The remaining prominent ^1H NMR signals were at δ 3.68 (s, 3 H) and 5.21 (s, 2 H) assigned to a methyl ester and an $ArCH_2OCO$ group that must be incorporated into a lactone ring since the carbonyl signal is at δ 170.8 in the ^{13}C NMR spectrum (Table I). A two-dimensional ^{13}C–^1H correlation experiment (COLOC)[4] revealed three-bond couplings from H-16 to C-12, C-14 and C-15, from COO-CH_3 to C-6, from H-12 to C-14, from H-11 to C-13, and C-8 and from CH_3-20 and H-5 to C-9. These data require that the lactone ring be attached to the aromatic ring with the methylene group at C-13 and the carbonyl group at C-14. Although the stereochemistry at C-7 is not rigid as in aplysulphurin (4) it is significant that membranolide shows similar nuclear Overhauser enhancements between H_3-20 and H-7 and between CH_3-18 and CH_3-17 suggesting that both molecules have the same geometry at C-7 and adopt similar conformations in solution. The relative stereochemistry of membranolide (2) was determined by reduction of 2 with lithium aluminum hydride in ether to obtain the triol 5 that was dehydrated using *p*-toluenesulfonic acid in benzene to obtain the known isobenzopyran 6[5] as the only product. Membranolide (2) must therefore have the 7*R**,10*S** relative stereochemistry.

Both 9,11-dihydrogracilin A (1) and membranolide (2) inhibited the growth of *Bacillus subtilis* at 100 μg/disk and membranolide (2) was also mildly active against *S. aureus*. We have not been able to assay the effects of either compound on the major Antarctic spongivores, the sea stars *Perknaster fuscus antarticus* and *Acodontaster conspicuus*.[1] However, there is an increasing body of circumstantial data to suggest that the spongian diterpenes are distasteful to all but specialized nudibranch predators.

Experimental Section

Two collections of *Dendrilla membranosa* were obtained from different sites [-40 m] at McMurdo Sound, Antarctica, and were stored separately in 2-propanol for 6 months. Each collection was examined separately as follows. The 2-propanol was decanted, the solvent evaporated, and the residue partitioned between dichloromethane and water. The dichloromethane extract was dried over sodium sulfate and the solvent evaporated. The smaller sample of *D. membranosa* (82-102B, 1.4 g dry weight) gave a brown oil (169 mg) that was purified by flash chromatography on silica gel (40–63 μm) using a hexane–ether gradient elution followed by LC on Partisil using 1:1 ether/hexane as eluant to obtain 9,11-dihydrogracilin A (1, 33 mg, 2.3% dry weight) as a colorless oil.

The larger sample of *D. membranosa* (85-102A, 30.1 g dry weight) gave a dichloromethane extract (1.77 g) that inhibited the growth of *S. aureus* and *B. subtilis*. The extract was chromatographed on Sephadex LH-20 using 1:1 dichloromethane/methanol as eluant and the active fractions were combined. The active material was purified by flash chromatography on silica gel using first a hexane–ethyl acetate gradient and then ether–hexane (1:1) to obtain membranolide (2, 65 mg, 0.2% dry weight) as a colorless oil.

9,11-Dihydrogracilin A (1): $[\alpha]_D$ -11.0° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 1740, 1235, 990, 935 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 0.89 (s, 3 H), 0.96 (s, 3 H), 1.03 (s, 3 H), 1.16 (br d, 1 H, *J* = 14 Hz), 1.65 (d, 3 H, *J* = 6.9 Hz), 1.85 (m, 1 H), 2.08 (s, 3 H), 2.10 (s, 3 H), 2.36 (ddd, 1 H, *J* = 9, 7.7, 7.5 Hz), 2.42 (dd, 1 H, *J* = 6.8, 4.2 Hz), 3.14 (dd, 1 H, *J* = 7.5, 5.5 Hz), 5.65 (q, 1 H, *J* = 6.9 Hz), 5.97 (s, 1 H), 6.44 (d, 1 H, *J* = 5.5 Hz); ^{13}C NMR (50.3 MHz, $CDCl_3$) see Table I; EIMS, *m/z* (relative intensity) 272 (M^+ - 2 AcOH, 3), 208 (7), 148 (100), 125 (27), 83 (18), 69 (69), 57 (14); CIMS (NH_3), *m/z* (relative intensity) 410 ($M + NH_4^+$, 44), 290 ($M + NH_4^+$ - 2AcOH, 100); HRMS, obsd *m/z* 272.2122, $C_{19}H_{28}O$, (M - 2AcOH) requires 272.2141.

