H$_2$CH$_3$), 97.67 (1 C), 104.46 (1 C), 112.56 (1 C, pyrrole-*C*H), 127.95 (1 C), 158.85 (1 C, C═O), 171.12 (1 C, O—C═O); IR (KBr) 3156, 3121, 1709, 1634, 1567, 1326, 1294, 1237 cm^{-1}; UV λ_{max} (EtOH) 250 nm (3.52, sh), 276 (4.07); EI-MS m/z (rel intensity) 366/368/370 (22/59/24) [M$^+$], 321/323/325 (12/22/13) [M$^+$-OEt], 320/322/324 (9/21/11), 292/294/296 (36/66/30), 250/252/254 (55/100/46); Anal. calcd for $C_{10}H_{12}N_2O_2Br_2$: C, 32.64; H, 3.29; N, 7.61. Found: C, 32.67; H, 3.34; N, 7.74.

*Synthesis of {3-[1-(4,5-Dibromo-1*H*-pyrrol-2-yl)-methanoyl]-amino}-propionic Acid (**12**).* To a solution of the ethyl ester **26** (3.80 g, 10.32 mmol) in ethanol (5 ml) was added 2 N NaOH (10 ml). After stirring for 30 min at room temperature, 2 N HCl was added as long as precipitation occurred. The product was filtered off, yielding a colorless solid (3.22 g, 91%): mp 198–200°C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.49 (t, J = 7.0 Hz, 2H, NHCH$_2$CH_2), 3.40 (dt, J = 6.8, 5.8 Hz, 2H, NHCH_2), 6.91 (s, 1H, pyrrole-C*H*), 8.19 (t, J = 5.5 Hz,

1H, amide-NH), 12.50 (br s, 2H, pyrrole-NH); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 33.82 (1 C), 34.93 (1 C), 97.70 (1 C, CBr), 104.30 (1 C, CBr), 112.67 (1 C, pyrrole-*C*H), 128.07 (1 C), 158.81 (1 C, C═O), 171.67 (1 C, O—C═O); IR (KBr) 3435, 1616, 1570, 1523, 1229, 1207 cm^{-1}; UV λ_{max} (EtOH) 252 nm (3.66 sh), 274 (4.07); EI-MS *m/z* (rel intensity) 338/340/342 (30/69/32) [M$^+$], 292/294/296 (32/72/34), 250/252L254 (54/100/46), 249/251/253 (23/46/24), 222/224/226 (10/20/10); Anal. calcd for $C_8H_8N_2O_2Br_2$: C, 28.26; H, 2.37; N, 8.24. Found: C, 28.14; H, 2.47; N, 8.16.

*Synthesis of 4,5-Dibromo-1*H*-pyrrole-2-carboxylic Acid (2-Hydroxy-ethyl) Amide (**13**).* To a solutionof 4,5-dibromo-1*H*-pyrrol-2-yltrichloromethyl ketone (2.0 g, 5.4 mmol) in dry DMF (5 ml) was added 2-aminoethanol (0.33 ml, 5.4 mmol). After 6 hr of stirring at room temperature, the solution was concentrated in vacuo and diluted with water (100 ml). The precipitate was collected by filtration, to yield the product as a colorless solid (1.06 g, 85%): mp 172–175°C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 12.64 (s, 1H, CBrN*H*), 8.09 (t, *J* = 5.5 Hz, 1H, CON*H*), 6.92 (s, 1H, C*H*), 4.73 (m, 1H, O*H*), 3.46 (m, 2H, CH_2OH), 3.26 (m, 2H, NHCH_2); ^{13}C NMR (90.6 MHz, DMSO-d_6) δ 159.12 (*C*O), 128.33 (CH*C*CO), 112.70 (*C*H), 104.43 (*C*Br), 97.83 (*C*Br), 59.90 (*C*H$_2$OH), 40.61 (N*C*H$_2$); IR (KBr) 3237, 1525 cm^{-1}; UV λ_{max} (MeOH) 274 and 201 nm; EI-MS *m/z* (rel intensity) = 310/312/314 (15/27/19) [M$^+$], 266/268/270 (27/36/19), 250/252/254 (44/100/40); HR-EI-MS calcd for $C_7H_7N_2O_2{}^{79}Br^{81}Br$ 311.8938, found 311.8932.

