acetate–benzene afforded 52 mg (83%) of the nitrile **13** as a pale yellow oil (a mixture of epimers, ratio ca. 2:1 by NMR): MS, *m/e* (relative intensity) 485 (M^+, 35), 340 (100).

To a solution of 52 mg (0.11 mmol) of the above nitrile **13** in 7.5 mL of ethanol were successively added 13 mL of 30% KOH and 2.8 mL of 30% H_2O_2, and the mixture was heated at 40 °C for 1 h and then at reflux for 2 h. After being cooled at 0 °C, the mixture was acidified with 10% HCl and extracted with ethyl acetate. The extract was washed with water and dried. Removal of the solvent gave a pale yellow oil, which was subjected to preparative TLC ($CHCl_3$–MeOH, 9:1) to afford 33 mg (61%) of the acid **14** as a colorless oil: $[\alpha]^{25}_D$ +48.0° (*c* 0.2, $CHCl_3$); IR (neat) 3600–2500, 1725 cm^{-1}; NMR δ 1.21 (d, 3 H, *J* = 6 Hz, CH_3), 1.54 (s, 3 H, CH_3), 1.76 (s, 3 H, CH_3), 2.96 (s, 3 H, CH_3OCH_2), 2.70–3.20 (m, 4 H, H-4 and H-11), 3.86 (s, 3 H, OCH_3), 4.36 (d, 1 H, *J* = 6 Hz, OCH_2O), 4.42 (m, 1 H, H-6′), 4.50 (m, 1 H, H-3), 4.55 (d, 1 H, *J* = 6 Hz, OCH_2O), 4.79 (d, 1 H, *J* = 4 Hz, H-3′), 6.84 (d, 1 H, *J* = 8 Hz, Ar H), 7.44 (t, 1 H, *J* = 8 Hz, Ar H), 7.59 (d, 1 H, *J* = 8 Hz, Ar H); MS, *m/e* (relative intensity) 504 (M^+, 67), 359 (100); exact mass calcd for $C_{26}H_{32}O_{10}$ 504.1993, found 504.1976.

9,10-(Isopropylidenedioxy)-5-methoxy-3,4-dihydro-1*H*-naphtho[2,3-*c*]pyran-1(*S*)-spiro-2′-[4′(*S*)-acetoxy-3′(*S*)-hydroxy-6′(*S*)-methyltetrahydropyran]-3(*R*)-ylacetic Acid (15). A solution of 45 mg (0.09 mmol) of the acid **14** in 6 mL of acetic anhydride and 8 mL of pyridine was allowed to stand at room temperature for 4 days. The reaction mixture was diluted with water and extracted with $CHCl_3$. The extract was washed with water and dried. Removal of the solvent gave a yellow oil, which was dissolved in a mixture of 2 mL of 10% HCl and 20 mL of dimethoxyethane and stirred at 50 °C for 5 h. The reaction mixture was partitioned between ethyl acetate and water, and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with water and dried. Removal of the solvent afforded a yellow oil, which was subjected to preparative TLC ($CHCl_3$–MeOH, 9:1) to give 26 mg (58%) of the acid **15** as a pale yellow oil: $[\alpha]^{25}_D$ +29.0° (*c* 0.54, $CHCl_3$); IR (neat) 3600–2500, 1735, 1715 cm^{-1}; NMR δ 1.18 (d, 3 H, *J* = 6 Hz, CH_3), 1.59 (s, 3 H, CH_3), 1.72 (s, 3 H, CH_3), 2.15 (s, 3 H, $COCH_3$), 2.70 (dd, 1 H, *J* = 16, 2 Hz, H-4), 2.80–3.00 (ABX system, 2 H, H-11), 3.22 (dd, 1 H, *J* = 16, 2 Hz, H-4), 3.88 (s, 3 H, OCH_3), 4.30 (m, 1 H, H-6′), 4.62 (ABX system, 1 H, H-11), 4.80 (d, 1 H, *J* = 4 Hz, H-3′), 5.34 (q, 1 H, *J* = 4 Hz, H-4′), 6.85 (d, 1 H, *J* = 8 Hz, Ar H), 7.44 (t, 1 H, *J* = 8 Hz, Ar H), 7.59 (d, 1 H, *J* = 8 Hz, Ar H); MS, *m/e* (relative intensity) 502 (M^+, 27), 359 (93), 358 (100); exact mass calcd for $C_{26}H_{30}O_{10}$ 502.1837, found 502.1820.

(+)-Griseusin A (2). To a stirred solution of 26 mg (0.05 mmol) of the acid **15** in 1.5 mL of THF were successively added 62 mg (0.5 mmol) of AgO and 0.15 mL of 6 N HNO_3 at room temperature. After being stirred for 10 min, the mixture was filtered. The filtrate was partitioned between ethyl acetate and water, and the aqueous layer was extracted with ethyl acetate. The combined extracts were washed with water and dried. Removal of the solvent afforded an orange solid, which was subjected to preparative TLC ($CHCl_3$–MeOH, 9:1) to give 19 mg (82%) of (+)-griseusin B (**16**): mp 208–210 °C (MeOH); $[\alpha]^{25}_D$ +318° (*c* 0.071, MeOH); IR (KBr) 3600–2500, 1730, 1640 cm^{-1}; NMR δ 1.21 (d, 3 H, *J* = 6 Hz, CH_3), 1.80–2.15 (m, 2 H, H-5′), 2.12 (s, 3 H, $COCH_3$), 2.43 (dd, 1 H, *J* = 19, 12 Hz, H-4), 2.74 (dd, 1 H, *J* = 16, 8 Hz, H-11), 2.85 (dd, 1 H, *J* = 16, 4 Hz, H-11), 2.94 (dd, 1 H, *J* = 19, 3 Hz, H-4), 4.30 (dqd, 1 H, *J* = 13, 6, 2 Hz, H-6′), 4.56 (m, 1 H, H-3), 4.81 (m, 1 H, H-3′), 5.29 (q, 1 H, *J* = 4 Hz, H-4′), 7.30 (m, 1 H, Ar H), 7.62 (m, 2 H, Ar H).