Membranolide (2): $[\alpha]_D$ -28.8° (*c* 2.25, $CHCl_3$); IR ($CHCl_3$) 1753, 1737, 1597 cm^{-1}; UV (MeOH) 211 nm (ϵ 20 300), 231 (5800), 285 (1600), 293 (1600); ^1H NMR (360 MHz, $CDCl_3$) δ 0.47 (s, 3 H), 0.94 (s, 3 H), 1.37 (s, 3 H), 1.53 (d, 1 H, *J* = 14 Hz), 1.75 (d, 3 H, *J* = 7 Hz), 2.08 (br d, 1 H, *J* = 14 Hz), 2.28 (br d, 1 H, *J* = 14 Hz), 3.68 (s, 3 H), 4.61 (q, 1 H, *J* = 7 Hz), 5.21 (s, 2 H), 7.31 (d, 1 H, *J* = 8.3 Hz), 7.81 (d, 1 H, *J* = 8.3 Hz); ^{13}C NMR (50.3 MHz, $CDCl_3$) see Table I; EIMS, *m/z* (relative intensity) 344 (M^+, 9), 313 (28), 312 (100), 297 (41), 284 (11), 269 (19), 230 (13), 229 (77), 228 (12), 219 (16), 213 (31), 201 (25), 200 (20), 189 (18), 187 (13), 83 (13), 69 (35), 57 (18), 55 (14); HRMS, *m/z* 344.1985, $C_{21}H_{28}O_4$ requires 344.1988.

Conversion of Membranolide (2) into Isobenzopyran 6. A solution of membranolide (2, 19 mg, 0.055 mmol) in dry ether (2.0 mL) was added to a stirred suspension of lithium aluminum hydride (24 mg) in dry ether (4.0 mL) under an atmosphere of dry nitrogen. The mixture was heated under reflux for 1 h, cooled to 0 °C, and quenched with ethyl acetate, followed by 2 M hydrochloric acid (3 mL). The mixture was extracted with ethyl acetate (2 × 10 mL), and the combined organic extracts were washed with dilute sodium bicarbonate solution (2 mL), dried over Na_2SO_4, and evaporated to give a clear oil. The oil was purified by chromatography on silica gel (1:1 ethyl acetate/hexanes, then 1:10:10 2-propanol/ethyl acetate/hexanes) to obtain the triol 5 (9.3 mg, 52%): IR ($CHCl_3$) 3600–3400 (br), 3010, 2880, 1600 cm^{-1}; UV (MeOH) 218 nm (δ 7500), 269 nm (290), 270 nm (190); ^1H NMR ($CDCl_3$) δ 0.37 (s, 3 H), 0.90 (s, 3 H), 1.43 (s, 3 H), 1.44 (d, 3 H, *J* = 6.8 Hz), 2.16 (d, 1 H, *J* = 14.5 Hz), 2.28 (bd, 1 H, *J* = 15 Hz), 3.86 (dd, 1 H, *J* = 11, 7.9 Hz), 3.94 (dd, 1 H, *J* = 11, 8.1 Hz), 4.13 (ddq, 1 H, *J* = 8.1, 7.9, 6.8 Hz), 4.57 (d, 1 H, *J* = 11.9 Hz), 4.79 (d, 1 H, *J* = 12.6 Hz), 4.82 (d, 1 H, *J* = 11.9 Hz), 4.85 (d, 1 H, *J* = 12.6 Hz), 7.18 (d, 1 H, *J* = 8.3 Hz), 7.42 (d, 1 H, *J* = 8.3 Hz); EIMS, (relative intensity) 302 ($M^+ - H_2O$, 20), 284 (17), 273 (16), 272 (58), 271 (100), 257 (15), 243 (43), 42 (18), 241 (88), 176 (38), 175 (25), 171 (21), 161 (22), 157 (26), 151 (27), 69 (32), 55 (16); HRMS, *m/z* 302.2242, $C_{20}H_{30}O_2$ requires 302.2245.