*Synthesis of 4,5-Dibromo-1*H*-pyrrole-2-carboxylic Acid-(prop-2-enyl) Amide (**15**).* To a solution of the ketone **22** (2 g, 5.4 mmol) in dry DMF (5 ml) was added 3-aminoprop-1-ene (339 mg, 0.45 ml, 5.9 mmol). After 6 hr of stirring at room temperature, the solution was concentrated in vacuo and diluted with water (100 ml). the precipitate was collected by filtration, to yield **15** as a pale brown solid (1.45 mg, 87%): mp 158–160°C; ^{1}H NMR (360 MHz, DMSO-d_6) δ 12.66 (s, 1H, CBrN*H*), 8.30 (t, *J* = 5.16 Hz, 1H, CON*H*), 6.97 (s, 1H, CBrC*H*C), 5.80 (ddt, *J* = 17.19, 10.31, 5.16 Hz, C*H*CH$_2$), 5.16 (d, *J* = 17.19 Hz, 1H, CHC*H*H), 5.10 (d, *J* = 10.31, 1H, CHCH*H*), 3.86 (dd, *J* = 5.16 Hz, 2H, NHCH_2); ^{13}C NMR (90.6 MHz, DMSO-d_6) δ 158.74 (*C*O), 135.33 (*C*H), 128.10 (CH*C*CO), 115.22 (CH*C*H$_2$), 112.66 (*C*H), 104.59 (*C*Br), 97.88 (*C*Br), 40.86 (N*C*H$_2$); IR (KBr) 3287, 3147, 1604, 1560 cm^{-1}; UV λ_{max} (MeOH) 274, 201 nm; EI-MS *m/z* (rel intensity) = 306/308/310 (5/10/5) [M$^+$], 250/252/254 (5/11/5) [M$^+$-C_3H_7N], 57 (100); HR-EI-MS calcd for $C_8H_8N_2O^{79}Br^{81}Br$ 307.8983, found 307.8983.

*Synthesis of (*E/Z*)-3-[3-(2,5-Dioxo-imidazolidin-4-yliden)-propyl]-isoindol-1,3-dione (**29**).* Solution ethoxide was prepared from sodium (0.41 g, 1.80 mmol) and dry ethanol (40 ml), followed by addition of the phosphonate **28** (4.21 g, 17.84 mmol). After 5 min, 3-phthalimido propionaldehyde (**27,** 3.05 g, 15.00 mmol) was added in one portion and the mixture was stirred at room temperature. After 2 hr, the mixture was neutralized with acetic acid, followed by filtra-

tion. The solid was recrystallized from ethanol/water to obtain **29** as a colorless solid (3.45 g, 80%): mp 240–244°C; ^{1}H NMR (300 MHz, $CF_3COOH/CDCl_3$ 3 : 1, *Z*/*E*-isomer ~ 2 : 1) δ 2.68/3.12 (dt, J = 7.3, 7.6 Hz/6.5, 8.2 Hz, 2H, $CH_2CH{=}$), 3.91 (t, J = 6.8/6.8 Hz, 2H, NCH_2), 6.09/5.94 (t, J = 8.0/8.0 Hz, 1H, $CH_2CH{=}$), 7.76–7.89/7.76–7.89 (m, 4H, arom. H), 8.95/8.46 (br s, 1H, NH), 9.27/9.21 (br s, 1H, NH); ^{13}C NMR (62.9 MHz, DMSO-d_6, *Z*/*E*-Isomer) δ 25.42, 24.45 (1 C, CH_2), 36.15, 36.75 (1 C, NCH_2), 107.34, 112.36 (1 C, $CH_2CH{=}$), 122.80, 122.71 (2 C, $C_{ar}{-}H$), 131.28, 130.34 (1 C), 134.17, 134.10 (2 C, $C_{ar}{-}H$), 154.57, 154.54 (1 C, C=O), 163.91, 164.23 (1 C, C=O), 167.53, 167.52 (2 C, C=O); IR (KBr) 1772, 1700, 1398 cm^{-1}; UV λ_{max} (EtOH) 274 and 226 nm; EI-MS *m*/*z* (rel intensity) 285 (8) [M$^+$], 160 (100), 138 (24); HR-EI-MS calcd for $C_{14}H_{11}N_3O_4$ 285.0750, found 285.0750. Anal. calcd for $C_{14}H_{11}N_3O_4$: C, 58.95; H, 3.98; N, 14.73. Found: C, 59.17; H, 4.19; N, 14.46.

Synthesis of (E/Z)*[[3-(2,5-Dioxo-imidazolidin-4-yliden)-propyl]amine (**17**).* The phthalimide **29** (4.00 g, 14.02 mmol) was suspended in dry ethanol (200 ml). After addition of hydrazine monohydrate (0.73 ml, 750 mg, 15.00 mmol) at 0°C, the mixture was stirred at 0°C for 4 hr and at room temperature for 3 hr. The solvent was evaporated, and the resulting residue was diluted with aqueous HCl (2 *N*, 100 ml). The solution was heated to 50°C for 20 min. After 1 hr at room temperature, the mixture was filtered and the precipitate was washed with water. The mother liquid was concentrated to dryness and the residue was diluted with aqueous HCl (1 *N*, 100 ml). The aqueous phase was washed with ethyl acetate (3 × 50 ml) and again evaporated to dryness. Crystallisation from ethanol/water gave **17** as colorless crystals (1.50 g, 56%): mp 260°C (dec); ^{1}H NMR (300 MHz, DMSO-d_6, *E*/*Z* isomer ~ 1 : 10) δ 2.50–2.57 (m, 2H, $CH_2CH{=}$), 2.87–2.93 (m, 2H, H_3NCH_2), 5.54 (t, J = 7.8 Hz, 1H, $CH_2CH{=}$), 8.23 (br s, 3H, NH_3), 10.36 (br s, 1H, NH), 11.06 (br s, 1H, NH); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 24.20 (1 C, $CH_2CH{=}$), 37.67 (1 C, H_3NCH_2), 106.14 (1 C, $CH_2CH{=}$), 132.23 (1 C), 154.75 (1 C, C=O), 164.15 (1 C, C=O); IR (KBr) = 3088, 1764, 1739, 1689 cm^{-1}; UV λ_{max} (EtOH) 272 nm; EI-MS *m*/*z* (rel intensity) 155 (31) [M$^+$], 127 (13) [M$^+$-CO], 126 (100) [M$^+$-CHO], 84 (11), 83 (12), 67 (10), 56 (19), 55 (81), 54 (99); HR-EI-MS calcd for $C_6H_9N_3O_2$ 155.0695, found 155.0695. Anal. calcd for $C_6H_{10}ClN_3O_2$: C, 37.61; H, 5.26; N, 21.93. Found: C, 37.70; H, 5.53; N, 21.64.