A solution of 19 mg of **16** in 2 mL of pyridine was allowed to stand at room temperature for 15 h. Removal of the solvent afforded an orange residue, which was subjected to preparative TLC (ethyl acetate–benzene, 1:1), giving 12 mg (63%) of (+)-griseusin A (**2**): mp 161–163 °C (MeOH); $[\alpha]^{25}_D$ +166° (*c* 0.038, EtOH); NMR δ 1.22 (d, 3 H, *J* = 6 Hz, CH_3), 1.91 (td, 1 H, *J* = 11, 4 Hz, H-5′$_{ax}$), 2.10 (ddd, 1 H, *J* = 11, 4, 2 Hz, H-5′$_{eq}$), 2.12 (s, 3 H, $COCH_3$), 2.47 (d, 1 H, *J* = 12 Hz, OH), 2.72 (d, 1 H, *J* = 17 Hz, H-11), 3.07 (dd, 1 H, *J* = 17, 5 Hz, H-11), 4.18 (dqd, 1 H, *J* = 11, 6, 2 Hz, H-6′), 4.81 (dd, 1 H, *J* = 5, 3 Hz, H-3), 4.95 (dd, 1 H, *J* = 12, 4 Hz, H-3′), 5.29 (q, 1 H, *J* = 4 Hz, H-4′), 5.31 (d, 1 H, *J* = 3 Hz, H-4), 7.33 (m, 1 H, Ar H), 7.70 (m, 2 H, Ar H), 11.94 (s, 1 H, OH); CD (EtOH) $[\theta]_{525}$ 0, $[\theta]_{500}$ +755, $[\theta]_{465}$ +3750, $[\theta]_{400}$ +1480, $[\theta]_{358}$ 0, $[\theta]_{300}$ −9800, $[\theta]_{287}$ −11 470, $[\theta]_{276}$ 0, $[\theta]_{270}$ +14 000.

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Registry No. **2**, 85922-69-6; **4**, 83312-78-1; **5**, 78284-30-7; **6**, 85883-50-7; **7**, 52431-65-9; **8**, 85883-42-7; **9**, 85883-43-8; *β*-**10**, 85883-44-9; *α*-**10**, 85922-65-2; **11**, 85883-45-0; **12a**, 85883-46-1; **12b**, 85922-66-3; *α*-**13**, 85883-47-2; *β*-**13**, 85922-67-4; **14**, 85883-48-3; **15**, 85883-49-4; **16**, 85922-68-5; 3-butenoic acid, 625-38-7.

Defensive Metabolites from Three Nembrothid Nudibranchs[1]

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The nembrothid nudibranchs *Tambje abdere*, *T. eliora*, and *Roboastra tigris* all contain tambjamines A–D (4–7). The aldehydes 1–3, produced during extraction with methanol, were key compounds in the structural elucidation. The tambjamines were traced to a food source, the bryozoan *Sessibugula translucens*, and were implicated in the chemical defense mechanism of the *Tambje* species.

Roboastra tigris Farmer 1978[2] is a large carnivorous nembrothid nudibranch that is known to prey on two smaller nembrothid nudibranchs, *Tambje eliora* (Marcus and Marcus, 1967)[3] and *Tambje abdere* Farmer 1978.[2] Methanolic extracts of all three nudibranchs contained the same group of biologically active bipyrroles **1–7** although the aldehydes **1–3** were subsequently shown to be artifacts of the extraction procedure. The tambjamines A–D (**4–7**) were traced to a dietary source, the ectoproct (bryozoan) *Sessibugula translucens* Osburn 1950,[4] and were impli-

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(2) Farmer, W. M. *Veliger* **1978**, *20*, 375.
(3) Marcus, E.; Marcus, E. *Stud. Trop. Oceanogr.* **1967**, *6*, 194–6.
(4) Osburn, R. C. *Allan Hancock Pacific Exped.* **1950**, *14*, 1.

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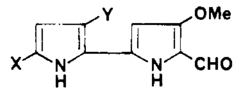
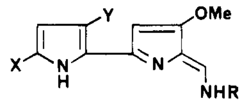
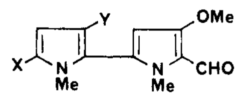


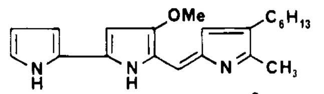
1 X = H Y = H
2 X = Br Y = H
3 X = H Y = Br



4 X = H Y = H R = H
5 X = Br Y = H R = H
6 X = H Y = H R = *i*-Bu
7 X = H Y = Br R = *i*-Bu



9 X = H Y = H
10 X = Br Y = H
11 X = H Y = Br



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cated in a chemical defense mechanism. In this paper we describe the structural elucidation of the tambjamines A–D (**4–7**) and discuss their complex biological roles.