A solution of the triol 5 (0.8 mg) in benzene (0.5 mL) was stirred with a small crystal of *p*-toluenesulfonic acid at 30 °C for 19 h. TLC showed two spots due to the presence of a trace of starting material and a single new nonpolar compound. The mixture was eluted through a short column of silica gel with 1:1 ethyl acetate/hexanes to afford the isobenzopyran 6 (0.2 mg), which had identical IR and NMR data with those reported by Karuso et al.[5]

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PD 125375, a Novel Metabolite Coproduced with Tomaymycin

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In our search for new antitumor agents, a *Streptomyces* sp. was found that produces the known antibiotic tomaymycin (**1a**)[1] and oxotomaymycin (**1b**).[2] During the course of isolating these two compounds, a third metabolite, PD

(1) Arima, K.; Kohsaka, M.; Tamura, G.; Imanaka, H.; Sakai, H. *J. Antibiot.* **1972**, *25*, 437.
(2) Kariyone, K.; Yazawa, H.; Kohsaka, M. *Chem. Pharm. Bull.* **1971**, *19*, 2289.

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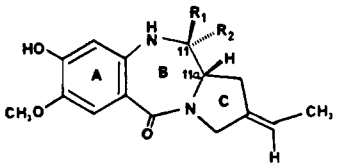

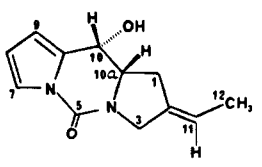

1a R_1 = H, R_2 = OCH_3

1b R_1R_2 = O

2

125375, was obtained. This new compound is devoid of antitumor and antimicrobial activity. The current interest[3] in tomaymycin, a highly potent antitumor agent, prompted us to examine PD 125375 to determine if it is structurally related to tomaymycin.

Results and Discussion

PD 125375 is a neutral, optically active compound with a molecular weight of 218 corresponding to $C_{12}H_{14}N_2O_2$. Its ^{13}C NMR spectrum (Table I) exhibits a single carbonyl signal (at δ 158.3) and six olefinic/aromatic carbon signals. Four of these carbon atoms bear one proton. Additional signals show that one methyl, two methylene, and two methine groups account for the remaining carbon atoms. The methylene carbon signals at δ 49.4 and 29.4 are consistent with $-CH_2N<$ and $-CH_2C=C<$, respectively. The ^1H NMR spectrum (Table I) of PD 125375 reveals the presence of a vinylic methyl group at δ 1.71 coupled to a vinyl proton at δ 5.52. The remaining ^1H NMR signals correspond to one exchangeable proton, and two methinyl protons (signals at δ 5.38 and 4.08), in addition to the two methylene groups mentioned above. The methinyl protons are coupled to each other and the broad O-methinyl proton signal at δ 5.38 sharpens after exchange with D_2O. The above spectral information indicated that PD 125375 has the same structural unit as the ring C portion of tomaymycin (**1a**). However, PD 125375 has three aromatic protons which are all part of the same spin system (signals at δ 5.94, 6.32, and 6.85; $J < 4$ Hz) and are not present in the tomaymycin structure. Instead, these signals suggested the presence of a substituted pyrrole bearing three adjacent protons which led to the tentative assignment of structure **2** to PD 125375.

The placement of the hydroxyl group (absorbing in the IR at 3200 cm^{-1}) at C-10 was established by ^1H–^1H homonuclear correlation (COSY) experiments which, in addition to a strong correlation between H-10 and H-10a, clearly revealed four-bond correlations between the protons at C-1, C-3, and C-11.[4] A correlation was also observed between H-3 and H-10a. No correlations between H-10 and the vinyl or H-3 protons were observed. The relative stereochemistry of PD 125375 at C-10 and C-10a was determined by a ^1H-^1H NOE correlated (NOESY) experiment. A strong NOE correlation was found between H-10 and H-10a which establishes a cis relationship between these protons. An NOE correlation was also observed between H-10 and one of the C-1 protons. From the above information, the carbonyl group, absorbing in the IR at 1630 cm^{-1}, must be at position-5, providing a cyclic urea functionality. The remaining spectral assignments (Table I) for PD 125375 were made by examining the data obtained from a ^1H–^{13}C heteronuclear correlation experiment.

Table I. NMR Data for PD 125375 (2) in $CDCl_3$[a]

position	^{13}C	^1H
1	29.4 t	2.80 dddq (15.5, 8.5, 1.5, 0.7)
		2.94 dddq (15.5, 10.0, 2.5, 2.0)
2	122.6 s	
3	49.4 t	4.03 ddq (15.2, 0.3, 2.0)
		4.28 ddq (15.2, 2.1, 2.0)
5	158.3 s	
7	122.7 d	6.85 dd (2.7, 1.5)
8	110.8 d	5.94 dd (3.9, 2.7)
9	113.0 d	6.32 dd (3.9, 1.6)
9a	133.4 s	
10	76.9 d	5.38 br d
10a	61.3 d	4.08 ddd (7.4, 7.4, 3.1)
10-OH		6.62 d (5.5)
11	117.9 d	5.52 ddq (6.9, 3.2, 1.9)
12	14.5 q	1.71 m (6.9, 2.0)

[a] Chemical shifts (δ) are in ppm downfield from internal Me_4Si. J values, in parentheses, are in hertz. See Experimental Section for details.