Laboratory Feeding Assays. Aquarium assays were performed as described in Pawlik et al. (1995) on natural products and synthetic compounds. Assays were performed on board the research vessel *Seward Johnson* or in aquaria at the University of North Carolina at Wilmington by employing a common predatory reef fish, the bluehead wrasse *Thalassoma bifasciatum*. The advantages of using this species for aquarium bioassays have previously been detailed (Pawlik et al., 1987, 1995). Groups of three fish (one terminal phase male, two females) were held in each of 15–20 separate, opaque-sided compartments in flow-through

laboratory aquaria. Groups of 10 fish were chosen out of 15 at random during feeding assays and randomly offered either a treated or control food pellet, followed by the other choice. When the second pellet was a treated pellet and was rejected, a third pellet was offered as a control to determine whether the fish had ceased feeding. Groups of fish that did not eat control pellets were considered satiated and were not used in the experiment; therefore, all 10 control pellets were eaten in all experiments. A food pellet was considered rejected if one or more fish took the pellet into their mouths and explled it at least three times, or if the pellet was approached and ignored. The significance of differences in the consumption of treated vs. control pellets was evaluated with the Fisher exact test (Zar, 1984). For any single assay of 10 replicates, a compound was significantly deterrent if four or more of the pellets were rejected ($P \leq 0.043$, one-tailed test); therefore, a sample was considered deterrent if the number of pellets eaten was less than or equal to six.

Food pellets for laboratory assays were made by mixing lyophilized, macerated squid (5 g) with alginic acid (3 g) and water (100 ml), followed by addition of the compound or compounds in a carrier solvent (for treated pellets) or solvent alone (for control pellets). The mixture was vigorously stirred to remove lumps and then loaded into a 5-ml syringe. The tip of the syringe was then dipped into a beaker filled with an aqueous solution of $CaCl_2$ (0.25 M) and the contents of the syringe slowly expelled to form a long strand. After a few minutes, the strand was removed, rinsed in seawater, and chopped into 4-mm-long pieces with a razor blade to form uniform pellets.

RESULTS

The results of fish feeding assays of naturally occurring sponge metabolites and synthetic derivatives are shown in Figure 1a and 1b. Of 21 compounds tested, 12 (**1–6**, **8–12**, **14**) were deterrent at 10 mg/ml of alginate food, 8 (**1–4**, **8–11**) at 5 mg/ml, and 2 (**4**, **8**) at 1 mg/ml. Nine compounds did not deter feeding by *Thalassoma bifasciatum* at any concentration tested (**7**, **13**, **15–21**). Among the seven compounds previously isolated as natural products from *Agelas* sp., only *rac*-longamide A (**7**) was not active at 10 mg/ml, while 4,5-dibromopyrrole-2-carboxylic acid (**5**) and the corresponding amide **6** were not deterrent at 5 mg/ml. The only natural product that was deterrent at 1 mg/ml was midpacamide (**4**).

Although midpacamide (**4**) was the most deterrent natural product, more potent feeding deterrency was exhibited by the alkylidene hydantoin **8**. This compound is a hydrolysis product of dispacamide A (**1**), but has not been identified as a natural product. Its di- and trimethylated derivatives **9** and **10** are less deterrent with increasing methylation. Of similar deterrent activity is the

