Limazepines A−F, Pyrrolo[1,4]benzodiazepine Antibiotics from an Indonesian *Micrococcus* sp.

Serge Fotso,[†] T. Mark Zabriskie,[†] Philip J. Proteau,[†] Patricia M. Flatt,[†,¶] Dwi Andreas Santosa,[‡,§] Sulastri,[§] and Taifo Mahmud*[,†]

Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, 203 Pharmacy Building, Corvallis, Oregon 97331-3507, Indonesian Center for Biodiversity and Biotechnology, ICBB-Complex, Jl. Cilubang Nagrak No. 62, Situgede, Bogor 16115, Indonesia, and Department of Soil Science and Land Resource, Faculty of Agriculture, Bogor Agricultural University, Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, Indonesia

Received December 29, 2008

In our screening of Indonesian microorganisms for novel bioactive natural products we have isolated seven new compounds, designated as limazepines A, B1 and B2 (isolated as an isomeric mixture), C, D, E, and F, from the culture broth of *Micrococcus* sp. strain ICBB 8177. In addition, the known natural products prothracarcin and 7-*O*-succinylmacrolactin A, as well as two previously reported synthetic compounds, 2-amino-3-hydroxy-4-methoxybenzoic acid methyl ester and 4-ethylpyrrole-2-carboxaldehyde, were obtained from the extract. Chemical structures were determined by spectroscopic methods and by comparison with the NMR data of structurally related compounds. The limazepines belong to the growing group of the pyrrolo[1,4]benzodiazepine antitumor antibiotics isolated from various soil bacteria. Limazepines B1/B2 mixture, C, and E were active against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli.* Limazepine D was also active against *S. aureus,* but was not active against *E. coli*. Interestingly, only the limazepines B1/B2 mixture and D were active against *Pseudomonas aeruginosa*.

Microorganisms are capable of producing a broad spectrum of secondary metabolites, and over the past five decades, the prokaryotic actinomycetes have been the most fruitful source of antibiotics, yielding 65−70% of all discovered antibiotics.[1] Therefore, screening of microorganisms for the production of new antibiotics continues to be an important approach in modern drug discovery programs.

In our continuing efforts to discover novel bioactive natural products from Indonesian microorganisms, we explored the active metabolites of the crude extract of the isolate ICBB 8177 that exhibited antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Isolation and structure elucidation of the active metabolites resulted in the identification of seven new pyrrolo[1,4] benzodiazepines (PBDs). The PBDs are a family of naturally occurring antitumor antibiotics that exert their cytotoxic activity by binding covalently to the C-2 amino group of guanine residues within the minor groove of DNA.[2,3] PBDs are tricyclic molecules, which in most cases contain a stereogenic center at C-11a (Figure 1). Several natural PBDs such as anthramycin (**1a**),[4] mazethramycin (**1b**),[5] porothramycin A (**1c**),[6] sibiromycin (**2**),[7] tomaymycin (**3a**),[8] oxotomaymycin (**3b**),[9] and prothracarcin (**4**)[10] have been reported as potent antitumor agents. Their inherent antitumor activity is due to their ability to regulate gene expression by recognizing and binding with DNA GC base pairs.[11,12] PBDs have been reported to possess antibacterial activity against some Gram-positive and acid-fast bacteria[13] and to also exhibit antileishmania[14] and herbicidal[15] activities. Therefore, there is currently an interest in this class of compounds in relation to their diverse biological activities. Herein we describe the isolation, structure elucidation, and biological activity of the new pyrrolo[1,4]benzodiazepines we have termed the limazepines.

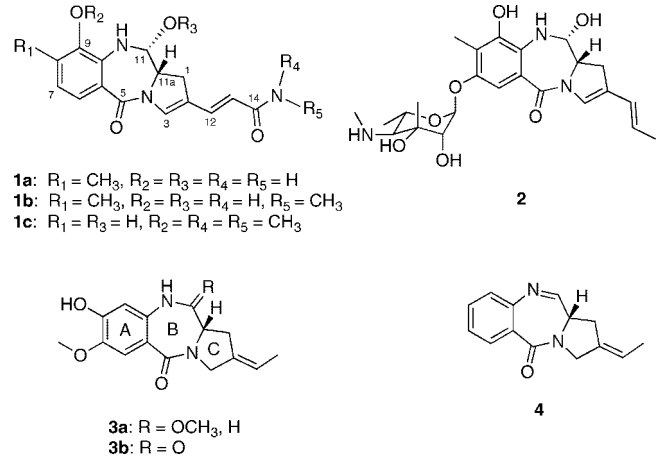


Figure 1. Chemical structures of various pyrrolo[1,4]benzodiazepine antitumor antibiotics.

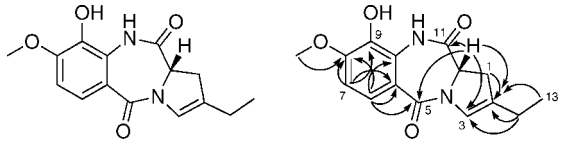
Results and Discussion

Micrococcus sp. strain ICBB 8177 was cultured in 5 L of M_2 medium at 28 °C for 6 days. The yellowish culture broth was filtered to separate the mycelia from the culture broth. The mycelia pellet was extracted first with EtOAc and subsequently with MeOH, and these organic extracts were concentrated to dryness. The culture broth was passed over Diaion HP-20 resin, and adsorbed compounds were eluted with MeOH. The methanolic fraction was concentrated to the point that only residual water remained, and then this material was extracted with EtOAc. Bioactivity tests of the extracts revealed that only the EtOAc extract of the culture broth possessed antibacterial activity. Bioassay-guided isolation of the active extract yielded the known compound prothracarcin (**4**) along with seven new pyrrolo[1,4]benzodiazepines, which we have named limazepines A (**5**), B1 and B2 (**6a** and **6b**), C (**7**), D (**8**), E (**9**), and F (**10**).

Limazepine A (**5**) was obtained as yellowish needles. Its ^{1}H NMR spectrum showed an exchangeable proton signal as a broad singlet at δ 9.40 and two aromatic *ortho* proton doublets at δ 7.28 (J = 8.7 Hz) and 6.95 (J = 8.7 Hz), indicating the presence of a 1,2,3,4-tetrasubstituted aromatic ring. In the aliphatic region, a methoxy singlet at δ 3.90 and a methine doublet of doublets at δ 4.53 (H-11a) were observed. The latter signal shows couplings in the ^{1}H,^{1}H COSY spectrum with two doublets of doublets at δ 3.30 and 2.72 assigned to the methylene protons at C-1. Further couplings were

* Corresponding author. Phone: 1-541-737-9679. Fax: 1-541-737-3999. E-mail: Taifo.Mahmud@oregonstate.edu.
[†] Oregon State University.
[‡] Indonesian Center for Biodiversity and Biotechnology.
[§] Bogor Agricultural University.
[¶] Present address: Department of Chemistry, Western Oregon University.

