Modified Phenazines from an Indonesian *Streptomyces* sp.†

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Fractionation of the extract from the Indonesian *Streptomyces* sp. ICBB8198 as directed by the antibacterial activity delivered the known phenazine antibiotics griseoluteic acid (**1a**) and griseolutein A (**1b**), as well as two new phenazine derivatives (**2** and **3**). In addition, the known compounds spirodionic acid, dihydrosarkomycins, and 6-ethyl-4-hydroxy-3,5-dimethyl-2*H*-pyran-2-one (**4a**), along with the new pyrone 3,6-diethyl-4-hydroxy-5-methyl-2*H*-pyran-2-one (**4b**), were isolated. We report here the isolation, structure elucidation, and antibiotic activity of the new metabolites as well as a hypothetical pathway for the formation of the new phenazine derivatives.

Phenazine antibiotics have been known since 1950, with the original discovery of griseolutein from *Streptomyces griseoluteus*.[1] Numerous members of this structural family have been described, with many being ester derivatives of griseoluteic acid, the base hydrolysis product of griseolutein A. Phenazine antibiotics are now known from a variety of microorganisms, including *Streptomyces* spp., *Pelagiobacter variabilis*,[2] *Pantoea agglomerans*,[3] *Pseudomonas* spp.,[4] and a *Vibrio* sp.[5] The diverse biological actions of these compounds include cytotoxicity, antibacterial, antiparasitic, and antimalarial activities.[6] Herein we report the discovery of phenazines that have a new type of modification to the main core structure.

In the continuation of our drug discovery program focused on microorganisms isolated from the Black Water Ecosystem in Kalimantan, Indonesia,[7,8] the extract of *Streptomyces* sp. ICBB8198 indicated activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The extract was separated using various chromatographic methods including silica gel, Sephadex LH-20, and HPLC. The main antibacterial action was correlated to the presence of griseoluteic acid (**1a**),[2,9] which was identified along with the related griseolutein A[10] (**1b**) by an Antibase[11] search and comparison with reported spectroscopic data.[2] In addition to these known phenazines, two new phenazine derivatives (**2** and **3**) were also isolated and characterized.

Compound **2** was obtained as a yellow solid. The molecular weight was deduced from the pseudomolecular ions at *m/z* 467 $[M + H]^+$ and 465 $[M - H]^-$, and the HRMS experiments supported $C_{25}H_{26}N_2O_7$ as the molecular formula. The aromatic region of the ^{1}H NMR spectrum of **2** is similar to those of **1a** and **1b** and exhibited evidence of two spin systems consisting of three consecutive protons at δ 8.95 (dd, $J = 7.2$, 1.5 Hz), 8.49 (dd, $J = 8.8$, 1.5 Hz), and 8.04 (dd, $J = 8.8$, 7.2 Hz) and two protons present as *ortho*-coupled doublets, suggesting that **2** possessed the same phenazine chromophore as in **1a/1b**. The aliphatic region was more complex than those of **1a/1b**. It exhibited two methoxy signals at δ 4.17 and 3.60 instead of one. Further signals observed in the aliphatic region were an ethyl group [δ 1.06 (t) and 2.58 (q)], two methyl signals at δ 1.41 (d) and 1.31 (s), a methine, and a methylene multiplet at δ 4.02, but all of these signals appeared doubled. Confirming the observations in the ^{1}H NMR spectrum, the ^{13}C NMR spectrum indicated signals as pairs in the 8−60 ppm range, except for the quaternary carbon at δ 62.0, the methoxy carbon at δ 56.7, and the methyl carbon at δ 7.9. Four carbonyl carbons were revealed in the HMBC spectrum, including two ketone carbonyls (δ 207.0 and 205.0) and carbons at δ 172.6 and 166.3, suggestive of ester and carboxylic acid carbons, respectively. The doubling of the carbonyl carbons could not be established from the ^{13}C NMR spectrum because these carbon resonances were not clearly observed.

