Three New Pseudodistomins, Piperidine Alkaloids from the Ascidian *Pseudodistoma megalarva*

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Received February 28, 1997[⊗]

Bioassay-guided fractionation of the MeOH−CH_2Cl_2 extract of the Micronesian ascidian *Pseudodistoma megalarva* yielded three new piperidine alkaloids, pseudodistomins D−F (**3**−**5**) and the previously reported pseudodistomins B and C (**1** and **2**). The structure and stereochemistry of these compounds were established by interpretation of spectral data. Pseudodistomins B−F were found to be active in a cell-based assay for DNA damage induction, but the activity was due to an alternative mechanism.

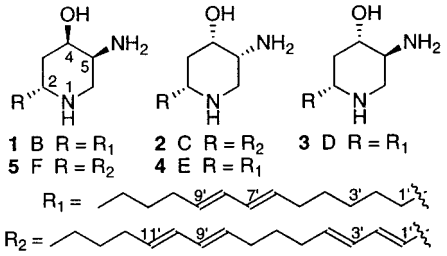
Piperidine alkaloids are among the most abundant metabolites of terrestrial plants,[1] but there are relatively few examples isolated from marine organisms. To our knowledge, there are only two reports to date of the isolation and identification of piperidine alkaloids from marine organisms. One of these reports described pseudodistomins A and B (**1**), which were isolated from the Okinawan tunicate *Pseudodistoma kanoko*.[2] Their proposed structures were revised,[3] and their absolute configurations[4] were subsequently determined. More recently, Kobayashi *et al*.[5] reported the isolation and synthesis of pseudodistomin C (**2**) from the same tunicate.

Because effective antitumor agents can act through many mechanisms which result in DNA damage, we initiated a high throughput screen to evaluate natural product extracts in a yeast-based assay for DNA damaging activity.[6] An extract of the ascidian *Pseudodistoma megalarva* Monniot and Monniot (Polyclinidae) collected in Palau, showed differential activity in a DNA repair-deficient yeast mutant and was therefore selected for fractionation. We now report the isolation and structure determination of three new piperidine alkaloids, pseudodistomin D−F (**3**−**5**), which were found in the presence of the known alkaloids pseudodistomins B (**1**) and C (**2**).

The freeze-dried tunicate was extracted with MeOH−CH_2Cl_2, and the resulting extract was chromatographed on a Sephadex LH-20 column to yield several fractions that demonstrated potent activity in DNA repair-deficient yeast mutants. Further purification of these active fractions by extensive preparative TLC led to the isolation of pseudodistomins B−F (**1**−**5**). The identities of pseudodistomins B (**1**) and C (**2**) were determined by complete spectral analysis and comparison of the spectral data with literature data.[2−5]

Results and Discussion

Pseudodistomin D (**3**), $[\alpha]_D$ +5°, was isolated as a colorless gum and possessed a molecular weight of 294 Da, which corresponded to a molecular formula of $C_{18}H_{34}N_2O$, indicating that it was an isomer of **1** with three degrees of unsaturation. An ND_3 DCIMS indicated that there was a total of four exchangeable hydrogens. The IR spectrum contained a strong hydroxyl band at 3260 cm^{-1}, and the UV absorption at 234 nm confirmed the presence of a conjugated diene in the side chain of **3**, similar to that found in **1**.



As with compound **1**, the MeOH-d_4 ^{1}H-NMR spectrum of **3** (see Table 1) contained four contiguous olefinic multiplets resonating between δ 5.96 and δ 5.53, and the ^{13}C-NMR spectrum (see Table 2) showed four corresponding tightly spaced olefinic methine carbons between δ 133.1 and δ 131.8, thus accounting for the presence of two double bonds. The absence of any further olefinic signals indicated that the final degree of unsaturation was due to the piperidine ring. COSY and HMBC data confirmed that the conjugated double bonds were situated in a 13 carbon straight chain as was the case for compound **1**.