10.1021/np800827w CCC: $40.75
Published on Web 03/18/2009



5

Figure 2. Chemical structure of limazepine A (**5**) and selected HMBC correlations in **5**.

seen between the C-1 methylene proton signals and a broad sp^2 singlet at δ 6.61. Finally, signals characteristic of an isolated ethyl group were observed as a quartet at δ 2.13 ($J = 7.4$ Hz) and a triplet at δ 1.06 ($J = 7.4$ Hz).

The molecular mass of limazepine A (**5**) was determined by HRESIMS, suggesting a molecular formula of $C_{15}H_{16}N_2O_4$. Fifteen carbon signals were observed in the ^{13}C NMR spectrum and sorted by a DEPT experiment as one methyl, two methylene, one sp^3 methine, one methoxy, three sp^2 methine, and seven quaternary carbons, including two carbonyls, presumed to belong to amides based on their chemical shifts and the molecular formula. The HMBC experiments revealed correlations from the methoxy protons at δ 3.90 and the two aromatic protons at δ 7.28 and 6.95 (H-6 and H-7) to C-8 (δ 149.9). The H-6 proton exhibited further couplings with C-9a (δ 124.6), C-5a (δ 120.3), and C-5 (δ 161.1), and H-7 showed additional correlations with C-5a and C-9. Together, these data and the chemical shifts of C-9 and C-9a allowed the placement of one nitrogen atom at C-9a. Key correlations were seen between H-11a (δ 4.53) and C-11 (δ 168.5), C-2 (δ 128.9), and C-5 (δ 161.1). These observations and the chemical shift of H-11a support the placement of the second nitrogen atom between C-11a and the C-5 carbonyl (δ 161.1). The protons on C-1 (δ 3.30 and 2.72) also correlated with C-11 and C-2. Further coupling observed between C-2 and protons H-12 and H-13 facilitated positioning of the ethyl group and finalize the second spin system. On the basis of the above data, a pyrrolobenzodiazepine skeleton was constructed for limazepine A (**5**) as shown in Figure 2. Compound **5** has a large positive specific rotation ($[\alpha]^{25}_D$ +480) similar to that of other natural PDBs which possess the *S* configuration at C-11a, which suggests the C-11a*S* configuration as depicted in Figure 2.[16]

Limazepines B1 and B2 (**6a** and **6b**) were isolated as an inseparable mixture of 11*R* and 11*S* isomers (Figure 3). High-resolution mass spectrometry of **6a** and **6b** gave a molecular formula of $C_{15}H_{18}N_2O_4$ with an index of hydrogen deficiency of eight, instead of nine as in limazepine A (**5**). The ^1H NMR spectrum of **6a** and **6b** showed two sets of signals, which can be resolved using 2D NMR (COSY, HSQC, and HMBC). The NMR data are generally similar to those of **5**, particularly in the aromatic region, where the two doublets suggested the presence of a 1,2,3,4-tetrasubstituted aromatic ring. In the aliphatic region, characteristic signals for an isolated ethyl group and a methoxy group were observed in each of the signal sets. The ^{13}C NMR spectrum of **6a** and **6b** also contained two sets of signals, each of which confirmed the absence of the carbonyl signal at δ 168.5 seen in **5**. The methine signals at δ 88.4 (δ_H 4.65) for **6a** and at δ 95.3 (δ_H 4.63) for **6b** confirmed that the C-11 carbonyl in **5** was reduced. The remainder of the structures of **6a** and **6b** was deduced based on comparisons of the data with those for limazepine A (**5**) and related compounds.

The relative configuration of C-11 and C-11a in **6a** was determined by interpretations of the ^1H NMR spectrum, in which the H-11 methine signal (δ 4.65) appeared as a singlet, indicating that there is no coupling between H-11 and H-11a. The lack of coupling between the two protons is commonly observed in PBDs, especially in the anthramycin and tomaymycin groups that have the 11*R*, 11a*S* configuration (Figure 3).[17] On the other hand, H-11 of **6b** appeared as a doublet with a coupling constant of 6.1 Hz, suggesting the opposite 11*S* configuration for **6b**.

Compounds **6a** and **6b** are relatively unstable and under mildly acidic conditions (e.g., in $CHCl_3$) equilibriate with limazepine C (**7**), which is a dehydrated form of **6a** and **6b** (Figures 3 and 4). Limazepine C (**7**) was also isolated from the culture broths of *Micrococcus* sp. ICBB 8177, although it is unclear whether **7** is the precursor or a product of **6a** and **6b**. However, more recent studies on the biosynthesis of anthramycin suggested that the seven-membered hemiaminal benzodiazepine skeleton of anthramycin, which corresponds to compounds **6a** and **6b**, is a direct cyclization product of a dipeptide intermediate.[18] Therefore, it is tempting to assume that other limazepine analogues, e.g., **5** and **7**, are derived from **6a** and/or **6b**. Also, in CH_3OH solution, **6a** and **6b** can convert to their corresponding methoxy derivatives (**6c** and **6d**), most likely through **7** (Figure 4C).

The proton resonances of **6c** and **6d** are almost identical to those of **6a** and **6b**, respectively. In addition, two singlets (δ 3.38, 3.50) corresponding to the C-11 methoxy groups of **6c** and **6d** were also observed (Figure 4C). High-resolution mass spectrometry of **6c** and **6d** gave a molecular formula of $C_{16}H_{20}N_2O_4$, confirming the additional methoxy group in these compounds.

Limazepine C (**7**) was isolated as a yellowish oil. The ^1H NMR spectrum was similar to that of **6a** and **6b**, but lacked the C-11 hydroxy group and contained a second sp^2 methine doublet at δ 7.92 ($J = 3.9$ Hz), suggesting **7** contained an additional double bond. The high-resolution EIMS for **7** yielded a molecular formula of $C_{15}H_{16}N_2O_3$, indicating the loss of a hydroxy group relative to **6a** and **6b**. HMBC correlations from H-11 to C-1, C11a, and C-9a confirmed the presence of a new double bond between N-10 and C-11. On the basis of the comparison of the positive specific rotation of **7** ($[\alpha]^{23}_D$ +160) with that of anhydromazethramycin ($[\alpha]^{22}_D$ +194) obtained by dehydration of mazethramycin (**1b**),[16] the configuration at C-11a is proposed to be *S*.

Upon prolonged storage (~2 months) in $CDCl_3$, limazepine C (**7**) can be completely transformed into a more stable product, limazepine D (**8**). The latter compound is more abundantly present in the culture broths of *Micrococcus* sp. ICBB 8177 (Figure 3).

The molecular formula of limazepine D (**8**) was established to be $C_{15}H_{14}N_2O_3$ by high-resolution mass spectrometry. The ^1H NMR spectrum indicated the presence of two aromatic *ortho* protons and a methoxy and an ethyl group, similar to those observed in **7**. The major difference was the absence of signals for H-11a and H-1 in **8**. In addition, three singlets and one exchangable proton were observed in the aromatic region, suggesting the presence of a pyrrole, instead of a pyrroline ring.