Samples of the three species of nudibranch were collected from the Gulf of California and were extracted by soaking in cold methanol. The dichloromethane-soluble material from each methanolic extract was fractionated in an identical manner by using several different chromatographic systems to obtain pure samples of the tambjamines A–D (**4–7**) and their hydrolysis products, the aldehydes **1–3** (Table I). Because of the hydrolysis reaction, comparison of the yields of individual compounds for each species is inappropriate. However, the combined yields of bipyrroles (expressed a precent dry weight) were similar for the two *Tambje* species but almost an order of magnitude lower for *Roboastra*.

The structural elucidation of the tambjamines was simplified when one of the hydrolysis products was identified as a known[5,6] compound, 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**1**): mp 263–265 °C; UV λ_{max} 364 nm. Rapoport and Holden[6] synthesized 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde [**1**: mp 265 °C (dec); UV λ_{max} 363 nm] during studies of the structural elucidation and synthesis of prodigiosin (**8**). Although we have not compared natural and synthetic samples directly, all spectral data, particularly the D_2O-exchanged ^{1}H NMR data, were consistent with this structural assignment. Methylation of bipyrrole **1** gave 1,1′-dimethyl-4-methoxy-2,2′-bipyrrole-5-carboxaldehyded (**9**), to which the bipyrroles **2–7** were subsequently related.

The remaining hydrolysis products 5′-bromo-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**2**) and 3′-bromo-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**3**) had the molecular formula $C_{10}H_9BrN_2O_2$. The position of the bromine atom in each compound was determined from the magnitude of the coupling constants of signals due to protons on the brominated pyrrole ring. Thus, the proton signals at δ 6.57 (d, 1 H, J = 3.9 Hz) and 6.20 (d, 1 H, J = 3.9 Hz) in the deuterium-exchanged ^{1}H NMR spectrum of bipyrrole **2** were assigned to protons on a 2,5-disubstituted pyrrole ring while signals at δ 6.74 (d, 1 H, J = 2.7 Hz) and 6.33 (d, 1 H, J = 2.7 Hz) were assigned to a 2,3-disubstituted pyrrole ring in bipyrrole **3**.[7] Methylation of the bipyrroles **2** and **3** gave the *N,N′*-dimethyl derivatives **10** and **11**, respectively. Both *N,N′*-dimethyl derivatives **10** and **11** were debrominated by hydrogenation over 10% palladium on charcoal catalyst to give the same product, *N,N′*-dimethyl-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**9**).

Tambjamine A (**4**) had the molecular formula $C_{10}H_{11}N_3O$, corresponding to a bipyrrole similar to the aldehyde **1** but having carboximine in place of the aldehyde group. The ultraviolet spectrum [397 nm (ε 20 000), 255 (4600)] was consistent with that assignment, and the infrared bands at 1675 and 1605 cm^{-1} could be assigned to C=N and C=C stretching. The ^{1}H NMR spectrum contained signals at δ 3.92 (s, 3 H) due to the methoxyl group, at δ 5.95 (s, 1 H), 6.30 (m, 1 H), 6.78 (m, 1 H), and 7.09 (m, 1 H) assigned to four pyrrole protons, and at δ 7.49 (m, 1 H), 9.20 (br, 2 H), and 11.3 (br, 1 H). On exchange with deuterium oxide, the signals at δ 6.30, 6.78, and 7.09 all sharpened to double doublets typical of protons on a 2-substituted pyrrole ring, the signals at δ 9.20 and 11.3 were exchanged, and the signal at δ 7.49 sharpened to a singlet. These data implied that the pyrrole ring having the carboximine group at C-5 was enolized to produce the enamine **4** as the more stable tautomer.

Tambjamine B (**5**) had the molecular formula $C_{10}H_{10}BrN_3O$. The ^{1}H NMR spectrum was similar to that of enamine **4** except for the absence of a pyrrole proton at δ ~7.10. After exchange with deuterium oxide, the pyrrole proton signals occurred at δ 5.91 (s, 1 H), 6.23 (d, 1 H, J = 3.9 Hz), and 6.67 (d, 1 H, J = 3.9 Hz), indicating bromination at C-5′. Methylation of a mixture of enamines **4** and **5** with methyl iodide and potassium carbonate in anhydrous acetone gave the aldehydes **9** and **10**. Hydrolysis of a 3:1 mixture of enamines **4** and **5** by using aqueous methanolic potassium hydroxide solution gave a 3:1 mixture of aldehydes **1** and **2**.

Tambjamine C (**6**) had the molecular formula $C_{14}H_{19}N_3O$. The ultraviolet spectrum [405 nm (ε 23 000), 258 (5700)] was consistent with an *N*-alkyl derivative of enamine **4** while the infrared spectra of the two compounds contained many similar bands. The ^{1}H NMR spectra revealed the presence of an isobutylamine residue that gave rise to signals at δ 1.03 (d, 6 H, J = 6.6 Hz), 2.03 (m, 1 H), and 3.29 (d, 2 H, J = 6.3 Hz). The remaining signals were remarkably similar to those of the enamine **4** except that only two NH proton signals were observed at δ 9.46 (br, 1 H) and 10.7 (br, 1 H) and that the signal due to the proton on the enamine carbon appeared as a doublet at δ 7.29 (d, 1 H, J = 15 Hz) that collapsed to a sharp singlet in the deuterium-exchanged spectrum. The large coupling constant between the proton on the enamine carbon and the NH proton suggested that the two protons were held in a trans-antiplanar conformation.