The piperidine ring contained five aliphatic carbon atoms, three of which were methines and two of which were methylenes, and these signals were easily recognized by their chemical shifts and correlation data. The COSY and HMQC data confirmed the ring assignments with H-2 at δ 2.50, methylene H-3 at δ 1.96 and δ 1.07, H-4 at δ 3.20, H-5 at δ 2.50, and methylene H-6 at δ 3.04 and δ 2.31. The chemical shift of the C-4 methine carbon at δ 75.5 indicated that it bore a hydroxyl substituent, while two of the methine (C-2 and C-5) and one of the methylene (C-6) carbons possessed chemical shifts between δ 51.9 and δ 56.7, suggesting that they were attached to nitrogen atoms. Because the molecular formula indicated the presence of a total of two nitrogen atoms, one oxygen atom, and four exchangeable hydrogens, the C-5 methine bore a primary amine substituent, and C-2 and C-6 were attached to the same nitrogen atom within the piperidine ring. The H-2 multiplet on the piperidine ring also shared COSY and HMBC correlations to H-1′ and C-1′ in the side chain,

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[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.

S0163-3864(97)00143-2 CCC: $14.00

Table 1. ^{1}H-NMR Assignments for Pseudodistomins B (**1**), D (**3**), E (**4**), and F (**5**) in CD_3OD at 400 MHz

^{1}H	pseudodistomin B (**1**)	pseudodistomin D (**3**)	pseudodistomin E (**4**)	pseudodistomin F (**5**)
2	2.87 (m)	2.50 (m)	2.44 (m)	3.45 (m)
3	1.90 (ddd, 4.6, 4.6, 13.8)	1.96 (ddd, 4.6, 4.6, 12.1)	1.68 (dddd, 0.8, 2.7, 4.0, 12.7)	1.84 (ddd, 2.7, 4.0, 14.0)
	1.30 (ddd, 2.4, 11.3, 13.8)	1.07 (ddd, 11.1, 11.3, 12.1)	1.21 (ddd, 11.4, 11.7, 12.7)	1.50 (ddd, 2.5, 11.6, 14.0)
4	3.91 (m)	3.20 (ddd, 4.6, 9.5, 11.1)	3.67 (ddd, 4.0, 4.8, 11.7)	3.91 (m)
5	2.74 (m)	2.50 (m)	2.90 (m)	2.69 (ddd, 2.4, 5.1, 9.8)
6	2.78 (dd, 10.9, 12.1)	3.04 (dd, 4.5, 12.1)	2.98 (dd, 2.5, 13.3)	2.77 (dd, 9.8, 11.0)
	2.72 (dd, 4.5, 12.1)	2.31 (dd, 10.9, 12.1)	2.73 (dd, 2.5, 13.3)	2.73 (dd, 5.1, 11.0)
1′	1.35 (2H, m)	1.35 (2H, m)	1.35 (2H, m)	5.55 (dm, 14.4)
2′	1.35 (2H, m)	1.35 (2H, m)	1.35 (2H, m)	6.17 (dm, 14.4)
3′	1.35 (2H, m)	1.35 (2H, m)	1.35 (2H, m)	6.05 (dm, 14.4)
4′	1.35 (2H, m)	1.35 (2H, m)	1.35 (2H, m)	5.67 (dm, 14.4)
5′	2.04 (2H, dt, 7.0, 7.1)	2.04 (2H, dt, 7.0, 7.1)	2.05 (2H, dt, 7.0, 7.1)	2.08 (2H, dt, 7.1, 7.1)
6′	5.53 (dm, 14.4)	5.53 (dm, 14.4)	5.52 (dm, 14.4)	1.47 (2H, m)
7′	5.96 (dm, 14.4)	5.96 (dm, 14.4)	5.96 (dm, 14.4)	2.05 (2H, dt, 7.1, 7.1)
8′	5.96 (dm, 14.4)	5.96 (dm, 14.4)	5.96 (dm, 14.4)	5.55 (dm, 14.4)
9′	5.53 (dm, 14.4)	5.53 (dm, 14.4)	5.52 (dm, 14.4)	6.00 (dm, 14.4)
10′	2.04 (2H, dt, 7.0, 7.1)	2.04 (2H, dt, 7.0, 7.1)	2.05 (2H, dt, 7.0, 7.1)	6.00 (dm, 14.4)
11′	1.35 (2H, m)	1.35 (2H, m)	1.35 (2H, m)	5.55 (dm, 14.4)
12′	1.35 (2H, m)	1.35 (2H, m)	1.35 (2H, m)	2.05 (2H, dt, 7.0, 7.1)
13′	0.90 (3H, t, 7.1)	0.90 (3H, t, 7.1)	0.90 (3H, t, 7.1)	1.36 (2H, m)
14′				1.36 (2H, m)
15′				0.90 (3H, t, 7.1)