The ^{13}C NMR spectrum of **8** showed 15 carbon signals, including one carbonyl at δ 161.3. Five sp^2 methine signals were observed instead of the four seen in **7**, confirming the presence of an additional double bond in **8**. The HMBC data indicated correlations of H-3 (δ 8.10) and H-1 (δ 7.00) with the methylene carbon C-12 (δ 20.2). In addition, H-12, H-13, H-3, and H-1 all showed couplings with C-2 (δ 128.3). Correlations between H-6 and the C-5 carbonyl (δ 161.3), as well as between H-11 and C-9a, provided evidence that support the structure of compound **8** with an aromatic C-ring, as shown in Figure 3.

Limazepine E (**9**) was isolated as a yellowish solid, and the molecular formula was determined by HREIMS to be $C_{15}H_{16}N_2O_3$, which is identical to that of **7**. The ^1H NMR spectrum of **9** also showed great similarities to that of **7**, particularly in the aromatic region. However, signals for the isolated ethyl group observed in **7** were substituted by an sp^2 methine at δ 6.62 (m, H-12) and a methyl group at δ 1.76 (d, $J = 6.8$ Hz, H-13), respectively, in **9**. In addition, the two multiplets for H-1a (δ 2.99) and H-1b (δ 3.20) of **7** appeared as a broad doublet at δ 3.00 in **9**, suggesting a minor structural change in the C-ring. Interpretation of the HMBC spectrum revealed correlations between H-11a (δ 3.98) and C-1 (δ 31.5) as well as between H-13 and both C-2 (δ 132.8) and C-12 (δ

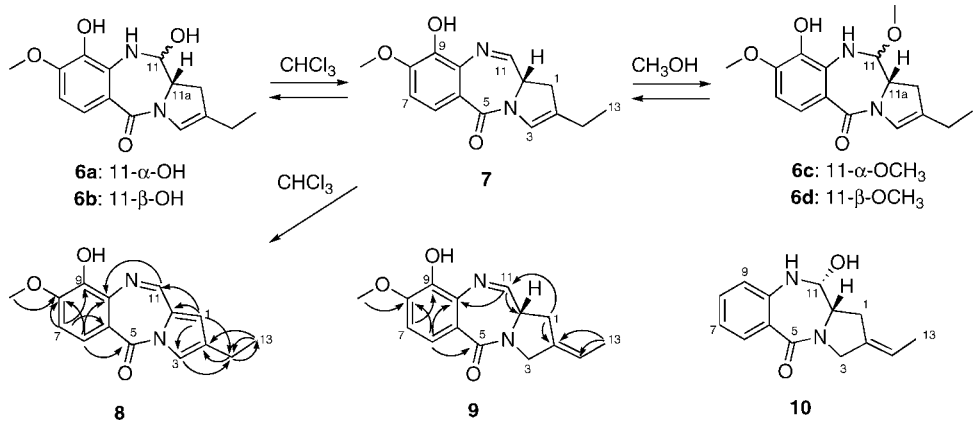


Figure 3. Chemical structures of limazepines B1−B2 (**6a**−**6b**), their methoxy derivatives (**6c**−**6d**), and limazepines C−F (**7**−**10**), and selected HMBC correlations observed for limazepines D (**8**) and E (**9**).

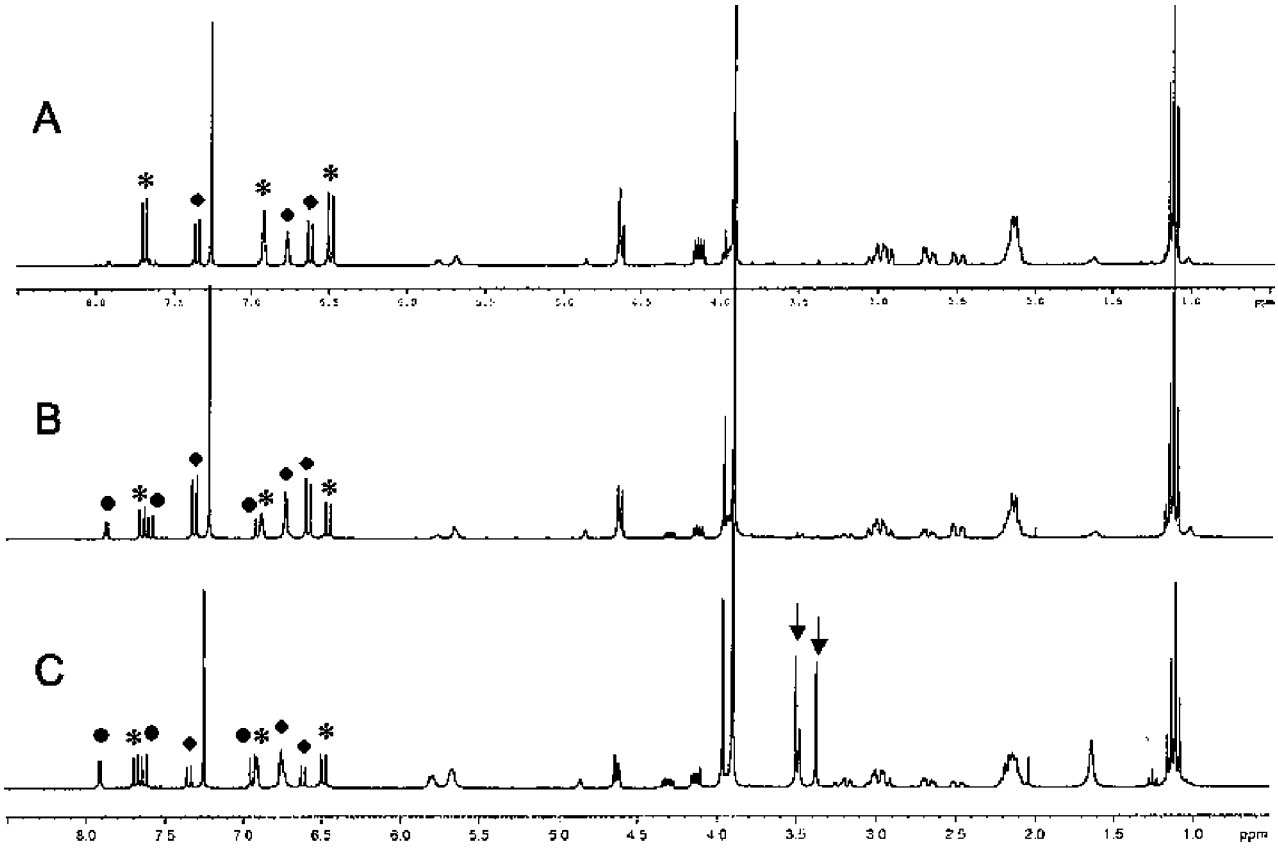
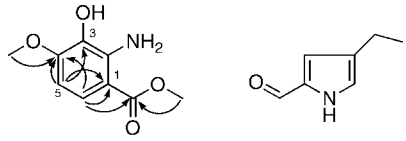


Figure 4. ^{1}H NMR spectra of limazepines B1 and B2 and their derivatives. (A) ^{1}H NMR spectrum of B1/B2 mixture recorded immediately after dissolving the compounds in $CDCl_3$; (B) ^{1}H NMR spectrum of B1/B2 mixture recorded after 3 days in $CDCl_3$; (C) ^{1}H NMR spectrum of B1/B2 mixture recorded after 5 days in $CDCl_3$, then transferred to CH_3OH, concentrated, and redissolved in $CDCl_3$. * indicates proton signals of limazepine B1 in the aromatic/olefinic region, ◆ indicates proton signals of limazepine B2, ● indicates proton signals of limazepine C, arrows indicate two new methoxy group signals of **6c** and **6d**.