Tambjamine D (**7**) had the molecular formula $C_{14}H_{18}BrN_3O$. Comparison of the spectral data of isobutylamine **7** with isobutylamine **6** revealed many similarities. However, comparison of the ^{1}H NMR spectra of isobutylamine **7** with those of enamine **5** and aldehydes **2** and **3** suggested that the bromine atom in isobutylamine **7** was at the 3′ atom. This was confirmed by methylation of the isobutylamines **6** and **7** with methyl iodide and potassium carbonate in anhydrous acetone to give the aldehydes **9** and **11**, respectively, and hydrolysis of a 3:1 mixture of isobutylamines **6** and **7** to yield a 3:1 mixture of aldehydes **1** and **3**. Tambjamine D (**7**) was synthesized by treating the aldehyde **3** with isobutylamine in chloroform solution, removing water by using molecular sieves.

In order to show that the methoxyl group in each of the bipyrroles was not derived from the methanol extraction solvent, small samples of *Tambje* were extracted with anhydrous acetone shortly after collection. The ^{1}H NMR spectrum of the crude extracts contained signals due to

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(6) Rapoport, H.; Holden, K. G. *J. Am. Chem. Soc.* **1962**, *84*, 635.
(7) Jones, R. A.; Bean, G. P. "The Chemistry of Pyrroles"; Academic Press: New York, 1977.

Table I. Distribution of Bipyrroles 1-7 in *T. abdere*, *T. eliora*, *R. tigris*, Bryozoan *S. translucens*, the Exudate from *T. abdere*, and a Slime Trail Produced by *T. abdere*

	T. abdere	*T. eliora*	*R. tigris*	bryozoan	exudate	trail
no. of animals	90	120	70		1	2
dry weight, g	26.6	10.9	48.2	42.0		
crude extract	3.0 g	1.0 g	1.5 g	0.47 g	4.8 mg	3.1 mg/50 cm
aldehyde 1, mg	55	27	30	10		
aldehyde 2, mg	45	12	10	30		
aldehyde 3, mg	125	20	20	60		
Tambjamine A (4)	200 mg	58 mg	39 mg	25 mg	0.85 mg	0.06 μg/cm
Tambjamine B (5)	85 mg	30 mg	24 mg	35 mg	0.80 mg	0.02 μg/cm
Tambjamine C (6)	160 mg	26 mg	38 mg	10 mg	0.79 mg	0.13 μg/cm
Tambjamine D (7)	240 mg	61 mg	25 mg	20 mg	0.56 mg	0.15 μg/cm
total	910 mg	234 mg	186 mg	190 mg	3.0 mg	17.7 μg/50 cm
wt/animal, mg	10.1	1.96	2.65			
% dry weight	3.42	2.15	0.39	0.45		

the methoxyl groups but did not contain aldehyde proton signals. This implied that the aldehydes had been formed by hydrolysis of the corresponding enamines and that only the enamines should be considered as natural products.

The bipyrroles 1–7 were screened for antimicrobial activity by using the disk assay method and as inhibitors of cell division in the fertilized sea urchin egg assay.[8] Tests were performed on semipurified mixtures of the aldehydes 1–3 (1, 60%; 2, 30%; 3, 10%), the enamines 4 and 5 (4, 40%; 5, 60%), and the isobutylamines (6, 30%; 7, 70%). The aldehydes 1–3 showed no antimicrobial activity but inhibited cell division at 1 μg/mL in seawater. The enamines 4 and 5 inhibited cell division at 1 μg/mL and showed moderate antimicrobial activity at 50 μg/disk against *Eschericia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Vibrio anguillarum*. The isobutylamines 6 and 7 inhibited cell division at 1 μg/mL, showed antimicrobial activity against *Candida albicans*, *B. subtilis*, *S. aureus*, and *V. anguillarum* at 5 μg/disk, and showed mild activity against *E. coli* at 50 μg/disk.

Most compounds isolated from nudibranchs have been traced to a dietary origin. Since the tambjamines inhibited microbial growth, we were able to locate the dietary source of the tambjamines using an antimicrobial screen coupled with collecting observations. The tambjamines 4–7 are the major secondary metabolites of a green ectoproct *Sessibugula translucens*.[9] Since the bipyrroles turn green gradually on standing and more rapidly during chromatography, we suspect that the green pigments of *S. translucens* are dimers of the bipyrroles, related to the blue pigment recently isolated from a compound ascidian.[10] Brominated nitrogenous compounds have previously been isolated from an ectoproct, *Flustra foliacea*.[11]

When attacked by *Roboastra tigris*, *Tambje abdere* produced a yellow mucus from goblet cells in the skin[2]. The defensive secretion that often caused the *Roboastra* to break off its attack contained relatively large quantities of the tambjamines (Table I). *Tambje eliora* did not appear to produce a defensive secretion but attempted to escape from *R. tigris* by using a vigorous writhing motion. In laboratory experiments, *R. tigris* preferred to eat *T. eliora* rather than *T. abdere*. Having observed that *R. tigris* could follow the slime trails of the *Tambje* species, we analyzed the slime trail produced by *T. abdere* and found low concentrations of the tambjamines 4–7. The function of the tambjamines in the slime trails is unknown. However, the presence of the tambjamines in a defensive secretion suggests that they are used to repel most potential predators. This general purpose chemical defense mechanism can be breached by the specialist preator *Roboastra tigris* that detects the chemicals in the slime trail and uses them to track its preferred prey. However, at higher concentrations, the same compounds may still act as a deterrent to the predator. Similar properties have been observed for ant trail pheromones.[12]