Table 2. ^{13}C-NMR Assignments for Pseudodistomins B (**1**), D (**3**), E (**4**), and F (**5**) in CD_3OD at 100 MHz

carbon	**1**	**3**	**4**	**5**
2	50.3	56.7	56.4	52.3
3	39.7	41.2	35.3	39.9
4	68.4	75.5	69.7	68.1
5	52.0	56.4	51.3	51.6
6	47.9	51.9	49.3	47.5
1′	36.7	37.2	36.9	133.2
2′	26.7	26.9	26.6	132.4
3′	30.2	30.3	30.2	131.4
4′	30.4	30.5	30.4	135.5
5′	33.2	33.3	33.3	33.2
6′	132.8	132.9	132.8	30.2
7′	131.9	132.0	132.0	33.0
8′	131.8	131.8	131.8	132.4
9′	133.0	133.1	133.1	132.2
10′	33.4	33.5	33.5	131.7
11′	32.8	32.8	32.8	133.0
12′	23.2	23.3	23.3	33.0
13′	14.2	14.3	14.3	32.8
14′				23.2
15′				14.2

thus establishing C-2 as the site on the piperidine ring to which the 13-carbon side chain was attached.

The length of the 13-carbon side chain of **3** was confirmed via the residual eight methylene, four olefinic methine, and one terminal methyl carbons observed in the ^{13}C-NMR spectrum. The side chain was unbranched because there were only aliphatic methylene and olefinic methine carbons present with no quaternary carbons. Thus, structurally, only the position of the two conjugated double bonds remained to be determined.

Both pairs of olefinic hydrogens shared 14.4 Hz couplings, indicative of the *E* geometry about the two double bonds, and the flanking methylene doublet of triplets at δ 2.04 shared 7.0 Hz coupling constants with the two outer olefinic protons at δ 5.53. On one side of the conjugated double bonds, the flanking methylene protons (H-10′) shared HMBC correlations with the vicinal carbon (C-11′) at δ32.8. The CH_3-13′ proton triplet at δ0.90, which capped the 13-carbon side chain, shared HMBC correlations with methylene C-12′ at δ 23.3 and C-11′ δ 32.8, the carbon previously shown to be two bonds removed from the conjugated double bonds. Thus, the conjugated double bonds in **3** were

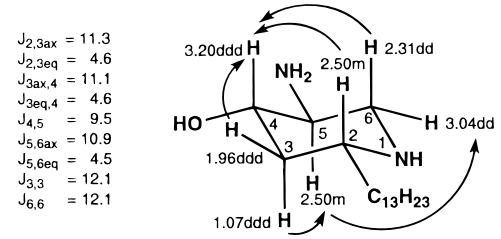

Figure 1. NOE and coupling constant data for pseudodistomin D (**3**).

situated at C-6′ through C-9′ as they were in **1**, a total of four carbons removed from the chain terminus. The flanking methylene protons at δ 2.04 on the other side of the double bonds (H-5′) shared HMBC correlations with methylene carbons at δ 30.5 (C-4′) and δ 30.3 (C-3′), both of whose protons resonated at δ 1.35. Other HMBC correlations between ring proton H-2 and methylene carbons at δ 37.2 (C-1′) and δ 26.9 (C-2′) completed the assignment. The ^{1}H- and ^{13}C-NMR assignments for **3** are summarized in Tables 1 and 2, respectively.