The HMBC spectrum indicated correlations between H-2 (δ 8.95) and C-3 (δ 134.9), C-10a (δ 138.6), and the carbonyl at δ 166.3, which suggested a carboxylic acid moiety at C-1. Furthermore, correlations were observed between H-3 and carbons C-1 (δ 125.5), C-2 (δ 136.9), and C-4a (δ 142.6), while the methoxy protons (δ 4.17), H-7 (δ 7.70), and H-8 (δ 7.12) were correlated to C-9 (δ 153.4). Observation of weak correlations from the methylene protons at δ 4.02 to carbons at δ 127.7 (C-6), 144.2 (C-5a), and 133.5 (C-7) suggested a benzylic methylene (C-1′) at C-6. The interpretation of these data confirmed the same phenazine core structure as seen in **1a** and **1b**.

The second part of structure **2** was determined by the interpretation of HMBC correlations of the aliphatic region: the ethyl group and the methyl doublet at δ 1.41 had correlations to the carbonyl at δ 207.0; the methyl singlet at δ 1.31 and the doublet at δ 1.41 showed cross-peaks to the carbonyl at δ 205.0; and the methoxy signal at δ 3.60 and the methyl singlet indicated correlations to the carbonyl at δ 172.6 and to the quaternary carbon at δ 61.9. All of this evidence identified a methyl 2,4-dimethyl-3,5-dioxoheptanoate fragment. This fragment was connected to the phenazine ring system through the C-1′ benzylic methylene, which had HMBC correlations to the carbonyl carbons at δ 205.0 and 172.6 and to the quaternary carbon at δ 61.9, establishing the planar structure of **2**. The duplication of the signals in the NMR spectra may be explained by the presence of an inseparable mixture of diastereomers, in which the methyl-bearing C-4′ readily epimerizes due to the acidity of H-4′. The configuration at C-2′ remains unassigned.

Compound **3** was isolated as a yellow solid. The (+)-ESIMS delivered the pseudomolecular ions *m/z* 437 $[M + H]^+$ and 459 $[M + Na]^+$, and the (−)-ESIMS gave *m/z* 435 $[M - H]^-$, providing a molecular weight of *m/z* 436. The HRMS experiment suggested the molecular formula $C_{24}H_{24}N_2O_6$, indicating a difference of CH_2O between the molecular formulas for **2** and **3**. The ^{1}H and ^{13}C NMR data revealed the presence of only a single methoxy group in **3**. In

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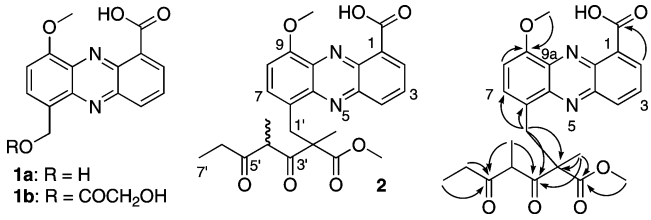

Figure 1. Structures of **1a**, **1b**, and **2** and selected HMBC correlations in **2**.

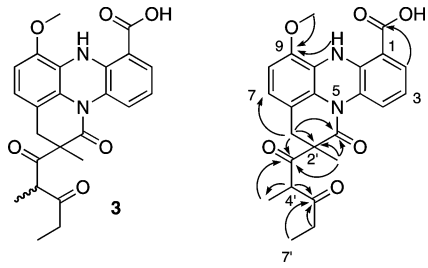

Figure 2. Structure and selected HMBC correlations in compound **3**.