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer. Ultraviolet spectra were measured on a Cary 219 double beam spectrophotometer. ^{1}H NMR spectra were measured on an instrument based on a 360-MHz Oxford narrow-bore magnet, a Nicolet 1180-E FT data system, and 293B pulse programmer; ^{13}C NMR spectra were measured on a Nicolet 200-MHz wide-bore FT spectrometer or a Varian CFT-20 spectrometer; all chemical shifts are reported with respect to Me_4Si (δ 0). High-resolution mass measurements were provided by the Bio-organic, Biomedical Mass Spectrometry Resource (A. L. Burlingame, Director), supported by NIH Grant RR00719, and low-resolution mass spectra were recorded on a Hewlett-Packard 5930A mass spectrometer. Melting points were measured on a Fisher-Johns apparatus and reported uncorrected. All solvents were either spectral grade or redistilled prior to use.

Collection Data. All specimens were collected by hand using SCUBA. Samples of *Tambje abdere*, *T. eliora*, and *Robastra tigris* were collected at Puerto Escondido, Baja California, Mexico (*T. abdere* and *T. eliora* only) in May 1980, at Bahia de los Angeles, Baja California, in May 1980 and April 1982, and at Isla Partida, Gulf of California (*T. eliora* only) in April 1982. The bryozoan was collected at Bahia de los Angeles and Isla Partida in April 1982. Samples were stored in chilled methanol or acetone. There was little or no variation in the chemical constituents of samples from different locations.

Typical Extraction and Chromatography Procedure. A total of 90 specimens of *Tambje abdere* (80-034) were soaked in methanol (1 L) at −10 °C for 6 months. The solvent was decanted, and the nudibranchs were washed with fresh methanol (1 L). The combined methanol extracts were evaporated to yield an aqueous suspension that was washed with dichloromethane (4 × 250 mL). The combined extracts were dried over sodium sulfate, and the solvent was evaporated to yield a khaki-colored gum (3.0 g, 11.3% dry weight, 33.3 mg/animal). *T. eliora*: 120 animals; 1.0 g of extract (9.2% dry weight, 8.3 mg/animal). *R. tigris*: 70 animals; 1.5 g of extract (3.1% dry weight, 21.4 mg/animal). Bryozoan: 42.0 g dry weight, 0.47 g of extract (1.12% dry weight).

The gum (3.0 g) was chromatographed on a column (110 × 2.5 cm diameter) of Sephadex LH-20 with methanol as the eluant to give seven major fractions as indicated by TLC. The seven fractions were assayed against *S. aureus* to yield three active

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(9) One hypothesis for the origin of bipyrroles in *S. translucens* is that they arise from the bipyrrole fragment of prodigiosin (8) produced by red marine bacteria of the genus *Benechea* (see: Giovannioni, S. J.; Margulis, L. *Microbios* 1981, *30*, 47) that are in the diet of the ectoproct.

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fractions. The last active fraction from the Sephadex column was separated by LC on μ-Porasil with 1:1 ether–dichloromethane as the eluant to yield 5′-bromo-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**2**; 45 mg, 0.17% dry weight, 0.5 mg/animal), 3′-bromo-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**3**; 125 mg, 0.47% dry weight, 1.39 mg/animal), and 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**1**; 55 mg, 0.21% dry weight, 0.61 mg/animal).

The fifth fraction from the initial separation was rechromatographed on Sephadex LH-20 with 1:1 methanol–dichloromethane as the eluant to yield four fractions, one of which was further chromatographed by LC on μ-Porasil with 20% 2-propanol in ethyl acetate to yield tambjamine A (**4**; 200 mg, 0.75% dry weight, 2.22 mg/animal) and tambjamine B (**5**; 85 mg, 0.32% dry weight, 0.94 mg/animal).

The fourth fraction from the initial separation was subjected to flash chromatography on TLC grade silica gel. The fraction eluted with 10% ethyl acetate in ether was further purified by LC on μ-Porasil with 1:1 ethyl acetate–dichloromethane to yield tambjamine D (**7**; 240 mg, 0.9% dry weight, 0.51 mg/animal). Similar treatment of the fraction eluted with 25% ethyl acetate in ether gave tambjamine C (**6**; 160 mg, 0.6% dry weight, 0.22 mg/animal).

The yields of material isolated from methanol extracts of *T. eliora*, *R. tigris*, and the bryozoan are given in Table I. The crude acetone extracts of all organisms were examined by ^{1}H NMR spectroscopy to determine that the aldehydes were not present, but no details of yields were obtained for these extracts.