The relative stereochemistry of the piperidine ring in pseudodistomin D (**3**) revealed a C-4 α hydroxyl group and a C-5 β primary amine based on coupling constants and NOE data. The H-3$_{ax}$ doublet of doublet of doublets at δ 1.07 shared a transperiplanar coupling with H-2 at δ 2.50 and with H-4 at δ 3.20, indicating that all three of these protons occupied axial positions with H-2 and H-4 on the opposite face of the piperidine ring from H-3$_{ax}$. By default the C-4 hydroxyl group occupied an α equatorial position on the same ring face as H-3$_{ax}$. The same type of transperiplanar coupling was observed for H-5 at δ 2.50 with H-4 and with H-6$_{ax}$ at δ 2.31, which implied that all three of these protons occupied axial positions with H-4 and H-6$_{ax}$ being situated on the same face of the piperidine ring as H-2 and with H-5 on the opposite face along with H-3$_{ax}$. Thus, the C-5 primary amine group occupied a β equatorial position on the same face of the ring as H-2, H-4, and H-6$_{ax}$. Supporting mutual NOE enhancements (shown as arrows in Figure 1) between H-2, H-3$_{eq}$, H-4, and H-6$_{ax}$ on one face and between H-3$_{ax}$, H-5, and H-6$_{eq}$ on the

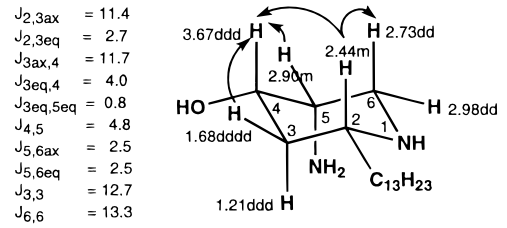

Figure 2. NOE and coupling constant data for pseudodistomin E (**4**).

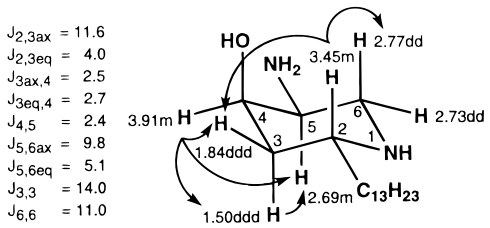

Figure 3. NOE and coupling constant data for pseudodistomin F (**5**).

other face of the piperidine ring confirmed the relative stereochemistry.

Pseudodistomin E (**4**), $[\alpha]_D$ −20.8°, was obtained as a colorless gum with a 294 Da molecular weight and a molecular formula of $C_{18}H_{34}N_2O$, indicating that it was a stereoisomer of **1** and **3** with three degrees of unsaturation. The IR and UV spectra of **4** were similar to those of **1** and **3.** The ^{1}H- and ^{13}C-NMR resonances from the piperidine ring in pseudodistomin E (**4**) were nearly identical to those of pseudodistomin C (**2**), but overall, there were four fewer olefinic methines and two more methylene groups present in **4** than in pseudodistomin C (**2**), suggesting that pseudodistomin E (**4**) consisted of a piperidine ring similar to that in **2** and a 13-carbon side chain similar to that in **3**. The COSY and HMBC correlations for the side chain resonances confirmed that the 13-carbon side chain in **4** possessed two conjugated double bonds at C-6′ through C-9′ as was the case for **1** and **3**. The complete ^{1}H- and ^{13}C-NMR assignments for **4** are presented in Tables 1 and 2, respectively.

The relative stereochemistry of the piperidine ring in pseudodistomin E (**4**) was identical to that in **2** with a C-4 α hydroxyl group and a C-5 α primary amine based on similar chemical shifts, coupling constants, and NOE data with **2**. The H-3_{ax} doublet of doublet of doublets at δ 1.21 shared a transperiplanar coupling with H-2 at δ 2.44 and with H-4 at δ 3.67, indicating that all three of these protons occupied axial positions with H-2 and H-4 on one face of the piperidine ring and with H-3_{ax} on the opposite face. By default the C-4 hydroxyl group occupied an α equatorial position on the same face as H-3_{ax}. H-5 at δ 2.90 was shown to occupy a β equatorial position by means of the small coupling constants it shared with the axial H-4 and with H-6_{ax} at δ 2.73, implying that the C-5 primary amine group was situated in an α axial position on the same face of the piperidine ring as H-3_{ax}. Mutual NOE enhancements (shown as arrows in Figure 2) between H-2, H-3_{eq}, H-4, H-5, and H-6_{ax} confirmed that these protons were all situated on the same face of the piperidine ring, thus placing both the C-4 hydroxyl and the C-5 primary amine in α positions.