addition to the loss of a methoxy group, differences in the UV spectra and shift variations observed in the aromatic region of the ^{1}H and ^{13}C NMR spectra indicated additional changes compared to **2**. For example, the ^{1}H NMR spectrum measured in DMSO-d_6 indicated two multiplets at δ 7.75 and 6.63 integrating for four and six protons, respectively, and a broad peak at δ 11.39 (exchangeable; absent in methanol-d_4). These shift differences and the exchangeable proton suggested a dihydrophenazine core rather than the phenazine. Griseolutein B, which is a dihydrophenazine derivative, has also been observed to have an exchangeable proton in its ^{1}H NMR spectrum, shifted downfield due to a hydrogen bond with the acid carbonyl at C-1.[12] For a better understanding of the aromatic spin system, additional ^{1}H NMR spectra were measured in $CDCl_3$ and in methanol-d_4, which provided greater resolution in the aromatic region. The ^{1}H,^{1}H−COSY spectrum indicated two aromatic spin systems (1,2,3-trisubstituted and 1,2,3,4-tetrasubstituted aromatic rings) as in **2**. Interpretation of the HMBC spectrum indicated correlations of H-7, H-8, the methoxy protons, and the protons at δ 11.39 to the carbon at δ 144.5 (C-9), confirming the position of the aromatic methoxy group and supporting an NH at N-10, consistent with a dihydrophenazine moiety (Figure 2). With the location of the aromatic methoxy secured, it is established that the methyl ester in **2** is no longer present in the structure of **3**. In the aliphatic region, three pairs of methyl protons, which appear as a singlet, a doublet, and a triplet, and a pair of methine protons at δ 2.27 and 2.36 were observed, similar to those seen in the ^{1}H NMR spectrum of **2**. The methylene protons at δ 3.22/δ 2.75, the methine at δ 4.24, and the methoxy at δ 3.77 were not split into paired signals. The ^{13}C NMR spectrum indicated all carbons as pairs of very close signals. Resonances for four carbonyl carbons, including two ketones, were clearly visible. A final connection in the structure was made between N-5 of the dihydrophenazine and the C-2′ carbonyl carbon (δ 168.2), resulting in the core structure of **3**. This linkage explains the absence of the methyl ester moiety in **3** and is consistent with no observable N-5 proton. Although there are few protons available in this lactam ring to verify this new structural element through standard NMR experiments, the shifts of the C-1′ methylene protons in **3** (δ 3.22/δ 2.75), compared to those in **2** (δ 4.02; overlapped), are consistent with the major change in magnetic environment that would occur upon cyclization into a rigid ring system. Because the protons signals are superimposable in the aromatic region and appear as pairs in the aliphatic region, **3** is also likely a mixture of epimers with the C-4′ configuration the source of variability. A D_2O exchange NMR

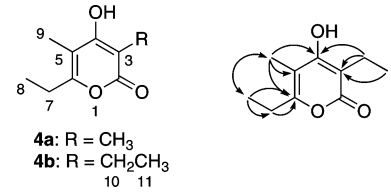

Figure 3. Structures of pyrones **4a** and **4b** and selected HMBC (single-headed arrows) and NOESY (double arrow) correlations in **4b**.

experiment supports this conclusion. The H-4′ signal of **3** disappeared after treatment with D_2O, but reappeared after re-equilibration with MeOH. All attempts to separate the diastereomers of **3** have failed. The configuration at C-2′ remains unassigned. Compounds **2** and **3** represent the first griseolutein derivatives that have a C−C linkage rather than a C−O linkage at C-1′. The tetracyclic nature of **3** has not previously been observed for griseolutein-derived compounds.

In addition to metabolites arising from the active fractions, two pyrones were isolated from inactive fractions. The known 6-ethyl-4-hydroxy-3,5-dimethyl-2*H*-pyran-2-one (**4a**) has been isolated previously from a *Streptomyces* sp.[13] and from the fungus *Emericella heterothallica*.[14] The minor compound **4b** was obtained as a colorless oil. HREIMS data in conjunction with NMR data supported the molecular formula $C_{10}H_{14}O_3$. It was closely related to **4a** on the basis of a comparison of the NMR data. The only difference was the presence of an additional methylene group in **4b**, representative of a second ethyl group in the structure. The extra methylene group can be introduced into **4b** at either C-3 or C-5.