119.2). These connections confirmed an exocyclic double bond on the C-ring of **9** like that found on prothracarcin (**4**).

The olefinic functionality in **9** was assigned the *E* configuration on the basis of comparison to NMR data reported for synthetic prothracarcin (**4**) and its isomer possessing a *Z* ethylidene moiety at C-2. Direct evidence for the configuration of **4** could not be obtained from NOE experiments, and the synthesis of both isomers helped confirm the natural product possessed the *E* configuration.[19,20] The key observation was that the olefinic methyl group induced an upfield shift on the *syn* methylene carbon of the pyrrolidine. Thus, the chemical shift of C-1 was seen at δ 31.1 in *E*-prothracarcin (**4**) and δ 35.3 in the unnatural *Z*-prothracarcin.[20] Similarly, C-3 of **4** resonated at δ 51.6 but was shifted upfield to 48.3 in the *Z* isomer. The major difference in the ^{1}H NMR spectrum is observed for the H-1 signal, which appears in **4** as broad doublet at δ 2.98 (J = 5.2 Hz) and in *Z*-prothracarcin as an AB quartet or multiplet at δ 2.85−3.20.[19,20] The corresponding shifts in **9** are δ 31.5 for C-1, δ 51.9 for C-3, and δ 3.00 (br d, J = 5.8 Hz) for H-1, which is consistent with the *E* configuration. Interestingly, a NOESY spectrum of **9** failed to reveal correlations between H-1 and the C-13 methyl or between H-3 and H-12. It should also be noted that the *E* configuration of the olefin in **3a** was established by X-ray analysis and confirmed by synthesis.[21,22]

Limazepine F (**10**) was obtained as a minor component. The ^{1}H NMR spectrum of **10** was indicative of a PBD derivative possessing four contiguous aromatic protons on the basis of a spin system of



11 **12**

Figure 5. Structures of compounds **11** and **12** and selected HMBC correlations in **11**.

two doublets and two triplets. The NMR spectra also indicated the presence of two methylenes and an exocyclic olefin as seen in **9**. The chemical structure of **10** was deduced mainly from the HREIMS data and comparisons of the spectroscopic data with those for related compounds.

In addition to the limazepines, we also isolated 2-amino-3-hydroxy-4-methoxybenzoic acid methyl ester (**11**), 4-ethylpyrrole-2-carboxaldehyde (**12**), and 7-*O*-succinylmacrolactin A[23] from the culture broth of strain ICBB 8177. Compound **11** was obtained as a yellowish oil. Its (+)-ESIMS indicated a pseudo-molecular ion at *m/z* 198 $[M + H]^+$, and the HRMS experiments supported a molecular formula of $C_9H_{11}NO_4$. The ^{1}H NMR spectrum of **11** revealed two methoxy signals at δ 3.91 and 3.85 in the aliphatic region, two *ortho* protons (H-5, H-6) at δ 7.47, 6.29 (d, $J = 9.0$ Hz) in the aromatic region, and a broad singlet for two exchangeable protons at δ 5.72. The ^{13}C NMR spectrum showed nine carbon signals including one carbonyl at δ 168.6, two *O*-methyl, two sp^2 methine, and four quaternary carbons. The key data for arranging the substituents on the aromatic ring were found in the HMBC spectrum (Figure 5). Correlations were observed for H-5, H-6, and the methoxy at δ 3.91 with the carbon at δ 149.1 (C-4). The correlation of the methyl signal at δ 3.85 and the doublet at δ 7.47 to the carbonyl at δ 168.6 placed a methyl ester at C-1 (δ 106.1). Three-bond couplings for H-5 to the quaternary carbons C-3 (δ 139.9) and C-1 and from H-6 to C-2 (δ 131.7) completed the structure of **11** (Figure 5). The chemical structure of **11** was independently confirmed by the fact that **11** is a degradation product of limazepine D (**8**) (see below). It is also noteworthy that compound **11** was previously reported as a synthetic compound, although no spectroscopic data were provided.[24]

The ^{1}H NMR spectrum of **12** contained only a triplet and a quartet at δ 1.22 and 2.53, respectively, in the aliphatic region, attributed to an ethyl group. There were also two singlets for aromatic protons at δ 6.92 and 6.82 and an aldehyde proton at δ 9.44 in the downfield region. The ^{13}C NMR and HSQC spectra confirmed the presence of the aldehyde group at δ 179.2 and confirmed there were six other carbon signals. The structure of compound **12** was elucidated as 4-ethylpyrrole-2-carboxaldehyde. Similar to **11**, compound **12** has been previously reported as a synthetic compound.[25] There are no reports on the isolation of either **11** or **12** from a natural source.

Compounds **11** and **12** may be degradation products of the pyrrolo[1,4]benzodiazepines in strain ICBB 8177. In fact, when limazepine D (**8**) was stirred overnight in MeOH with silica gel, in an attempt to generate a limazepine B analogue, **11** was the major product isolated, suggesting it and **12** are most likely artifacts of the isolation process. Nonetheless, the anthranilic acid derivative **11** may be a biosynthetic precursor to the limazepines. Early studies on the biosynthesis of the benzodiazepines using isotopically labeled precursors established that the anthranilic acid moiety leading to the A-ring is derived from tryptophan and that the C-ring is derived from tyrosine.[26] Recently, Bachmann and co-workers identified the anthramycin biosynthesis gene cluster from *S. refuineus* and demonstrated that exogenous 3-hydroxyanthranilic acid, but not 4-methyl-3-hydroxyanthranilic acid, was able to restore anthramycin formation in several gene deletion mutants where the targeted genes were proposed to function in the conversion of tryptophan to anthranilic acid.[18] The composition of the anthramycin cluster led these authors to propose an NRPS-mediated condensation of the anthranilate and "dehydroproline acrylamide" precursors followed by a reductive release from the NRPS, leading to the pyrrolo[1,4]benzodiazepine skeleton.[18]