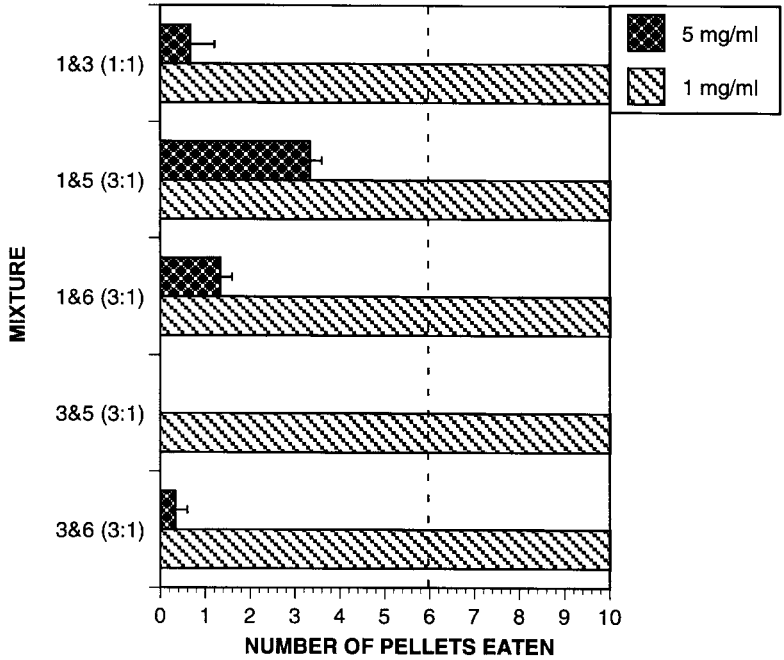

FIG. 3. Consumption by *Thalassoma bifasciatum* of food pellets (mean + SE) containing a mixture of two of four selected alkaloids found naturally in the tissues of *Agelas* spp. Fish consumed all 10 control pellets in all cases. The number of replicate assays performed at each concentration is shown in parentheses. For any individual assay, compounds were considered deterrent if the number of pellets eaten was ≤6 (Fisher exact test, one-tailed) as indicated by the dotted line on the graphs.

histamine derivative **11**. None of the compounds that lacked the pyrrole ring (**17–21**) were active in this assay at any of the concentrations tested.

In an effort to test for additive or synergistic effects, four of the natural products, dispacamide A (**1**), oroidin (**3**), 4,5-dibromopyrrole-2-carboxylic acid (**5**), and 4,5-dibromopyrrole-2-carboxylic acid amide (**6**), were assayed in combinations at overall concentrations of 1 and 5 mg/ml (Figure 3). All of the combinations were active at 5 mg/ml, but none were active at 1 mg/ml.

DISCUSSION

Systematic studies of structure–activity relationships in feeding deterrency have rarely been conducted, as they are limited by the small quantities of compounds usually available by isolation from natural sources. To our knowledge,

the comparative analysis of feeding deterrency of 21 pure natural and synthetic products presented in this paper represents the first of its kind in marine chemical ecology, although structure–function relationships have been addressed in previous studies in which a multiplicity of pure compounds were assayed (e.g., Pawlik and Fenical, 1992; Vervoort et al., 1998; Hay et al., 1998). In the field of terrestrial chemical ecology, a recent investigation of insect feeding deterrents employed partly synthetic drimane sesquiterpenoids as terrestrial crop protection agents (Messchendorp et al., 1998, and references cited therein), aiming at the discovery of more effective antifeedant molecules. Other studies have dealt with insect antifeedant activities of diterpenoids (e.g., see Enriz et al., 1994, and references cited therein; Mullin et al., 1997; Gonzalez-Coloma et al., 1998).

Oroidin (**3**) and 4,5-dibromopyrrole-2-carboxylic acid (**5**) were discovered to be responsible for the chemical defense of *Agelas clathrodes* and five other species of *Agelas* from tropical Caribbean coral reefs (Chanas et al., 1996). The abundance of pyrrole–imidazole alkaloids in the tissues of sponges of the genus *Agelas* worldwide (for the occurrence of the natural products discussed in this paper, see Table 1) suggested that these compounds may have been evolutionarily retained as chemical defenses, but the relative activities of the natural products, and the concentrations necessary to elicit a behavioral response in potential predators were not known. Our program on the synthesis of these exclusively marine alkaloids (Lindel and Hoffmann, 1997a,b; Lindel and Hochgürtel, 1998) has provided sufficiently large amounts of pure, structurally varied compounds to permit replicate feeding assays. Further analyses of dimeric pyrrole–imidazole alkaloids of the sceptrin and ageliferin type that are found in high concentrations of some species of *Agelas* (Cafieri et al., 1997) are the subject of forthcoming reports (Assmann et al., 2000).

Of the seven natural products tested (**1–7**), the first six, which each possess a conformationally flexible pyrrolyl–carbonyl bond and permit a transoid conformation of the amide bond, deterred fish feeding at 10 mg/ml (Figure 1a). In addition, the natural products bearing the imidazole group (**1–4**) were more potently deterrent and active at 5 mg/ml. Only midpacamide (**4**) was active at 1 mg/ml, while *rac*-longamide A (**7**) was not significantly deterrent at any of the assay concentrations.

Analyzing the activities of the natural products (**1–7**, Figure 1a) and of the synthetic compounds (**8–21**, Figure 1b) provides the following insights regarding the relationship between molecular structure and fish feeding deterrency, which are summarized in Figure 4.

Pyrrole. The pyrrole moiety was required for deterrent activity in the fish feeding assay. None of the compounds lacking the pyrrole moiety (**17–21**) were active, even when the imidazole moiety was present (**17**). Methylation of the pyrrole ring did not alter compound activity (**4**, **9**, **10**).