Data from HMBC and NOESY experiments determined the position of this second ethyl group (Figure 3). The protons H_2-10 and H_3-11 showed HMBC correlations to C-3 (δ 105.1), and cross-peaks were seen from H_2-10 and H_3-9 to C-4 (δ 169.1). Further correlations were observed between H_2-7, H_3-8, H_3-9, and δ 161.3 (C-6/C-2 overlap) and from H_2-7 and H_3-9 to C-5 (δ 109.4), suggesting the structure of **4b** has an ethyl group at C-3. Further structure confirmation was obtained from the NOESY spectrum, where distinct correlations were seen between H_3-9 and H_2-7, H_3-8, respectively. The structure was assigned as 3,6-diethyl-4-hydroxy-5-methyl-2*H*-pyran-2-one (**4b**).

The presence of the pyrones along with metabolites **2** and **3** suggested a potential route for the formation of the new phenazines. Condensation of **1b** and **4a** could lead to intermediate **A**, which could be attacked by MeOH to provide **2** directly (Figure 4) or by H_2O to form the carboxylic acid, which would require conversion to the methyl ester **2**. Compound **3** could arise by intramolecular attack of N-5 of **A** onto the pyrone carbonyl, followed by reduction of the central ring of the tricyclic system. Alternatively, **3** may be formed from **2** by similar N-5 attack on the methyl ester. Methanolysis of **3** to form **2** is another possibility, but this seems unlikely, as **3** is stable in the methanol-d_4 NMR solvent. The potential formation of **2** via methanolysis of intermediate **A** suggests that **2** may be an isolation artifact. In order to test this hypothesis, an effort was made to detect intermediate **A** in a fresh culture by extracting with acetone, rather than an alcoholic solvent mixture. Under these conditions, **3** was observed, but no **2**, nor putative intermediate **A**, was detected. This experiment supports the formation of **2** during the original isolation procedure and also argues against the formation of **3** from **2**. While **2** apparently is not a natural product, its ease of isolation when the common extraction solvent MeOH was used suggests that **2** is an isolable surrogate either for precursor **A** or for the free carboxylate equivalent of **2**. The specific rotations determined for **2** (+150) and **3** (−17) support an enzymatic condensation between griseolutein A and pyrone **4a**, forming the C-2′ asymmetric center.

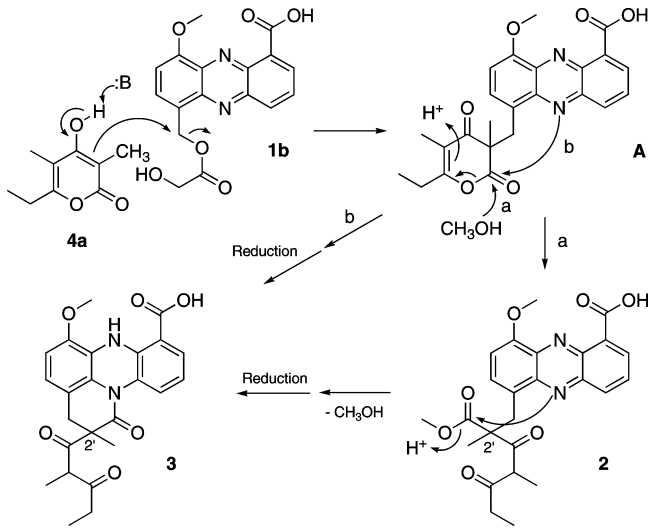

Figure 4. Hypothetical formation of compounds **2** and **3** from **1b** and **4a**.