Table 1. Antibacterial Activity of Limazepines, Inhibition Diameter (mm)

		inhibition diameter (mm)		
compound	concentration (μg/disk)	*E. coli*	*Staph. aureus*	*Ps. aeruginosa*
prothracarcin (**4**)	30			
limazepine A (**5**)	40			
limazepines B1/B2 (**6a/6b**)	40	15	18	16
limazepine C (**7**)	40	10	18	
limazepine D (**8**)	30		7	9
limazepine E (**9**)	40	10	12	
limazepine F (**10**)	30			
11	30		11	
12	24			
ampicillin	1	25	13	30

Limazepines A−F belong to the growing group of the pyrrolo[1,4]benzodiazepine antitumor antibiotics isolated from actinomycetes, particularly from the genus *Streptomyces*. The limazepines are the first pyrrolo[1,4]benzodiazepines isolated from a *Micrococcus* species. Limazepines A−E are distinguished from other members of the class by the unique oxidation pattern at C-8 and C-9 of the A-ring rather than the known C-7, C-7/C-8, or C-7/C-9 patterns. Limazepine F and the co-occurring prothracarcin are among the few examples of PBDs with an unsubstituted A-ring. Viewed in light of the findings by Hu et al. for anthramycin biosynthesis,[18] the A-ring of limazepines A−E likely arises from incorporation of 3-hydroxyanthranilic acid followed by subsequent hydroxylation and *O*-methylation at C-8. The isolation of the minor components **4** and **10** from this *Micrococcus* sp. would suggest that low-level incorporation of anthranilic acid also occurs without further oxidative tailoring.

Biological Activities. The antimicrobial activity of the isolated compounds was evaluated against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans,* and *Mucor miehei*. The results revealed that pyrrolo[1,4]-benzodiazepines **6a/6b**, **7**, **8**, and **9** were active against *S. aureus,* whereas **6a/6b**, **7**, and **9** were also active against *E. coli* (Table 1). Concerning the results of the benzodiazepine mixture, it is unknown whether the activity was due to **6a** and/or **6b** or their readily formed derivatives **6c**, **6d**, and **7**. Compounds **4**, **5**, and **10** did not show antibacterial activity against any of the organisms at the concentrations tested. The C-11 carbonyl in **5** and the lack of the hydroxy and/or the methoxy groups in the A-rings of **4** and **10** may contribute to their lack of activity. However, prothracarcin (**4**) was reported to possess weak activities against Gram-positive and Gram-negative bacteria with MICs in the range 50−100 μg/mL.[10] Interestingly, only **6a/6b** and **8** were active against *P. aeruginosa,* and compound **11**, which represents the A-ring of the pyrrolo[1,4]-benzodiazepines, showed some activity against *S. aureus* (Table 1). None of the compounds showed antibacterial activity against *B. subtilis* or antifungal activity against *C. albicans* or *M. miehei*.

Experimental Section

General Experimental Procedures. Melting points were measured on a Thomas-Hoover Uni-Melt melting point apparatus. Optical rotations were measured on a Jasco P1010 polarimeter (10 and 100 mm cells were used). UV−vis spectra were recorded on a Beckmann DU 640 B spectrophotometer, and IR spectra were recorded on a Nicolet IR100 FT-IR spectrophotometer. All NMR spectra were measured on a Bruker Unity 300 (300.15 MHz) spectrometer. ESIMS were recorded on a ThermoFinnigan LCQ Advantage system, and HRESI mass spectra were obtained on a Waters/Micromass LCT spectrometer. HREIMS and HRCIMS were measured on a JEOL HMS-600H MS route magnetic sector instrument. Preparative RP-HPLC was performed using

a 250 × 15 mm column (Luna RP-100 C18, 5 μm, Phenomenex) with UV detection at 254 nm. Column chromatography was carried out on silica gel (230−400 mesh). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Organism Collection and Identification. Samples containing the isolate ICBB 8177 were taken from soil of the Black Water River, Pangkoh Lima, located on the border between Maliku and Kanamit subdistrict, Pulang Pisau Regency, Central Kalimantan Province, Indonesia. This river is part of the unique Black Water ecosystem in Kalimantan and lies in a remote area about 150 km from the coast of South Kalimantan. Soil samples were stored at room temperature. Details of the isolation of the bacterial strain were as previously described.[27]

ICBB strain 8177 was identified as a *Micrococcus* sp. through comparisons of the genomic 16S rDNA sequence with the NCBI BLAST database. The Wizard Genomic DNA isolation kit (Promega) and the GoTaq Polymerase System (Promega) were used to conduct the PCR amplification of the 16S rDNA sequence. Manufacturer's specified guidelines were followed with an addition of 4% DMSO in the PCR reaction mixture. The bacterial-specific degenerate primers EubB[28] and 1492R[29] were used to amplify a 1 kb fragment of the 16S rDNA. The 16S rRNA gene sequence of *Micrococcus* sp. ICBB 8177 was found to be 99% identical over the 928 base-sequenced region to that of *Micrococcus luteus* strain JC2 isolated from the Manhattan Konza Prairie and *Micrococcus* sp. MOLA 4, isolated from a coastal northwest Mediterranean ecosystem. The sequence was submitted to GenBank under accession number FJ194442. The strain ICBB 8177 was deposited at ICBB-CC (Indonesian Center for Biodiversity and Biotechnology, Culture Collection of Microorganisms), Bogor, Indonesia.

Fermentation and Isolation. The isolate ICBB 8177 was cultured in M_2-medium (4 g glucose, 4 g yeast extract, and 10 g malt extract, pH adjusted to 7.4 with 2 N NaOH prior to sterilization) on a 5 L scale in 20 1-L baffled flasks containing 250 mL of liquid media, and were shaken on a rotary shaker at 28 °C for 6 days. The yellowish crude extract was filtered over Celite and the filtrate passed over Diaion HP20, while the mycelium was extracted first with EtOAc and then with MeOH. Both organic fractions were concentrated, and two extracts were obtained, EAM (ethyl acetate mycelium; 400 mg) and MM (methanol mycelium; 600 mg). The HP20 column was washed with MeOH, and the eluent was concentrated. The residual water was extracted with EtOAc and then *n*-BuOH, which after evaporation of the organic solvents delivered respectively EAW (ethyl acetate/water; 500 mg) and NBE (*n*-butanol extract; 700 mg). All four extracts were tested against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Bacillus subtilis*, *Mucor miehei*, and *Mycobacterium smegmatis,* but only the EAW extract exhibited activity against *E. coli* and *S. aureus*. This extract was separated on silica gel using a gradient of increasing MeOH (2−15%) in CH_2Cl_2, and three fractions, I−III, were obtained. Bioassay placed the active components in fraction I. Trituration of fraction I in MeOH delivered limazepine A (**5**, 15 mg). Chromatography of the MeOH-soluble material on Sephadex LH-20 (MeOH) followed by preparative TLC (8:92 MeOH/CH_2Cl_2) and RP-HPLC using a gradient from 20:80 MeOH/H_2O to MeOH in 30 min delivered limazepines E (**9**, 5 mg) and prothracarcin (**4**, 2 mg).