Bromination. The influence of the bromination of the pyrrole ring was not

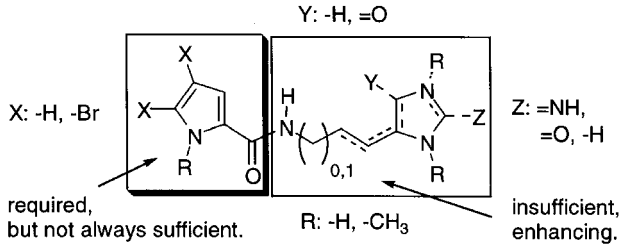

FIG. 4. Summary of the structure–activity relationships of fish feeding deterrency by pyrrole-imidazole alkaloids from marine sponges of the genus *Agelas*. For the influence of the groups R, X, Y, and Z see text.

studied systematically, but 4,5-dibromopyrrole-2-carboxylic acid (**5**) was somewhat more deterrent than its debrominated analog **14** at 10 mg/ml. Similarly, the monobrominated keramadine (**2**) was also somewhat less deterrent than the dibrominated oroidin (**3**).

Imidazole. Presence of the imidazole group in addition to the pyrrole and chain section enhanced feeding deterrent activity. Compounds bearing both the imidazole and pyrrole moieties were the most potent feeding deterrents (**1–4**, **8–11**), but variously functionalized imidazoles alone were not deterrent (**17–21**). the oxidation state of the imidazole ring at the four-position (*Y* = —H or =O; Figure 4) had no influence on activity, because there was no difference in the activity of compounds **2**, **3**, and **11** and the higher oxidized derivatives **1**, **4**, and **8–10**. Similarly, the functionalization of the imidazole 2-position (*Z* = =NH, =O, —H; Figure 4) did not affect activity. Removal of the imidazole moiety with retention of the chain resulted in loss of activity (**13**, **15**), except for the acid **12** at 10 mg/ml.

Polarity and N-*Methylation.* Among the similar, active compounds, increasing polarity appeared to enhance feeding deterrent activity, with the activity of **8** exceeding the di- and tri-*N*-methylated **9** and **10**, respectively. Similarly, among compounds not containing an imidazole group, the activity of **12** exceeded the activity of **13**.

Among the natural products that have been isolated from *Agelas* sp. (Table 1), combinations of compounds appeared to have an additive impact on the activit in feeding assays (Figure 3), and there was no evidence of synergy. The assays that resulted in Figure 3 were all performed with one group of fishes in one locality (unlike the replicate assays that resulted in Figure 1), which may explain the lack of variability in response to mixtures of compounds assayed at 1 mg/ml (all of which were eaten). The threshold of activity in all of these assays was very abrupt. In a previous study, we determined that the mixture of oroidin (**3**) and 4,5-dibromopyrrol-2-carboxylic acid (**5**) in the tissues of *Age-*

TABLE 1. PYRROLE–IMIDAZOLE ALKALOIDS ISOLATED FROM *Agelas* spp. AND OTHER MARINE SPONGES

Natural product	Citation	Species	Concentration
Dispacamide A (**1**)	Cafieri et al. (1996)	*A. conifera*, *A. dispar*, *A. clathrodes*, *A. longissima*	1–5% (BuOH extr.)
	Cafieri et al. (1997)	As above	0.8–3.5% (dry wt)
Keramadine (**2**)	Nakamura et al. (1984)	*A.* sp.	0.0014% (wet wt)
	Cafieri et al. (1998)	*A. dispar*	not reported
Oroidin (**3**)	Forenza et al. (1971)	*A. oroides*	2.3% (dry wt)
	Walker et al. (1981)	*A. sceptrum*	0.5% (dry wt)
	de Nanteuil et al. (1985)	*Pseudaxinyssa cantharella*,	0.01% (H_2O extr.)
	Chanas et al. (1996)	*A. clathrodes*	(1.4 ± 1.1) mg/ml
	Cafieri et al. (1997)	*A. conifera*, *A. dispar*, *A. clathrodes*, *A. longissima*	2.1–4.2% (dry wt)
	König et al. (1998)	*A. oroides*	0.073% (CH_2Cl_2 extr.)
Midpacamide (**4**)	Chevolot et al. (1977)	unidentified	0.064% (dry wt)
	Fathi-Afshar and Allen (1988)	*A. mauritiana*	0.05% (wet wt)
	Jiménez and Crews (1994)	*A. mauritiana*	0.003% (wet wt)
5	Forenza et al. (1971)	*A. oroides*	0.21% (dry wt)
	Gunasekera et al. (1989)	*A. flabelliformis*	unclear
	Chanas et al. (1996)	*A. clathrodes*	1/3 of oroidin (**3**)
	König et al. (1998)	*A. oroides*	0.025% (CH_2Cl_2 extr.)
6	Forenza et al. (1971)	*A. oroides*	trace
	Tsukamoto et al. (1996)	*Pseudoceratina purpurea*	0.005% (wet wt)
	Manchini et al. (1997)	*Acanthella carteri*	0.009% (EtOH extr.)
Longamide A (**7**)	Cafieri et al. (1995)	*A. longissima*	0.08% (dry wt)
	Umeyama et al. (1998)	*Homaxinella* sp.	0.009% (wet wt)

las clathrodes was approximately 3 : 1, with a mean concentration of 1.4 mg/ml (range = 0.4–5.2 mg/ml) (Chanas et al., 1996), suggesting that sponges may be producing these defensive metabolites at the lower limit of their effectiveness. Cafieri et al. (1997) reported concentrations of dispacamide A (**1**, 0.8–3.5% of the dry weight, mixture with dispacamides B and C) and of oroidin (**3**, 2.1–4.2%) in *A. conifera*, *A. dispar*, *A. clathrodes*, and *A. longissima*. Using the dry mass-to-volume conversions provided in Chanas and Pawlik (1995) for *A. conifera*, *A. dispar*, and *A. clathrodes*, the approximate range of volumetric concentrations reported by Cafieri et al. (1997) of dispacamides is 1.1–6.3 mg/ml and those of