4′-Methoxy-2,2′-bipyrrole-5-carboxaldehyde (1): mp. 263–265 °C dec; UV (MeOH) 364 nm (ϵ 6200), 252 (3600); IR ($CHCl_3$) 3325, 2975, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.93 (s, 3 H), 6.02 (d, 1 H, J = 2.5 Hz), 6.28 (m, 1 H), 6.67 (m, 1 H), 6.96 (m, 1 H), 9.19 (s, 1 H), 11.7 (br, 1 H), 11.9 (br, 1 H); ^{1}H NMR ($CDCl_3$ + D_2O) δ 3.93 (s, 3 H), 6.02 (s, 1 H), 6.28 (dd, 1 H, J = 3.5, 2.7 Hz), 6.67 (dd, 1 H, J = 3.5, 1.5 Hz), 6.96 (dd, 1 H, J = 2.7, 1.5 Hz), 9.19 (s, 1 H); high-resolution mass measurement, obsd m/z 190.0741, $C_{10}H_{10}N_2O_2$ requires 190.0742.

5′-Bromo-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (2): mp 235–238 °C dec; UV (MeOH) 364 nm (ϵ 7200), 256 (4700); IR ($CHCl_3$) 3325, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.94 (s, 3 H), 5.98 (d, 1 H, J = 2.4 Hz), 6.20 (dd, 1 H, J = 3.9, 2.6 Hz), 6.57 (dd, 1 H, J = 3.9, 2.6 Hz), 9.20 (s, 1 H), 11.8 (br, 1 H), 12.3 (br, 1 H); ^{1}H NMR ($CDCl_3$ + D_2O) δ 3.94 (s, 3 H), 5.98 (s, 1 H), 6.20 (d, 1 H, J = 3.9 Hz), 6.57 (d, 1 H, J = 3.9 Hz), 9.20 (s, 1 H); high-resolution mass measurement, obsd m/z 267.9841, $C_{10}H_9{}^{79}BrN_2O_2$ requires 267.9847.

3′-Bromo-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (3): mp 243–245 °C dec; UV (MeOH) 357 nm (ϵ 6800), 247 (3700); IR ($CHCl_3$) 3325, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3 H), 6.33 (t, 1 H, J = 2.7 Hz), 6.74 (d, 1 H, J = 2.6 Hz), 6.89 (t, 1 H, J = 2.7 Hz), 9.24 (s, 1 H), 11.7 (br, 1 H), 12.0 (br, 1 H); ^{1}H NMR ($CDCl_3$ + D_2O) δ 3.97 (s, 3 H), 6.33 (d, 1 H, J = 2.7 Hz), 6.74 (s, 1 H), 6.89 (d, 1 H, J = 2.7 Hz), 9.24 (s, 1 H); high-resolution mass measurement, obsd m/z 267.9846, $C_{10}H_9{}^{79}BrN_2O_2$ requires 267.9847.

Tambjamine A (4): oil; UV (MeOH) 397 nm (ϵ 20 000), 255 (4600); IR ($CHCl_3$) 3635, 3480, 1675, 1605, 1535 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3 H), 5.95 (s, 1 H), 6.30 (m, 1 H), 6.78 (m, 1 H), 7.09 (m, 1 H), 7.49 (m, 1 H), 9.20 (br, 2 H), 11.30 (br, 1 H); ^{1}H NMR ($CDCl_3$ + D_2O) δ 3.92 (s, 3 H), 5.95 (s, 1 H), 6.30 (dd, 1 H, J = 3.7, 2.6 Hz), 6.78 (dd, 1 H, J = 3.7, 1.3 Hz), 7.09 (dd, 1 H, J = 2.6, 1.3 Hz), 7.49 (s, 1 H); high-resolution mass measurement, obsd m/z 189.0894, $C_{10}H_{11}N_3O$ requires 189.0902.

Tambjamine B (5): oil; UV (MeOH) 397 nm (ϵ 20 000), 255 (4600); IR ($CHCl_3$) 3635, 3480, 1675, 1605, 1535 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3 H), 5.91 (s, 1 H), 6.23 (m, 1 H), 6.67 (m, 1 H), 7.51 (m, 1 H), 9.6 (br, 2 H), 12.10 (br, 1 H); ^{1}H NMR ($CDCl_3$ + D_2O) δ 3.91 (s, 3 H), 5.91 (s, 1 H), 6.23 (d, 1 H, J = 3.9 Hz), 6.67 (d, J = 3.9 Hz), 7.51 (s, 1 H); mass spectrum, m/z 269, 267 (peaks obscured by PFK standard in HRMS).

Tambjamine C (6): oil; UV (MeOH) 405 nm (ϵ 23 000), 258 (5700); IR ($CHCl_3$) 3200, 1665, 1610, 1530 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.03 (d, 6 H, J = 6.6 Hz), 2.03 (m, 1 H), 3.29 (m, 2 H), 3.93 (s, 3 H), 5.94 (s, 1 H), 6.28 (m, 1 H), 6.73 (m, 1 H), 7.07 (m, 1 H), 7.29 (d, 1 H, J = 15 Hz), 9.46 (br, 1 H), 10.7 (br, 1 H); ^{1}H NMR ($CDCl_3$ + D_2O) δ 1.03 (d, 6 H, J = 6.6 Hz), 2.03 (m, 1 H), 3.29 (d, 2 H, J = 6.3 Hz), 3.93 (s, 3 H), 5.94 (s, 1 H), 6.28 (m, 1 H), 6.73 (m, 1 H), 7.07 (m, 1 H), 7.29 (s, 1 H); high resolution mass measurement, obsd m/z 245.1536, $C_{14}H_{19}N_3O$ requires 245.1528.