A second fermentation of the strain was conducted in the same medium but with Amberlite XAD-16 (5 g/250 mL) added prior to sterilization. After 6 days the culture broth was filtered, the XAD was washed with MeOH, and the extract was worked up as described above. The XAD and the EtOAc extracts were pooled on the basis of bioassay and TLC. Chromatography on Sephadex LH-20 (MeOH/CH_2Cl_2, 1:1) gave four fractions, A−D. The oily fraction A contains only fatty acids and was discarded. Fraction 2 was first chromatographed on silica gel using an increasing gradient of MeOH in CH_2Cl_2, followed by RP-HPLC (a gradient from 20:80 MeOH/H_2O to MeOH in 30 min), affording a mixture of limazepines B1 and B2 (**6a/6b**, 4 mg), limazepine C (**7**, 5 mg), and limazepine F (**10**, 1.5 mg). Preparative TLC of fraction III followed by RP-HPLC using a gradient from 20:80 MeOH/H_2O to MeOH gave the highly fluorescent methyl 2-amino-3-hydroxy-4-methoxybenzoate (**11**, 7 mg) and 4-ethylpyrrole-2-carboxaldehyde (**12**, 2 mg). Fraction IV yielded limazepine D (**8**, 10 mg) and 7-*O*-succinylmacrolactin A (6 mg).

Limazepine A (5): yellowish needles (from CH_2Cl_2/MeOH); mp 280−282 °C; $[\alpha]^{25}_D$ +480 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 303 (3.95), 280 (3.84), 265 (3.91) nm; IR (neat) ν_{max} 3130, 2915, 1694, 1629, 1601, 1571, 1512, 1439, 1346, 1290, 1248, 1211, 1091, 1020, 922 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.40 (1H, br s, H/D), 7.28 (1H, d, J = 8.7 Hz, H-6), 6.95 (1H, d, J = 8.7 Hz, H-7), 6.61 (1H, br s, H-3), 4.53 (1H, dd, J = 10.8, 3.6 Hz, H-11a), 3.90 (3H, s, 8-OCH_3), 3.30 (1H, H-1a)*, 2.72 (1H, dd, J = 16.0, 10.8 Hz, H-1b), 2.13 (2H, q, J = 7.4 Hz, H-12), 1.06 (3H, t, J = 7.4 Hz, H-13); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 168.5 (C-11), 161.1 (C-5), 149.9 (C-8), 136.7 (C-9), 128.9 (C-2), 124.6 (C-9a), 120.7 (C-6), 120.7 (C-3), 120.3 (C-5a), 108.2 (C-7), 56.12 (OCH_3), 56.03 (C-11a), 32.5 (C-1), 21.1 (C-12), 12.1 (C-13); (+)-ESIMS *m/z* (%) 289 ($[M + H]^+$, 14), 599 ($[2M+Na]^+$, 100); (−)-ESIMS *m/z* (%) = 287 ($[M - H]^-$, 100); HREIMS *m/z* 288.1106 (calcd for $C_{15}H_{16}N_2O_4$, 288.1110). *under the solvent signal.

Limazepines B1 and B2 (6a and 6b): yellowish oil; **6a**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.70 (1H, d, J = 9.1 Hz, H-6), 6.93 (1H, m, H-3), 6.50 (1H, d, J = 9.1 Hz, H-7), 4.65 (1H, s, H-11), 4.14 (1H, dd, J = 11.0, 5.7 Hz, H-11a), 3.92 (3H, s, 8-OCH_3), 3.00 (1H, m, H-1a), 2.69 (1H, dd, J = 16.3, 5.9 Hz, H-1b), 2.14 (2H, br q, J = 7.3 Hz, H-12), 1.12 (3H, t, J = 7.3 Hz, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 162.8 (C-5), 147.7 (C-8), 132.0 (C-9), 126.8 (C-2), 124.7 (C-6), 124.6 (C-3), 122.0 (C-9a), 111.1 (C-5a), 101.9 (C-7), 88.4 (C-11), 58.5 (C-11a), 56.3 (8-OCH_3), 38.2 (C-1), 22.0 (C-12), 12.3 (C-13). **6b**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.36 (1H, d, J = 8.7 Hz, H-6), 6.78 (1H, m, H-3), 6.63 (1H, d, J = 8.7 Hz, H-7), 4.63 (1H, d, J = 6.1 Hz, H-11), 3.96 (1H, dd, J = 3.8, 1.7 Hz, H-11a), 3.92 (3H, s, 8-OCH_3), 3.00 (1H, m, H-1a), 2.50 (1H, dd, J = 16.8 3.9 Hz, H-1b), 2.14 (2H, br q, J = 7.3 Hz, H-12), 1.12 (3H, t, J = 7.3 Hz, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 164.5 (C-5), 148.7 (C-8), 136.2 (C-9), 122.7 (C-9a), 127.5 (C-2), 122.9 (C-3), 121.8 (C-6), 122.3 (C-5a), 105.2 (C-7), 95.3 (C-11), 56.3 (8-OCH_3), 58.4 (C-11a), 38.6 (C-1), 22.0 (C-12), 12.3 (C-13); (+)-ESIMS *m/z* 291 $[M + H]^+$; (−)-ESIMS *m/z* = 290 $[M - H]^+$; (+)-HRESIMS *m/z* 291.1331 (calcd for $C_{15}H_{19}O_4N_2$, 291.1344).

6c/6d: yellowish oil; **6c**: ^1H NMR (300 MHz, MeOH-d_4) δ 7.46 (1H, d, J = 9.3 Hz, H-6), 6.78 (1H, m, H-3), 6.55 (1H, d, J = 9.3 Hz, H-7), 4.74 (1H, s, H-11), 4.09 (1H, dd, J = 11.1, 5.1 Hz, H-11a), 3.91 (3H, s, 8-OCH_3), 3.35 (3H, s, 11-OCH_3), 3.04 (1H, m, H-1a), 2.68 (1H, m, H-1b), 2.19 (2H, br q, J = 7.5 Hz, H-12), 1.14 (3H, t, J = 7.5 Hz, H-13); ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 165.4 (C-5), 150.3 (C-8), 135.2 (C-9a), 133.5 (C-9), 130.1 (C-2), 124.8 (C-6), 124.6 (C-3), 112.8 (C-5a), 103.3 (C-7), 89.5 (C-11), 56.6 (8-OCH_3), 54.8 (11-OCH_3), 60.3 (C-11a), 39.5 (C-1), 22.7 (C-12), 12.6 (C-13). **6d**: ^1H NMR (300 MHz, MeOH-d_4) δ 7.18 (1H, d, J = 8.8 Hz, H-6), 6.73 (1H, d, J = 8.8 Hz, H-7), 6.68 (1H, m, H-3), 4.65 (1H, d, J = 8.7 Hz, H-11), 3.90 (1H, under OMe peak, H-11a), 3.90 (3H, s, 8-OCH_3), 3.35 (3H, s, 11-OCH_3), 3.04 (1H, m, H-1a), 2.54 (1H, m, H-1b), 2.19 (2H, br q, J = 7.5 Hz, H-12), 1.15 (3H, t, J = 7.5 Hz, H-13); ^{13}C NMR (75.5 MHz, MeOH-d_4) δ 166.9 (C-5), 151.6 (C-8), 138.7 (C-9a), 133.4 (C-9a), 130.8 (C-2), 122.4 (C-3), 121.7 (C-6), 117.0 (C-5a), 106.7 (C-7), 97.0 (C-11), 56.7 (8-OCH_3), 54.8 (11-OCH_3), 60.2 (C-11a), 39.0 (C-1), 22.7 (C-12), 12.7 (C-13); (+)-ESIMS *m/z* (%) 305 ($[M + H]^+$, 100), 631 ($[2M+Na]^+$, 35); (+)-HRESIMS *m/z* 305.1493 (calcd for $C_{16}H_{21}N_2O_4$, 305.1501).