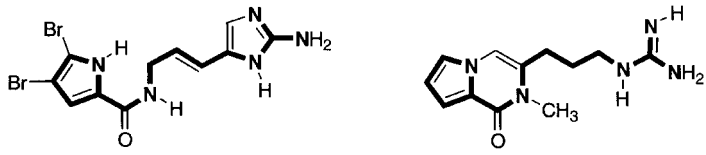

FIG. 5. Structural similarities of oroidin (**3**), a fish antifeedant metabolite from a marine sponge, and peramine (**30**), an insect antifeedant metabolite from a terrestrial fungus.

oroidin are 3.0–7.6 mg/ml. In addition to oroidin (**3**) (Chanas et al., 1996), dispacamide A (**1**) may also function as a primary defensive metabolite of *Agelas* sp. The other natural products, including keramadine (**2**), midpacamide (**4**), 4,5-dibromopyrrole-2-carboxamide (**6**), and longamide A (**7**) have been reported as minor secondary metabolites, with concentrations 100-fold lower than oroidin (**3**) or dispacamide A (**1**). Although active as chemical defenses, it appears that these minor compounds are not present in high enough concentrations to have much deterrent effect, and they may represent biosynthetic intermediates, artifacts, or have other biological functions.

Whereas for some natural products, such as the dialdehyde scalaradial (Rogers and Paul, 1991), fish feeding deterrency can be attributed to the presence of chemically reactive functional groups, other examples indicate more subtle mechanisms of action. For example, the strong feeding deterrency of didemnimide D appears to depend on bromination of an indole nucleus and *N*-methylation of an imidazole ring (Vervoort et al., 1998).

This investigation is a first step toward understanding the chemical basis of the ecological functions of pyrrole–imidazole alkaloids in marine sponges. Interestingly, the structurally related pyrrole–guanidinium alkaloid peramine (**30**) from the terrestrial microfungus *Acremonium lolii* acts as a strong antifeedant against certain insects (Figure 5) (Rowan et al., 1986). Oroidin (**3**) and peramine (**30**) possess a common substructure consisting of a pyrrole ring and a guanidinium group that is less basic in oroidin (**3**) due to its incorporation into the aromatic imidazole system. In the broadest sense, the ability of a molecule to take certain conformations, and thereby spatial arrangements of functional groups, may be as important for feeding deterrency as the presence of certain substructures. This observation has been made for the sweet taste of aspartame-like dipeptides and dipeptide mimetics (see Yamazaki et al., 1994, and references cited therein).

Acknowledgments—T. Lindel thanks the Deutsche Forschungsgemeinschaft for a Habilitandenstipendium (Li 597/2-3). T. Lindel, H. Hoffmann, and M. Hochgürtel thank Prof. Dr. Richard Neidlein for generous support. J. R. Pawlik thanks the National Science Foundation (OCE-9314145

and OCE-9711255) and the NOAA/National Undersea Research Program in Key Largo, Florida (UNCW9523 and UNCW9812) for financial support. Will O'Neal and Greg McFall kindly performed most of the fish feeding assays. Matthias Köck and Michael Assmann (Universität Frankfurt) very kindly provided us with a sample of oroidin and commented on earlier drafts of the manuscript. We express our appreciation to the Government of the Bahamas for permission to perform research in their territorial waters. Contribution #237 to UNCW's Center for Marine Science.

REFERENCES

ASSMANN, M., LICHTE, E., PAWLIK, J. R., and KÖCK, M. 2000. Chemical defenses of the Caribbean sponges *Agelas wiednemayeri* and *Agelas conifera*. Submitted to *Mar. Ecol. Prog. Ser.*

BAILEY, D. M., and JOHNSON, R. E. 1973. Pyrrole antibacterial agents. 2. 4,5-Dihalopyrrole-2-carboxylic acid derivatives. *J. Med. Chem.* 16:1300–1302.

BRAEKMAN, J. C., DALOZE, D., MOUSSIAUX, B., STOLLER, C., and DENEUBOURG, F. 1989. Sponge secondary metabolites: new results. *Pure Appl. Chem.* 61:509–512.

CAFIERI, F., FATTORUSSO, E., MANGONI, A., and TAGLIALATELA-SCAFATI, O. 1995. Longamide and 3,7-dimethylisoguanine, two novel alkaloids from the marine sponge *Agelas longissima. Tetrahedron Lett.* 36:7893–7896.

CAFIERI, F., FATTORUSSO, E., MANGONI, A., and TAGLIALATELA-SCAFATI, O. 1996. Dispacamides, anti-histamine alkaloids from Caribbean *Agelas* sponges. *Tetrahedron Lett.* 37:3587–3590.