Tambjamine D (7): oil; UV (MeOH) 401 nm (ϵ 23 000), 257 (6200); IR ($CHCl_3$) 3240, 1660, 1605, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.04 (d, 6 H, J = 6.6 Hz), 2.05 (m, 1 H), 3.32 (m, 2 H), 3.97 (s, 3 H), 6.33 (m, 1 H), 6.64 (s, 1 H), 7.00 (m, 1 H), 7.39 (d, 1 H, J = 14 Hz), 9.92 (br, 1 H), 11.26 (br, 1 H); ^{1}H NMR ($CDCl_3$ + D_2O) δ 1.04 (d, 6 H, J = 6.6 Hz), 2.05 (m, 1 H), 3.32 (d, 2 H, J = 6.8 Hz), 3.97 (s, 3 H), 6.33 (d, 1 H, J = 2.8 Hz), 6.64 (s, 1 H), 7.00 (d, 1 H, J = 2.8 Hz), 7.39 (s, 1 H); high-resolution mass measurement, obsd m/z 325.0600, $C_{14}H_{18}{}^{81}BrN_3O$ requires 325.0613.

Methylation of Bipyrroles 1–7. Methyl iodide (0.5 mL) was added to a solution of the pyrrole (~0.02 mmol) in dry acetone (10 mL) containing anhydrous potassium carbonate (500 mg), and the mixture was stirred at 80 °C under reflux for 18 h. The solvent was evaporated and the residue partitioned between water (10 mL) and dichloromethane (3 × 10 mL). The combined extracts were washed with water (2 × 10 mL) and dried over anhydrous sodium sulfate, and the solvent was evaporated to yield the corresponding *N,N′*-dimethyl-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde in 50–75% yield. 4-Methoxy-2,2′-bipyrrole-5-carboxaldehyde (**1**), tambjamine A (**4**), and tambjamine C (**6**) gave 1,1′-dimethyl-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**9**); 5′-bromo-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**2**) and tambjamine B (**5**) gave 5′-bromo-1,1′-dimethyl-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**10**); 3′-bromo-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**3**) and tambjamine D (**7**) gave 3′-bromo-1,1′-dimethyl-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**11**).

1,1′-Dimethyl-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (9): oil; UV (MeOH) 330 nm (ϵ 16 000); IR ($CHCl_3$) 2720, 1635, 1530 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.57 (s, 3 H), 3.75 (s, 3 H), 3.85 (s, 3 H), 5.79 (s, 1 H), 6.24 (m, 1 H, J = 3.6, 1.5 Hz), 6.25 (m, 1 H, J = 3.6, 2.5 Hz), 6.77 (dd, 1 H, J = 2.5, 1.5 Hz), 9.65 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 175.7 (d), 158.8 (s, C-4), 133.4 (s, C-5), 124.4 (d, C-5′), 122.6 (s, C-2′), 118.5 (s, C-2), 112.1 (d, C-3), 108.2 (d, C-4′), 95.6 (d, C-3′), 57.8 (q), 34.7 (q), 34.2 (q); mass spectrum, m/z 218.

5′-Bromo-1,1′-dimethyl-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (10): oil; UV (MeOH) 327 nm (ϵ 20 000); IR ($CHCl_3$) 1635, 1530 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.48 (s, 3 H), 3.72 (s, 3 H), 3.85 (s, 3 H), 5.79 (s, 1 H), 6.24 (d, 1 H, J = 3.8 Hz), 6.26 (d, 1 H, J = 3.8 Hz), 9.66 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 175.9 (d), 158.6 (s, C-4), 132.8 (s, C-5), 123.7 (s, C-2′), 118.6 (s, C-2), 112.5 (d, C-3), 110.7 (d, C-4′), 105.3 (s, C-5′), 96.0 (d, C-3′), 57.8 (q), 43.0 (q), 33.6 (q); mass spectrum, m/z 270, 268 (M^+).

3′-Bromo-1,1′-dimethyl-4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (11): oil; UV (MeOH) 311 nm (ϵ 21 000); IR ($CHCl_3$) 1640, 1530 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.48 (s, 3 H), 3.69 (s, 3 H), 3.87 (s, 3 H), 5.85 (s, 1 H), 6.26 (d, 1 H, J = 2.8 Hz), 6.72 (d, 1 H, J = 2.8 Hz), 9.70 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 176.3 (d), 158.4 (s, C-4), 130.6 (s, C-5), 124.2 (d, C-5′), 121.0 (s, C-2′), 118.9 (s, C-2), 111.1 (d, C-3), 99.8 (d, C-4′), 96.8 (s, C-3′), 57.8 (q), 35.6 (q), 34.2 (q); high-resolution mass measurement, obsd m/z 298.0154, $C_{12}H_{13}{}^{81}BrN_2O_2$ requires 298.0140.

Hydrogenation of Aldehydes 10 and 11. A solution of either aldehyde **10** or **11** (15 mg, 0.05 mmol) in anhydrous ether (10 mL) containing 10% palladium on charcoal catalyst (2 mg) was stirred at 25 °C under an atmosphere of hydrogen for 24 h. The catalyst was removed by filtration and the solvent removed under vacuum to yield aldehyde **9** (9 mg, 82% theoretical), identical in all respects with authentic material.