Limazepine C (7): yellowish oil; $[\alpha]^{23}_D$ +160 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 280 (4.00), 235 (4.09), 210 (4.07) nm; IR (neat) ν_{max} 3253, 2965, 2933, 1623, 1597, 1564, 1525, 1433, 1432 1439, 1362, 1211, 1136, 1092, 1012, 872, 759 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.92 (1H, d, J = 3.9 Hz, H-11), 7.63 (1H, d, J = 8.8 Hz, H-6), 6.94 (1H, d, J = 8.8 Hz, H-7), 6.75 (1H, m, H-3), 4.31 (1H, ddd, J = 9.2, 3.9, 1.3 Hz, H-11a), 3.97 (3H, s, 8-OCH_3), 3.20 (1H, m, H-1a), 2.99 (1H, m, H-1b), 2.17 (2H, q, J = 7.4 Hz, H-12), 1.14 (3H, t, J = 7.4 Hz, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 165.5 (C-11), 161.2 (C-5), 149.4 (C-8), 140.7 (C-9), 132.5 (C-9a), 127.6 (C-2), 122.7 (C-3), 122.3 (C-6), 120.4 (C-5a), 111.1 (C-7), 56.5 (OCH_3), 54.2 (C-11a), 38.0 (C-1), 22.0 (C-12), 12.2 (C-13); EIMS *m/z* (%) 272 (M^+, 100), 243 (20), 214 (20), 178 (42), 96 (42); HREIMS *m/z* 272.1154 (calcd for $C_{15}H_{16}N_2O_3$, 272.1161).

Limazepine D (8): yellow solid; UV (MeOH) λ_{max} (log ϵ) 403 (sh), 391 (3.46), 289 (3.80), 233 (sh) nm; IR (neat) ν_{max} 3300, 2853, 1630, 1610, 1597, 1432, 1290, 1091, 820, 720 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.97 (1H, br s, H/D), 8.28 (1H, s, H-11), 8.20 (1H, d, J = 9.2 Hz, H-6), 8.10 (1H, br s, H-3) 7.09 (1H, d, J = 9.2 Hz, H-7), 7.00 (1H, br s, H-1), 4.02 (3H, s, 8-OCH_3), 2.64 (2H, q, J = 7.6 Hz, H-12), 1.29 (3H, t, J = 7.6 Hz, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) 161.3 (C-5), 151.2 (C-8), δ 145.0 (C-11), 143.7 (C-9), 133.7 (C-9a), 132.7 (C-2), 128.3 (C-11a), 124.8 (C-6), 124.8 (C-3), 124.3 (C-1), 114.9 (C-5a), 111.7 (C-7), 56.5 (OCH_3), 20.2 (C-12), 14.6 (C-13); (+)-ESIMS

m/z (%) 271 ([M + H]$^+$, 100); (+)-APCIMS *m/z* (%) 271 ([M + H]$^+$, 100); HREIMS *m/z* 270.0997 (calcd for $C_{15}H_{14}N_2O_3$, 270.1005).

Limazepine E (9): yellow solid; $[\alpha]^{25}_D$ +73 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 302 (3.57), 269 (3.84), 235 (3.94), 212 (3.93) nm; IR (neat) ν_{max} 3356, 2924, 2853, 1622, 1566, 1521, 1429, 1361, 1287, 1211, 1134, 1248, 1211, 1134, 1076, 997, 760 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (1H, d, J = 4.3 Hz, H-11), 7.63 (1H, d, J = 8.9 Hz, H-6), 6.97 (1H, d, J = 8.9 Hz, H-7), 6.74 (1H, br s, 9-OH), 6.62 (1H, m, H-12), 4.28 (2H, br s, H-3), 3.98 (3H, s, 8-OCH_3), 3.98 (1H, m, H-11a), 3.00 (2H, br d, J = 5.8 Hz, H-1), 1.76 (3H, d, J = 6.8 Hz, H-13); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 165.7 (C-11), 164.9 (C-5), 149.1 (C-8), 140.4 (C-9), 132.8 (C-2), 132.0 (C-9a), 121.9 (C-6), 121.0 (C-5a), 119.2 (C-12), 111.1 (C-7), 56.5 (OCH_3), 54.5 (C-11a), 51.9 (C-3), 31.5 (C-1), 14.8 (C-13); EIMS *m/z* (%) 272 (M$^+$, 100), 241 (20). 193 (20), 176 (40), 112 (80), 96 (24); HREIMS *m/z* 272.1183 (calcd for C_{15} $H_{16}N_2O_3$, 272.1161).

Limazepine F (10): yellow solid; $[\alpha]^{25}_D$ +260 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 312 (3.26), 226 (3.93) nm; IR (neat) ν_{max} 3332, 2920, 2851, 1613, 1595, 1568, 1510, 1486, 1455, 1240, 999 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.68 (1H, dd, J = 8.1, 1.1 Hz, H-6), 7.46 (1H, d, J = 6.0 Hz, H/D), 7.20 (1H, br t, J = 8.1 Hz, H-8), 6.77 (1H, d, J = 8.1 Hz, H-9), 6.67 (1H, t, J = 8.1 Hz, H-7), 5.06 (1H, m, H-12), 4.91 (1H, d, J = 6.2 Hz, H-11), 4.02 (2H, AB, J = 16.4 Hz, H-3), 3.88 (1H, dd, J = 9.1, 3.5 Hz, H-11a), 2.73 (1H, m, H-1a), 2.30 (1H, m, H-1a), 1.10 (3H, d, J = 6.5 Hz H-13); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 165.3 (C-5), 143.5 (C-9a), 133.6 (C-2), 132.1 (C-6), 131.3 (C-7), 118.5 (C-5a), 117.9 (C-9), 116.6 (C-8), 115.2 (C-12), 83.5 (C-11), 58.0 (C-11a), 51.7 (C-3), 32.0 (C-1), 13.6 (C-13); EIMS *m/z* (%) 244 (M$^+$, 5), 226 (M $-$ H_2O, 100); HREIMS *m/z* 244.1262 (calcd for $C_{14}H_{16}N_2O_2$, 244.1212).