Pseudodistomin F (**5**), $[\alpha]_D$ −13.9°, was also obtained as a colorless gum with a 318 Da molecular weight and a molecular formula of $C_{20}H_{34}N_2O$ requiring five degrees of unsaturation, identical to that of pseudodistomin C (**2**). The IR and UV spectra of **5** were similar to those of **2.** As seen in Tables 1 and 2, the ^{1}H- and ^{13}C-NMR resonances from the piperidine ring in pseudodistomin F (**5**) were nearly identical to those of pseudodistomin B (**1**), except for the chemical shifts of H-2 and C-2, which appeared somewhat downfield in **5**. The spectral data suggested that there were fifteen carbons present in the side chain of **5**, with a total of four double bonds, as was the case for pseudodistomin C (**2**).

The 15-carbon length of the side chain of **5** was confirmed by the presence of the six methylene, eight olefinic methine, and one terminal methyl carbons observed in the ^{13}C-NMR spectrum, which were not part of the piperidine ring. As was the case for **2**, the side chain of **5** was unbranched because there were only aliphatic methylene and olefinic methine carbons present with no quaternary carbons.

The COSY data indicated that the eight olefinic protons were distributed into two distinct sets of dienes, all of which shared large coupling constants indicative of the *E* geometry. On one side of one set of conjugated double bonds, the flanking methylene protons (H-12′) shared HMBC correlations with the vicinal carbon (C-13′) at δ 32.8. The CH_3-15′ proton triplet at δ 0.90, which capped the 15-carbon side chain, shared HMBC correlations with methylene C-14′ at δ 23.2 and C-13′ δ 32.8, the carbon that was two bonds removed from the conjugated double bonds. Thus, one set of conjugated double bonds was situated at C-8′ through C-11′, four carbons removed from the chain terminus (as in **2**). The flanking methylene protons at δ 2.05 on the other side of these double bonds (H-7′) shared COSY correlations with H-6′ at δ 1.47, which in turn correlated to H-5′ at δ 2.08. A string of correlations, continuing on from H-5′ including olefins H-4′, H-3′, H-2′, and H-1′ revealed that the second pair of conjugated double bonds was situated between C-1′ and C-4′ at the extreme opposite end of the side chain. Reciprocal HMBC correlations between H-2 and C-1′ and between H-1′ and C-2 confirmed these assignments. The complete ^{1}H- and ^{13}C-NMR assignments for **5** are presented in Tables 1 and 2, respectively.

The relative stereochemistry of the piperidine ring in pseudodistomin F (**5**) was shown to be identical to that in **1** with a C-4 β hydroxyl group and a C-5 β primary amine based on similar chemical shifts, coupling constants, and NOE data. The H-3_{ax} doublet of doublet of doublets at δ 1.50 shared an 11.6-Hz transperiplanar coupling with H-2 at δ 3.45 and a 2.5-Hz coupling with H-4 at δ 3.91, indicating that H-3_{ax} and H-4 were situated on one face of the piperidine ring, while H-2 and the β axial hydroxyl group at C-4 were positioned on the opposite face. A set of mutual NOE enhancements (shown as arrows in Figure 3) between H-3_{ax}, H-4, and H-5 indicated that these protons were all situated on the same face of the piperidine ring, placing H-4 in an α equatorial position and H-5 in an α axial position. Consistent with the proposed relative stereochemistry shown above was the transperiplanar coupling between H-5 and H-6_{ax}.

The optical rotations of pseudodistomin F (**5**), −13.9°, and pseudodistomin B (**1**), −13.0°, were nearly identical, indicating that they possessed the same absolute stereochemistry. A similar observation was made for

Table 3. Activity of Pseudodistomins in Engineered Yeast Strains

	IC_{12} (μg/100 μL well) of yeast strain			
compound	wild-type	Δrad 52	Δrad52:Δ top1 GAL4:RAD52 in glucose	Δrad52:Δ top1 GAL4:RAD52 in galactose
pseudodistomin B	13.0	1.8	1.5	1.5
pseudodistomin C	38	20	12	13
pseudodistomin D	11	3.8	1.9	2.0
pseudodistomin E	12	4.3	1.8	2.3
pseudodistomin F	20	9.0	3.1	3.3
streptonigrin	>0.1	0.012	0.009	>0.1
amsacrine	>20	8.2	2.7	>20
rapamycin	0.00043	0.00057	0.00044	0.00074

pseudodistomins C (**2**) and E (**4**). The absolute stereochemistry of pseudodistomin D (**3**), $[\alpha]_D$ +5°, was not determined.