CAFIERI, F., CARNUCCIO, R., FATTORUSSO, E., TAGLIALATELA-SCAFATI, O., and VALLEFUOCO, T. 1997. Anti-histaminic activity of bromopyrrole alkaloids isolated from Caribbean *Agelas* spones. *Bioorg. Med. Chem. Lett.* 7:2283–2288.

CAFIERI, F., FATTORUSSO, E., and TAGLIALATELA-SCAFATI, O. 1998. Novel bromopyrrole alkaloids from the sponge *Agelas dispar. J. Nat. Prod.* 61:122–125.

CHANAS, B., and PAWLIK, J. R. 1995. Defenses of Caribbean sponges against predatory reef fish. II. Spicules, tissue toughness, and nutritional quality. *Mar. Ecol. Prog. Ser.* 127:195–211.

CHANAS, B., PAWLIK, J. R., LINDEL, T., and FENICAL, W. 1996. Chemical defense of the Caribbean sponge *Agelas clathrodes. J. Exp. Mar. Biol. Ecol.* 208:185–196.

CHEVOLOT, L., PADUA, S., RAVI, B. N., BLYTH, P. C., and SCHEUER, P. J. 1977. Isolation of 1-methyl-4,5-dibromopyrrole-2-carboxylic acid and its 3′-(hydantoyl)propylamide (midpacamide) from a marine sponge. *Heterocycles* 7:891–894.

DE NANTEUIL, G., AHOND, A., GUILHEM, J., POUPAT, C., TRANS HUU DAU, E., POTIER, P., PUSSET, M., PUSSET, J., and LABOUTE, P. 1985. Isolement et identification des metabolites d'une nouvelle espece de spongaire, *Pseudaxinyssa cantharella. Tetrahedron* 41:6019–6033.

DUFFY, J. E., and PAUL, V. J. 1992. Prey nutritional quality and the effectiveness of chemical defenses against tropical reef fishes. *Oecologia* 90:333–339.

ENRIZ, R. D., BALDONI, H. A., JAUREGUI, E. A., SOSA, M. E., TONN, C. E., and GIORDANO, O. S. 1994. Structure–activity relationship of clerodane diterpenoids acting as antifeeding agents. *J. Agric. Food Chem.* 42:2958–2963.

FATHI-AFSHAR, R., and ALLEN, T. M. 1988. Biologically active metabolites from *Agelas mauritiana. Can. J. Chem.* 66:45–50.

FAULKNER, D. J. 1998. Marine natural products. *Nat. Prod. Rep.* 15:113–158.

FORENZA, S., MINALE, L., and RICCIO, R. 1971. New bromopyrrole derivatives from the sponge Agelas oroides. *J. Chem. Soc. Chem. Commun.* 1971:1129–1130.

GONZALEZ-COLOMA, A., GUADANO, A., GUTIERREZ, C., CABRERA, R., DE LA PEÑA, E., DE LA FUENTE, G., and REINA, M. 1998. Antifeedant delphinium diterpenoid alkaloids. Structure–activity relationships. *J. Agric. Food Chem.* 46:286–290.

GUNASEKERA, S. P., CRANICK, S., and LONGLEY, R. E. 1989. Immunosuppressive compounds from a deep water marine sponge, *Agelas flabelliformis. J. Nat. Prod.* 52:757–761.

HAY, M. E., PIEL, J., BOLAND, W., and SCHNITZLER, I. 1998. Seaweed sex pheromones and their degradation products frequently suppress amphipod feeding but rarely suppress sea urchin feeding. *Chemoecology* 8:91–98.

HIXON, M. A. 1983. Fish grazing and community structure of coral reefs and algae: A synthesis of recent studies, pp. 79–87, *in* M. S. Reaka (ed.). The Ecology of Deep and Shallow Coral Reefs. Symposia series for undersea research, NOAA/NURP, Washington, DC.

JIMÉNEZ, C., and CREWS, P. 1994. Mauritamide A and accompanying oroidin alkaloids from the sponge *Agelas mauritiana. Tetrahedron Lett.* 35:1375–1378.

KÖNIG, G. M., WRIGHT, A. D., and LINDEN, A. 1998. Antiplasmodial and cytotoxic metabolites from the maltese sponge *Agelas oroides. Planta Med.* 64:443–447.

LINDEL, T., and HOCHGÜRTEL, M. 1998. The alkyne pathway to keramadine from the marine sponge *Agelas* sp. *Tetrahedron Lett.* 39:2541–2544.

LINDEL, T., and HOFFMANN, H. 1997a. Synthesis of dispacamide from the maine sponge *Agelas dispar. Tetrahedron Lett.* 38:8935–8938.

LINDEL, T., and HOFFMANN, H. 1997b. Synthesis of *rac*-midpacamide and the *spiro*-cyclization of its precursor. *Liebigs Ann./Recueil* 1997:1525–1528.

LINDEL, T., JUNKER, J., and KÖCK, M. 1999. 2D-NMR-guided constitutional analysis of organic compounds employing the computer program COCON. *Eur. J. Org. Chem.* 1999:573–577.

MANCINI, I., GUELLA, G., AMADE, P., ROUSSAKIS, C., and PIETRA, F. 1997. Hanishin, a semiracemic, bioactive C_9 alkaloid of the axinellid sponge *Acanthella carteri* from the Hanish Islands. A shunt metabolite? *Tetrahedron Lett.* 38:6271–6274.