Hydrolysis of Tambjamines A–D (4–7). A solution of a 3:1 mixture of enamines **4** and **5** (100 mg) in aqueous methanolic potassium hydroxide solution (10 mL; 1 mL of 5% potassium hydroxide solution in 9 mL methanol) was stirred at 25 °C for 1 h. The solution was diluted with water (10 mL) and the methanol evaporated to yield an aqueous suspension that was extracted with dichloromethane (3 × 20 mL). The combined extracts were dried over anhydrous sodium sulfate, and the solvent was evaporated to yield a 3:1 mixture of aldehydes **1** and **2** (65 mg), as judged by LC and ^{1}H NMR spectroscopy.

Under identical conditions, a 3:1 mixture of isobutylamines **6** and **7** (25 mg) gave a 3:1 mixture of the aldehydes **1** and **3** (15 mg).

Conversion of Aldehyde 3 into Tambjamine D. A solution of the aldehyde 3 (4.7 mg) and isobutylamine (1 drop) in chloroform (5 mL) was stirred at 22 °C over molecular sieves (Type 3A pellets) for 2 h. The reaction product was filtered through silica gel with ethyl acetate as the eluant to yield tambjamine D (7; 3.0 mg, 53% theoretical), identical in all respects with the natural product.

LC Analysis of Tambjamines A–D (4–7) in *T. abdere* Exudate and Slime Trail. A specimen of *R. tigris* was allowed to attack an average sized specimen of *T. abdere* in a dish containing "Instant Ocean" synthetic seawater (100 mL). The *Tambje* exuded copious amounts of a yellow exudation from glands all over the dorsal surface. The animals were separated and removed. The dish and its contents were extracted with dichloromethane (3 × 75 mL), the combined extracts were dried over anhydrous sodium sulfate, and the solvent was removed to yield a green oil (4.8 mg).

The concentrations of the tambjamines A–D (4–7) were determined by analytical LC by using known concentrations of pure compounds as standards. LC on an Alltech Spherisorb 5-μm C18-ODS column by using a linear gradient from 20% to 75% acetonitrile in 0.05 M pyridinium acetate buffer (pH 5.0) gave good separation of tambjamines A–D (retention times: A, 20.5 min; B, 13.5 min; C, 55.5 min; D, 61.0 min) that were detected by their UV absorption at 400 nm. Standard response curves of concentration vs. peak area (height × $W_{1/2}$) for each pure compound were used to calculate concentrations of the tambjamines in the exudate and slime trail (see Table I).

Two specimens of *T. abdere* were allowed to crawl over a bed of aquarium dolomite that had been washed with water, dichloromethane, deionized water, and synthetic seawater. The trials were marked with colored dolomite, the animals were carefully removed, and the dolomite on which the trail was laid was scooped up with a "lab scoop" spatula. The dolomite was washed with dichloromethane (3 × 200 mL), the combined extracts were dired over sodium sulfate, and the solvent was evaporated to yield a crude extract (3.1 mg) that was analyzed for tambjamines A–D as before (see Table I).

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Registry No. 1, 10476-41-2; 2, 85849-98-5; 3, 85849-99-6; 4, 85850-00-6; 5, 85850-01-7; 6, 85850-02-8; 7, 85850-03-9; 9, 85850-04-0; 10, 85850-05-1; 11, 85850-06-2; isobutylamine, 78-81-9.

Intramolecular Alkylation Route to the Bicyclo[3.3.1]nonane Ring System. A Total Synthesis of *dl*-Clovene

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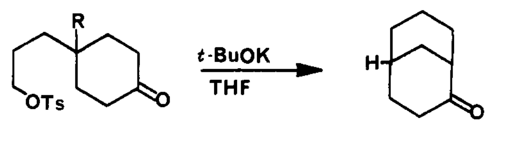
A formal total synthesis of *dl*-clovene (**9**) is described. The synthesis is highlighted by the efficient intramolecular alkylation of trisubstituted cyclohexenone **11b** to give 5-(2-ethylallyl)-1-methylbicyclo[3.3.1]non-2-en-4-one (**13b**) in 80% isolated yield. The preparation of **11b** follows an alkylation route starting with the enol ether **3** of cyclohexane-1,3-dione.

In 1966, Marvell and co-workers reported the first synthesis of a bicyclo[3.3.1]nonane by intramolecular enolate alkylation; e.g., **1a** → **2**.[1] The preparation of **1a** begins

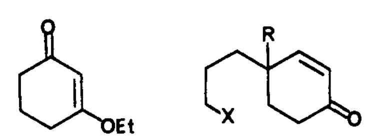


1a, R = H **2**

b, R = alkyl, aryl

by *p*-cyanoethylation of phenol and requires nine experimental steps. This strategy might not be readily adapted to synthesis of derivatives **1b** which have geminal ring disubstitution.[2]

The highly flexible 4,4-disubstituted cyclohexane ring synthesis developed by Stork and Danheiser[3] seems well suited to the preparation of cyclohexenones of type **4** from enol ether **3** of cyclohexane-1,3-dione. Cyclization of **4** would then provide bicyclo[3.3.1]nonanes with bridgehead substitution.



3 **4**

Cargill and Jackson report[4] that bicyclic enones such as **5a** give tricyclic enones (e.g., **6**) on internal α′-enolate alkylation.[5] This work was extended by Piers and coworkers to cyclizations of **5b,c**.[6] The Piers study is significant because experimental conditions were developed for nearly exclusive α′-alkylation to give α,β-enone **7** and α-alkylation to give β,γ-enone **8**.

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