As was evident from the data in Table 3, the pseudodistomins demonstrated potent inhibitory activity toward both DNA repair-competent and DNA repair-deficient strains of the yeast, *Saccharomyces cerevisiae*. In the strain in which both the RAD52 and TOP-1 genes had been deleted, there was a 6- to 9-fold hypersensitivity compared to the DNA repair-competent wild-type cells. Pseudodistomin C (**3**) showed minimal differential activity in the yeast strains. This pattern of differential activity is evident for inhibitors of topoisomerase II as exemplified by amsacrine. Hypersensitivity to such compounds is due to production of DNA damage by virtue of topoisomerase II-mediated DNA lesions; the deletion of topoisomerase I results in compensatory increases in topoisomerase II[6] and deletion of the RAD52 gene renders the cells incapable of repairing the DNA lesions that are produced.

A recent modification of our yeast-based screen, the incorporation of inducible expression of RAD52 from a plasmid, however, revealed that the pseudodistomins are false positives in the assay. The growth of the Δrad 52:Δtop 1 GAL4:RAD52 strain in glucose strongly represses expression of the RAD52 gene, which is under the control of the GAL4 promoter. Under these conditions there is sensitivity to known DNA-damaging agents as exemplified by amsacrine and streptonigrin. The same strain, when grown in galactose, expresses high levels of RAD52 and demonstrates loss of sensitivity to DNA-damaging agents. There is minimal change in response to antifungal agents that work by other mechanisms as exemplified by rapamycin, an inhibitor of signal transduction. The pseudodistomins demonstrated no change in activity in glucose vs. galactose in this strain, indicating that these compounds are killing yeast by some other mechanism than by production of DNA damage. The basis for the increased sensitivity of Δrad 52 and Δrad 52:Δtop 1 strains to pseudodistomins is unknown.

Experimental Section

General Experimental Procedures. The IR spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. All homonuclear and heteronuclear 1D and 2D NMR data were recorded on a Bruker AMX-400 spectrometer in CD_3OD. The LRDCIMS and HRDCIMS were acquired on a VG-70SE mass spectrometer. Analytical and preparative TLC were carried out on precoated Si gel G (Kiesel gel G_{254}) and reversed-phase (Whatman KC18F) plates. The UV spectra were recorded on a Beckman DV-7 spectrophotometer. Optical rotations were recorded on Perkin-Elmer 241 MC polarimeter. Reagent grade chemicals (Fisher and Baker) were used throughout.

Bioassays. The assay used for initial detection and fractionation was differential inhibition of DNA repair-deficient compared to congenic DNA repair-competent yeast strains.[7] Extracts of compounds were placed in wells punched in agar overlaid with appropriate yeast mutant, and zones of inhibition were measured after incubation for 48 h at 30 °C. Activity was quantified by linear regression of log concentration vs zone size to determine the concentration in μg per well that produced a 12-mm zone of inhibition (IC_{12}). A key strain used for evaluation of these compounds had the chromosomal TOP1 and RAD52 genes deleted and was transferred with a plasmid bearing the RAD52 gene under the control of the GAL4 promoter. This allowed for inducible expression of RAD52 by growing the cells in galactose instead of glucose as an energy source. The use of a single test strain precluded any minor phenotypic differences between test strains which may result in differential sensitivity that is not due to the targeted biochemical difference.