MEANWELL, N. A., ROTH, H. R., SMITH, E. C. R., WEDDING, D. L., and WRIGHT, J. J. K. 1991. Diethyl 2,4-dioxoimidazoline-5-phosphonates: Horner-Wadsworth-Emmons reagents for the mild and efficient preparation of C-5 unsaturated hydantoin derivatives. *J. Org. Chem.* 56:6897–6904.

MESSCHENDORP, L., GOLS, G. J. Z., and VAN LOON, J. J. A. 1998. Behavioral effects and sensory detection of drimane deterrents in *Myzus persicae* and *aphis gossypii* nymphs. *J. Chem. Ecol.* 24:1433–1446.

MOE, O. A., and WARNER, D. T. 1949. 1,4 Addition reactions. III. The addition of cyclic imides to α,β-unsaturated aldehydes. A synthesis of β-alanine hydrochloride. *J. Am. Chem. Soc.* 71:1251–1253.

MULLIN, C. A., GONZALEZ-COLOMA, A., GUTIERREZ, C., REINA, M., EICHENSEER, H., HOLLISTER, B., and CHYB, S. 1997. Antifeedant effects of some novel terpenoids on chrysomelidae beetles: Comparisons with alkaloids on an alkaloid-adapted an nonadapted species. *J. Chem. Ecol.* 23:1851–1866.

NAKAMURA, H., OHIZUMI, Y., KOBAYASHI, J., and HIRATA, Y. 1984. Keramadine, a novel antagonist of serotonergic receptors isolated from the Okinawan sea sponge *Agelas* sp. *Tetrahedron Lett.* 25:2475–2478.

PAWLIK, J. R. 1993. Marine invertebrate chemical defenses. *Chem. Rev.* 93:1911–1922.

PAWLIK, J. R., and FENICAL, W. 1992. Chemical defense of *Pterogorgia anceps*, a Caribbean gorgonian coral. *Mar. Ecol. Prog. Ser.* 87:183–188.

PAWLIK, J. R., BURCH, M. T., and FENICAL, W. 1987. Patterns of chemical defense among Caribbean gorgonian corals: A preliminary survey. *J. Exp. Mar. Biol. Ecol.* 108:55–66.

PAWLIK, J. R., CHANAS, B., TOONEN, R. J., and FENICAL, W. 1995. Defenses of Caribbean sponges against predatory reef fish. I. Chemical deterrency. *Mar. Ecol. Prog. Ser.* 127:183–194.

PENNINGS, S. C., PABLO, S. R., PAUL, V. J., and DUFFY, E. 1994. Effects of sponge secondary metabolites in different diets on feeding by three groups of consumers. *J. Exp. Mar. Biol. Ecol.* 180:137–149.

RANDALL, J. R., and HARTMAN, W. D. 1968. Sponge-feeding fishes of the West Indies. *Mar. Biol.* 1:216–225.

ROGERS, S. D., and PAUL, V. J. 1991. Chemical defenses of three *Glossodoris* nudibranchs and their dietary *Hyrtios* sponges. *Mar. Ecol. Prog. Ser.* 77:221–232.

ROWAN, D. D., HUNT, M. B., and GAYNOR, D. L. 1986. Peramine, a novel insect feeding deterrent from ryegrass infected with the endophyte *Acromonium lolii. J. Chem. Soc. Chem. Commun.* 1986:935–936.

TSUKAMOTO, S., KATO, H., HIROTA, H., and FUSETANI, N. 1996. Ceratinamides A and B: New antifouling dibromotyrosine derivatives from the marine sponge *Pseudoceratina purpurea. Tetrahedron* 52:8181–8186.

UMEYANA, A., ITO, S., YUASA, E., ARIHARA, S., and YAMADA, T. 1998. A new bromopyrrole alkaloid and the optical resolution of the racemate from the marine sponge *Homaxinella* sp. *J. Nat. Prod.* 61:1433–1434.

VERVOORT, H. C., PAWLIK, J. R., and FENICAL, W. 1998. Chemical defense of the Caribbean ascidian *Didemnum conchyliatum. Mar. Ecol. Prog. Ser.* 164:221–228.

WALKER, R. P., FAULKNER, D. J., VAN ENGEN, D., and CLARDY, J. 1981. Sceptrin, an antimicrobial agent from the sponge *Agelas sceptrum. J. Am. Chem. Soc.* 103:6772–6773.

WILSON, D. M., PUYANA, M., FENICAL, W., and PAWLIK, J. R. 1999. Chemical defense of the Caribbean reef sponge *Axinella corrugata* against predatory fishes. *J. Chem. Ecol.* 25:2811–2823.

YAMAZAKI, T., BENEDETTI, E., KENT, D., and GOODMAN, M. 1994. Konformationsvoraussetzungen für den süssen Geschmack von Dipeptiden und Dipeptidmimetica. *Angew. Chem.* 106:1502–1517.

ZAR, J. H. 1984. Biostatistical Analysis, 2nd ed. Prentice-Hall, Englewood Cliffs, New Jersey.