2-Amino-3-hydroxy-4-methoxybenzoic acid methyl ester (11): [24] yellowish oil; UV (MeOH) λ_{max} (log ϵ) 408 (1.60), 314 (2.12), 268 (2.44), 236 (2.51), 218 (2.49) nm; IR (neat) ν_{max} 3376, 2952, 1687, 1623, 1552, 1501, 1438, 1439, 1273, 1246, 1118, 1091, 887, 769, 729 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (1H, d, J = 9.0 Hz, H-6), 6.29 (1H, d, J = 9.0 Hz, H-5), 5.72 (2H, br s, H/D), 3.91 (3H, s, 4-OCH_3), 3.85 (3H, s, CO*OCH*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.6 (CO), 149.1 (C-4), 139.9 (C-3), 131.7 (C-2), 123.3 (C-6), 106.1 (C-1), 99.7 (C-5), 56.2 (4-O*CH*$_3$), 51.6 (O*CH*$_3$); (+)-ESIMS: *m/z* (%) 197.9 ([M + H]$^+$, 100%); HREIMS *m/z* 197.0690 (calcd for $C_9H_{11}NO_4$, 197.0688).

4-Ethylpyrrole-2-carboxaldehyde (12): [25] colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.44 (1H, d, J = 0.9 Hz, CHO), 6.92 (1H, br s, H-3), 6.82 (1H, br s, H-5), 2.53 (2H, q, J = 7.5 Hz, *CH*$_2$CH$_3$), 1.22 (3H, t, J = 7.5 Hz, CH$_2$*CH*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 179.2 (CHO), 129.8 (C-2)*, 129.5 (C-4)*, 123.9 (C-3), 120.4 (C-5), 19.9 (CH_2), 15.3 (CH_3); EIMS *m/z* (%) 123 (M$^+$, 80), 108 (100), 69 (60), 57 (40); HREIMS *m/z* 123.0684 (calcd for C_7H_9NO, 123.0684). *Interchangeable

Acknowledgment. We thank J. Morré for the high-resolution mass spectra recorded at the mass spectrometry facility of the Environmental Health Sciences Center at Oregon State University, which is supported in part by a grant from NIEHS (ES00210). This work was supported in part by the OSU College of Pharmacy Research and Scholarship Fund.

Supporting Information Available: ^{1}H NMR spectra of **5**−**12**, ^{13}C NMR spectra of **5**−**9** and **11**, HSQC and HMBC spectra of **5** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Berdy, J. *J. Antibiot.* **2005**, *58*, 1–26.
(2) Denny, W. A. *Curr. Med. Chem.* **2001**, *8*, 533–544.
(3) Hadjivassileva, T.; Thurston, D. E.; Taylor, P. W. *J. Antimicrob. Chemother.* **2005**, *56*, 513–518.
(4) Komatsu, N.; Kimura, K.; Abe, S.; Kagitani, Y. *J. Antibiot.* **1980**, *33*, 54–60.
(5) Kunimoto, S.; Masuda, T.; Kanbayashi, N.; Hamada, M.; Naganawa, H.; Miyamoto, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1980**, *33*, 665–667.
(6) Tsunakawa, M.; Kamei, H.; Konishi, M.; Miyaki, T.; Oki, T.; Kawaguchi, H. *J. Antibiot.* **1988**, *41*, 1366–1373.
(7) Brazhnikova, M. G.; Konstantinova, N. V.; Mesentsev, A. S. *J. Antibiot.* **1972**, *25*, 668–673.
(8) Arima, K.; Kosaka, M.; Tamura, G.; Imanaka, H.; Sakai, H. *J. Antibiot.* **1972**, *25*, 437–444.
(9) Kariyone, K.; Yazawa, H.; Kohsaka, M. *Chem. Pharm. Bull.* **1971**, *19*, 2289–2293.
(10) Shimizu, K.; Kawamoto, I.; Tomita, F.; Morimoto, M.; Fujimoto, K. *J. Antibiot.* **1982**, *35*, 972–978.
(11) Kamal, A.; Rao, M. V.; Laxman, N.; Ramesh, G.; Reddy, G. S. *Curr. Med. Chem. Anticancer Agents* **2002**, *2*, 215–254.
(12) Thurston, D. E. *Br. J. Cancer* **1999**, *80*, 65–85.
(13) Kaneko, T.; Wong, H.; Doyle, T. W. *J. Antibiot.* **1984**, *37*, 300–302.
(14) Clark, R. L.; Carter, K. C.; Mullen, A. B.; Coxon, G. D.; Owusu-Dapaah, G.; McFarlane, E.; Duong Thi, M. D.; Grant, M. H.; Tettey, J. N.; Mackay, S. P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 624–627.
(15) Guaciaro, M. A.; Harrington, P. M.; Karp, G. M. U.S. Patent 5438035 A 19950801, 1995.
(16) Hurley, L. H. *J. Antibiot.* **1977**, *30*, 349–370.
(17) Hochlowski, J. E.; Andres, W. W.; Theriault, R. J.; Jackson, M.; McAlpine, J. B. *J. Antibiot.* **1987**, *40*, 145–148.
(18) Hu, Y.; Phelan, V.; Ntai, I.; Farnet, C. M.; Zazopoulos, E.; Bachmann, B. O. *Chem. Biol.* **2007**, *14*, 691–701.
(19) Langley, D. J.; Higgins, D. A. *Microbios* **1987**, *49*, 97–105.
(20) Mori, M.; Uozumi, Y.; Kimura, M.; Ban, Y. *Tetrahedron* **1986**, *42*, 3793–3806.
(21) Arora, S. K. *J. Antibiot.* **1981**, *34*, 462–464.
(22) Tozuka, Z.; Takaya, T. *J. Antibiot.* **1983**, *36*, 142–146.
(23) Chutima, J.; Khanit, S.; Somboon, T.; Prasat, K.; Piamsak, M. *J. Nat. Prod.* **2000**, *63*, 984–986.
(24) Chao, S. W.; Kao, Y. S.; Chou, C. H.; Hsu, B. *Sci. Sin. (Engl. Ed.)* **1963**, *12*, 49–71.
(25) Singh, S. K.; Dekhane, M.; Le Hyaric, M.; Potier, P.; Dodd, R. H. *Heterocycles* **1997**, *44*, 379–391.
(26) Hurley, L. H. *Acc. Chem. Res.* **1980**, *13*, 263–269.
(27) Fotso, S.; Mahmud, T.; Zabriskie, T. M.; Santosa, D. A.; Sulastri, N. A.; Proteau, P. J. *J. Nat. Prod.* **2008**, *71*, 61–65.
(28) Cary, S. C.; Warren, W.; Anderson, E.; Giovannoni, S. J. *Mol. Mar. Biol. Biotechnol.* **1993**, *2*, 51–62.
(29) Edwards, U.; Rogall, T.; Blocker, H.; Emde, M.; Bottger, E. C. *Nucleic Acids Res.* **1989**, *17*, 7843–7853.

NP800827W