In addition to the phenazines and pyrones, a diastereomeric mixture of dihydrosarkomycins was also identified.[13,15] The collection of diverse metabolites in the ICBB8198 strain paralleled that of the spirodionic acid producer, *Streptomyces* sp. Tü6077, which produced griseoluteic acid, griseolutein, dihydrosarkomycin, and pyrone **4a**, in addition to spirodionic acid.[13] This similarity in metabolite production with the ICBB8198 strain led us to re-examine the extract for the presence of spirodionic acid. Indeed, after growing another culture of ICBB8198 and characterizing the extract, spirodionic acid was found (characterized by MS, NMR, and comparison with literature data[13]). While there is a significant overlap in metabolites, the two *Streptomyces* strains were isolated from soil samples from distinct geographical locations (Tü6077 from the east coast of Ghana and ICBB8198 from Kalimantan, Indonesia).

Biological Activities. Compounds **2** and **3** were tested against *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, the fungus *Mucor miehei*, and the yeast *Candida albicans* in the agar diffusion test, at concentrations of 24 and 56 μg/disk, while **1a** was tested at 14 μg/disk. Compound **2** indicated activity against *Staph. aureus* with an inhibition zone of 14 mm (at 24 μg/disk), and **1a** inhibits *Staph. aureus* and *E. coli* with inhibition zones of 25 and 20 mm, respectively. Griseoluteic acid (**1a**) was mainly responsible for the high activity of the extract. Compound **3** had no antimicrobial activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco P1010 polarimeter. UV spectra were recorded on a Beckmann DU 640 B spectrophotometer. IR spectra were recorded on a Nicolet IR100 FT-IR spectrometer. NMR spectra were measured on a Bruker Unity 300 MHz spectrometer. ESIMS data were recorded on a ThermoFinnigan LCQ Advantage system with a quaternary Rheos 4000 pump (Flux Instrument). HRESI mass spectra were recorded on a Waters/Micromass LCT spectrometer. HREIMS and HRCIMS were measured on a JEOL HMS-600H MS route magnetic sector instrument. Preparative HPLC was performed using an RP18 column (Phenomenex, RP 100-C18, 5 μm) with the detector set at 254 nm. Flash chromatography was carried out on silica gel (230−400 mesh). Thin-layer chromatography was performed on aluminum sheets with silica gel 60 F_{254} (EMD chemicals Inc.). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Organism Collection and Identification. The actinomycete isolation procedure was the same as previously reported.[7] The *Streptomyces* sp. ICBB8198 culture is deposited at ICBB-CC (Indonesian Center for Biodiversity and Biotechnology Culture Collection of Microorganisms) as 0.5 mL of a 20% glycerol stock stored at −20 °C.

The 16S rRNA gene sequence (GenBank GQ470680) of *Streptomyces* sp. ICBB8198 was found to have 99.5% identity over the sequenced region to *Streptomyces* sp. LJMUEG T4 (GenBank DQ989563), isolated from a Thailand soil sample.

Fermentation and Isolation. The *Streptomyces* sp. ICBB8198 was cultivated on 6 L scale using 1 L Erlenmeyer flasks containing 250 mL of GOT medium (glycerol 60 g/L, oatmeal 15 g/L, tomato paste 5 g/L, $CaCO_3$ 3 g/L; adjusted to pH 7.0 with 1 N NaOH prior to

Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) Data for Compounds **2** and **3**[a]