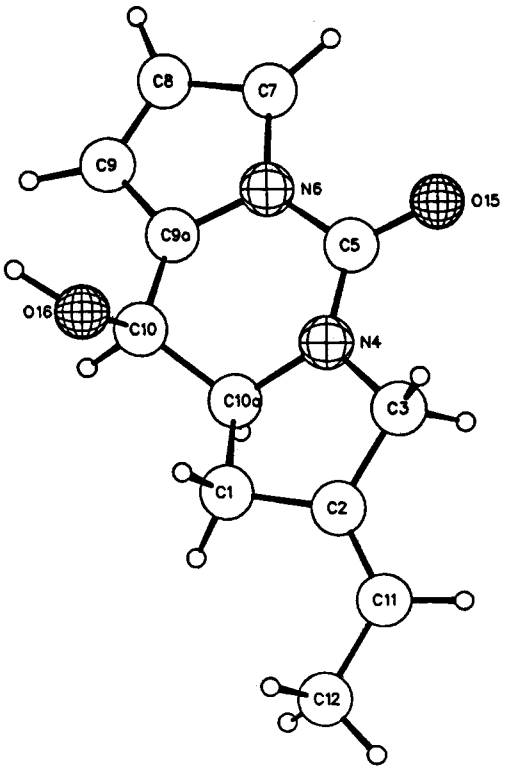

Figure 1. Computer-generated perspective drawing of the final X-ray model of PD 125375.

The assignment of structure **2** to PD 125375 was confirmed by single-crystal X-ray diffraction analysis. A computer-generated perspective drawing of the final X-ray model of PD 125375 is shown in Figure 1. This analysis verified the cis relationship of H-10 and H-10a and established the *E* configuration for the exocyclic double bond. These same stereochemical features are also present in tomaymycin. The X-ray analysis defined only the relative configuraton, so the enantiomer shown was arbitrarily selected to agree with the 11*R*,11a*S* stereochemistry assigned to tomaymycin.[5]

The biosynthesis of tomaymycin and related benzodiazepine antibiotics has been elucidated by Hurley and his co-workers.[6] Although the novel structure of PD 125375 includes the 3-ethylidinepyrrolidine moiety present

(3) Thurston, D. E.; Langley, D. R. *J. Org. Chem.* **1986**, 51, 705.

(4) The numbering system is based on the systemic name for the enantiomer shown by structure **2** which is (2*E*,10*R*,10a*S*)-2-ethylidine-2,3,10,10a-tetrahydro-10-hydroxy-1*H*,5*H*-dipyrrolo[1,2-*c*:2′1′-*f*]pyrimidin-5-one.

(5) Tozuka, Z.; Takaya, T. *J. Antibiot.* **1983**, *36*, 142.

(6) Hurley, L. H.; Thurston, D. E. *Pharm. Res.* **1984**, 52. Hurley, L. H. *Acc. Chem. Res.* **1980**, *13*, 263.

in tomaymycin, the biosynthetic pathway leading to PD 125375 is obscure.

Experimental Section

General Methods. ^{13}C and ^{1}H data were obtained at 75.4 and 300 MHz, respectively, on a Varian XL-300 NMR spectrometer. Quadrature mode detection was used for all NMR experiments. Data processing was performed with the Motorola VM02-based data system. COSY and NOESY spectra were obtained by recording a data matrix of 256 × 256 complex points. A spectral width of 1774.0 Hz was used in both domains. An incremented mixing period was used in the NOESY experiment to reduce the intensity of J-correlated cross peaks.[7] Pseudo-echo weighting was performed along both dimensions prior to Fourier transformation. The 2D data were symmetrized to remove false peaks. HETCOR spectra were obtained by recording a data matrix of 64 × 1024 complex points. Spectral widths of 1774.0 Hz and 11351.0 Hz were used along the t_1 (^{1}H) domain and the t_2 (^{13}C) domain, respectively. Exponentional broadening was applied along both dimensions. Prior to Fourier transformation the t_1 and t_2 domains were zero-filled to 128 points and 2048 points, respectively. All spectra were converted to an absolute value mode and then a contour-type plot was made. Carbon multiplicities were determined by performing a DEPTGL[8] experiment. The optical rotation of PD 125375 was measured with a Perkin-Elmer Model 141 polarimeter; the infrared spectrum was recorded on a Nicolet SX-60 FTIR spectrometer; and ultraviolet spectra were obtained with an IBM Model 9420 UV spectrometer. Chromatographic separations were monitored by HPLC using a Waters Assoc. μBondapak C-18 column (0.4 × 30 cm) and 0.05 M pH 6.8 NH_4OAc buffer-MeCN (65:35) as the mobile phase. At a flow rate of 1.5 mL/min, the retention times of oxotomaymycin, PD 125375, and tomaymycin are about 3.3, 3.6, and 5.5 min, respectively.