Collection, Extraction, and Isolation. The stalked ascidian (PAL93−002) was collected by hand using scuba at a depth of 10 m in the Rock Islands, Palau, in January of 1993, and specimens were frozen immediately and kept at −20 °C until extraction. A preserved voucher sample of the ascidian has been identified by Dr. Francois Monniot, Paris Museum of Natural History, as *Pseudodistoma megalarva* (family Polyclinidae). The voucher sample of this ascidian has been deposited in the Paris Museum of Natural History, sample number MNHN:A1Pse 35. The whole freeze-dried ascidians (58 g) were extracted with 1:1 MeOH−CH_2Cl_2 (3 × 250 mL, 14 h, 20 °C) to give a dark red residue (4.3 g) that was dissolved in H_2O (100 mL) and extracted with *n*-BuOH (3 × 75 mL) to yield a red oil (0.452 g). The DNA-damaging active *n*-BuOH-soluble portion was chromatographed on a Sephadex LH-20 column eluting with MeOH−CH_2Cl_2 (1:1) to provide fractions that were tested against a wild type of yeast strain, a strain deficient in double-stranded DNA repair (RAD52) and a strain deficient in both double-stranded DNA repair and topoisomerase I. Further purification of the residue (0.179 g) obtained from the combined active fractions using RP-18 (Whatman, ODS-3, H_2O−MeOH−20:80) followed by Si gel preparative TLC (MeOH−CH_2Cl_2−H_2O−NH_4OH−13:87:1.0:0.5) yielded three fractions (A−C). The Si gel preparative TLC of fraction A (34 mg) employing CH_2Cl_2−$(CH_3)_2CO$−MeOH−H_2O−NH_4OH

(70:15:15:1.0:1.5) afforded pseudodistomins C (**2**, 13.3 mg) and E (**4**, 11.6 mg). Reversed-phase preparative TLC of fraction B (64 mg) using MeOH−NH_4OH (95:5) followed by Si gel preparative TLC using MeOH−CH_2Cl_2−H_2O−NH_4OH (50:50:2.0:2.5) furnished pseudodistomins B (**1**, 43 mg) and D (**3**, 11.6 mg). Repeated preparative TLC of fraction C (28 mg) using MeOH−CH_2Cl_2−H_2O−NH_4OH (50:50:2:2.5) yielded pseudodistomin F (**5**, 18 mg) in pure form.

Pseudodistomin B (1): colorless gum, $[\alpha]^{25}_D$ −13° (*c* 0.87, MeOH); UV (MeOH) λ_{max} 222 nm; IR (neat) ν_{max} 3257, 3014, 3000−2800, 1565, 1457, 985 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; DCIMS, *m/z* 294; HRDCIMS *m/z* 294.2660 (calcd for $C_{18}H_{34}N_2O$, 294.2671).

Pseudodistomin D (3): colorless gum, $[\alpha]^{25}_D$ +5° (*c* 0.26, MeOH); UV (MeOH) λ_{max} 240 nm; IR (neat) ν_{max} 3260, 3018, 3000−2800, 1563, 1462, 982 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; DCIMS, *m/z* 294; HRDCIMS *m/z* 294.2662 (calcd for $C_{18}H_{34}N_2O$, 294.2671).

Pseudodistomin E (4): colorless gum, $[\alpha]^{25}_D$ −20.8° (*c* 0.39, MeOH); UV (MeOH) λ_{max} 239 nm; IR (neat) ν_{max} 3258, 3011, 3000−2800, 1569, 1460, 981 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; DCIMS, *m/z* 294; HRDCIMS *m/z* 294.2674 (calcd for $C_{18}H_{34}N_2O$, 294.2671).

Pseudodistomin F (5): colorless gum, $[\alpha]^{25}_D$ −13.9° (*c* 0.42, MeOH); UV (MeOH) λ_{max} 233 nm; IR (neat) ν_{max} 3257, 3014, 3000−2800, 1565, 1457, 985 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; DCIMS, *m/z* 318; HRDCIMS *m/z* 318.2664 (calcd for $C_{20}H_{34}N_2O$, 318.2671).

Acknowledgment. We would like to thank Dr. Carole A. Bewley for assistance during the collection of *Pseudodistoma megalarva*, Dr. Francois Monniot for identification of the ascidian, and the government and people of the Republic of Palau for permission to collect the marine organisms. We would also like to thank Mr. Gary Zuber for the IR spectra. This work was supported in part by a grant (CA-50771) from the National Institute of Health.

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NP9701438