	2[b]		**3**[c]	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	125.5, C		116.7/116.6, C	
2	136.9, CH	8.95, dd (7.2, 1.5)	129.0/128.95, CH	7.75, m
3	130.8, CH	8.04, dd (8.8, 7.2)	118.5/118.3, CH	6.63, m
4	134.9, CH	8.49, dt (8.8, 1.5)	124.9/124.86, CH	7.75, m
4a	142.6, C		123.5, C	
5a	144.2, C		123.3/123.2, C	
6	127.7,[d] C		124.4/124.2, C	
7	133.5, CH	7.72, dd (8.0, 3.5)	117.4/117.3, CH	6.63, m
8	108.4, CH	7.14, d (8.0)	107.6/107.55, CH	6.63, m
9	153.4, C		144.5/144.49, C	
9a	127.8,[d] C		n.d.	
10a	138.6, C		139.2, C	
1′	34.0/33.6, CH_2	4.02, m	33.4/33.3, CH_2	3.22, d (15.4); 2.75, d (15.4)
2′	61.9, C		57.7/57.5, C	
3′	205.0, C		205.7/205.6, C	
4′	57.8/57.7, CH	4.02, m	54.5/54.4, CH	4.24, q (7.0)
5′	207.0, C		206.2/206.0, C	
6′	33.3/32.7, CH_2	2.58, q (7.2)	33.2, CH_2	2.27, q (7.2)/2.36, q (7.3)
7′	7.9, CH_3	1.06, t (7.2)	7.5/7.4, CH_3	0.77, t (7.2)/0.86, t (7.0)
COOH	166.3, C		173.0, C	
9-OCH_3	56.6, CH_3	4.17, s	60.1, CH_3	3.77, s
CO_2CH_3	173.0, C			
CO_2*C*H_3	52.5/52.7, CH_3	3.60, s		
2′-CH_3	19.7/19.1, CH_3	1.31, s	20.3/20.0, CH_3	1.33, s/1.35, s
4′-CH_3	16.5/15.8, CH_3	1.41, d (7.0)	14.7/14.6, CH_3	1.09, d (7.0)/1.04, d (7.0)
10-NH				11.39, brs
*C*ON			168.2/168.1, qC	

[a] Dual entries for some positions indicate doubling of some resonances in the 1D spectra. [b] $CDCl_3$. [c] DMSO-d_6. [d] Assignments may be interchanged; n.d. = not detected.

sterilization) at 29 °C for 4 days on a rotary shaker (250 rpm). The brown culture broth was mixed with Celite and filtered under vacuum. The filtered medium was passed through an HP-20 Diaion column, the resin was washed with distilled H_2O, and compounds were eluted with 50% MeOH/50% acetone (EMA), followed by 100% MeOH. The residual H_2O from the methanolic extract was extracted with EtOAc (EAW). The mycelia were extracted sequentially with EtOAc (EAM) and then MeOH (MM). The extracts were evaporated to dryness separately. Four extracts were obtained, two from the mycelium (EAM, MM) and two from the broth (EAW, EMA). Bioassay of the four fractions was carried out against *Staph. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *My. smegmatis*, the fungus *Mu. miehei*, and the yeast *C. albicans*. While the extracts from the broth indicated high activity against *Staph. aureus* and *B. subtilis,* the mycelium extracts were active against *E. coli* and *B. subtilis*. Column chromatography of the extract EMA on Sephadex LH-20 (3% MeOH/CH_2Cl_2) gave fractions I−IV, with high activity in fraction III. Trituration of fraction III in MeOH delivered a red powdery mixture of two compounds, which were further purified by PTLC (3% MeOH/CH_2Cl_2) and identified as griseolutein A (**1b**, 5 mg) and griseoluteic acid (**1a**, 20 mg), which was responsible for the high activity against *Staph. aureus*. The purification on Sephadex LH-20 (3% MeOH/CH_2Cl_2) of the EAM extract from the mycelium gave seven fractions. Only fractions 6 and 7 indicated activity against all test organisms except the fungus *M. miehei*, and high activity was observed against *E. coli*. Fractions 6 and 7 were combined on the basis of TLC similarity, and the resulting material was separated on silica gel using a MeOH/CH_2Cl_2 gradient and afforded a diastereoisomeric mixture of dihydrosarkomycin (1D-, 2D-NMR and MS data), which was responsible for the activity against *E. coli* and *P. aeruginosa*, while the activity against the other organisms was attributed to traces of **1a**. All the other fractions were combined, and repeated column chromatography on silica gel with a CH_2Cl_2/MeOH gradient gave 5-ethyl-4-hydroxy-3,5-dimethyl-2*H*-pyran-2-one (**4a**, 6 mg). Preparative HPLC (20% MeOH/H_2O to 100% MeOH) yielded 3,6-diethyl-4-hydroxy-5-methyl-2*H*-pyran-2-one (**4b**, 1.5 mg, second culture 2.3 mg). The EAW extract was chromatographed on Sephadex LH-20 (50% MeOH/CH_2Cl_2) to provide fractions A−D. Only fraction D indicated activity against *Staph. aureus*, *B subtilis*, *E. coli*, *P. aeruginosa*, and *C. albicans*. Repeated separation on Sephadex LH-20 (100% MeOH) afforded **2** (5.1 mg) and **3** (7.5 mg) in addition to griseoluteic acid (**1a**).