Isolation of PD 125375 (2). Fermentation broth (24 L) was extracted with 1-butanol and the organic layer was concentrated in vacuo to afford 16 g of a residue. This product was chromatographed over alumina (300 g) using absolute ethanol, EtOH–H_2O (95:5), and MeOH–H_2O (80:20). The latter two eluents afforded, respectively, tomaymycin (127 mg) and oxotomaymycin (222 mg) as crystalline solids.[9] The residue (2.0 g) obtained from the initial absolute ethanol fractions was dissolved in 4 mL of MeOH–H_2O (1:1) and chromatographed over 160 g of C-18 silica gel using MeCN–H_2O (15:85) as the mobile phase. Several 200-mL fractions were collected and each was analyzed by HPLC. PD 125375 was present in fractions 8–11 (800 mL) which were combined and concentrated to 500 mL. Two extractions with 500-mL portions of ethyl acetate afforded 0.52 g of a partially crystalline residue. Recrystallization of this material from ethyl acetate yielded 232 mg of PD 125375 as colorless needles: mp 181–183 °C; MS, m/e 218 (M^+); UV λ^{MeOH}_{max} nm (ϵ) 231 (9505), 273 (12 100); IR ν_{max} (CCl_4) cm^{-1} 3200, 1630, 1560, 1475, 1440; $[\alpha]_D$ +89.8° (c 0.52, MeOH); NMR data are listed in Table I. Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.83. Found: C, 65.82; H, 6.33; N, 12.53.

Single-Crystal X-ray Diffraction Analysis of PD 125375. Preliminary X-ray photographs of **2** displayed orthorhombic diffraction symmetry, and accurate lattice constants of a = 12.479 (4), b = 12.421 (2), and c = 7.057 (1) Å were determined from a least-squares fit of 15 diffractometer measured 2θ values. Systematic extinctions, optical activity, and an approximate density of 1.33 g/cm^3 were uniquely accommodated by space group $P2_12_12_1$ with 1 molecule of formula $C_{12}H_{14}N_2O_2$ forming the asymmetric unit. All unique diffraction maxima with $2\theta < 114°$ were measured by using graphite-monochromated Cu Kα radiation (1.54178 Å) and variable speed, 1° ω-scans. Of the 885 reflections measured in this way, 732 (83%) were judged observed ($F_o > 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects.[10] The structure was solved routinely by using direct methods and all non-hydrogen atoms were clearly visible in the initial E-synthesis. Hydrogen atoms were located in a ΔF-synthesis following partial refinement. Block-diagonal least-squares refinements have converged to a conventional crystallographic residual of 0.086 for the observed data. Additional crystallographic details are available and are described in the paragraph entitled Supplementary Material Available at the end of this paper.

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Supplementary Material Available: Tables of fractional coordinates, thermal parameters, interatomic distances, and interatomic angles for PD 125375 (5 pages). Ordering information is given on any current masthead page.

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(10) All crystallographic calculations were done on a PRIME 9950 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were REDUCE and UNIQUE, data reduction programs by M. E. Leonowicz, Cornell University, 1978; MULTAN 80 and RANTAN 80, systems of computer programs for the automatic solution of crystal structures from X-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, University of York, England, 1980; BLS78A, an anisotropic block-diagonal least-squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a locally modified crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu and G. Van Duyne, Cornell University, 1985.

New Organocuprate-Induced Reduction of the Enol Phosphate Moiety in 1-[(Diethoxyphosphinyl)oxy]-*F*-1-alkene-1-phosphonates: An Efficient Synthesis of (*Z*)-1-Hydryl-*F*-1-alkene-1-phosphonates

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The replacement of an enol ester function, such as enol acetates, triflates, and phosphates, with a hydrogen or an alkyl group is an important reaction in organic synthesis. In the literature, there exist several methods for such a transformation, which involve a transition-metal-catalyzed cross-coupling reaction,[1] organocopper-mediated reaction,[2]

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