Compound 2: yellow solid; $[\alpha]_D$ +150 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 364 (3.25), 268 (3.44), 247 (sh), 208 (3.50) nm; IR (neat) ν_{max} 2923, 1719, 1700, 1540, 1457, 1173, 1106, 764 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75.5 MHz, $CDCl_3$) data, see Table 1; (+)-ESIMS *m/z* (%) 467 ($[M + H]^+$, 100), 489 ($[M + Na]^+$, 30), 955 ($[2M + Na]^+$, 85); (−)-ESIMS *m/z* (%) 465 ($[M - H]^+$, 100), 953 ($[2M - H + Na]^-$, 25); (+)-HRESIMS *m/z* 467.1850 $[M + H]^+$ (calcd for $C_{25}H_{27}N_2O_7$, 467.1818) and 489.1619 $[M + Na]^+$ (calcd for $C_{25}H_{27}N_2O_7Na$, 467.1638).

Compound 3: yellow powder; $[\alpha]_D$ −17 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 370 (2.75), 206 (3.13), 248 (sh), 274 (sh) nm; IR (neat) ν_{max} 2920, 2850, 1727, 1697, 1667, 1635, 1584, 1518, 1463, 1377, 1282, 1248 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75.5 MHz, DMSO-d_6) data, see Table 1; ^1H NMR (300 MHz, MeOH-d_4) (+)-ESIMS *m/z* (%) 437 ($[M + H]^+$, 7), 459 ($[M + Na]^+$, 11), 895 ($[2M + Na]^+$, 100); (−)-ESIMS *m/z* (%) 435 ($[M - H]^-$, 100), 893 ($[2M - H + Na]^+$,15); (+)-HREIMS *m/z* 436.16326 (calcd for $C_{24}H_{24}N_2O_6$, 436.16344).

3,6-Diethyl-4-hydroxy-5-methyl-2*H*-pyran-2-one (4b): colorless oil; UV (MeOH) λ_{max} (log ε) 279 (2.48), 207 (2.79) nm; IR (neat) ν_{max} 2971, 2936, 1670, 1565, 1453, 1407, 1220, 1169, 1126, 1077, 1038 cm^{-1}; ^1H NMR (300 MHz, MeOH-d_4) δ 2.58 (q, J = 7.6 Hz, 2H), 2.45 (q, J = 7.4 Hz, 2H), 1.97 (s, 3H, 3-CH_3), 1.21 (t, J = 7.6 Hz, 3H), 1.04 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75.5 MHz, MeOH-d_4) 169.2 (C, C-4), 161.3 (C, C-2/C-6), 109.4 (C, C-5), 105.1 (C, C-3), 25.3 (CH_2, C-7), 17.8 (CH_2, C-10), 13.2 (CH_3, C-11), 12.1 (CH_3, C-8), 10.0 (CH_3, C-9); (−)-ESIMS *m/z* (%) 181 ($[M - H]^-$, 100); HREIMS *m/z* 182.09404 (calcd for $C_{10}H_{14}O_3$, 182.09430).

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Supporting Information Available: ^1H and ^{13}C NMR, HSQC, and HMBC spectra for **2**, **3**, and **4b** and a protocol for obtaining the 16S rRNA gene sequence are available. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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