J. Antibiot. 61(7): 449–456, 2008

ORIGINAL ARTICLE

THE JOURNAL OF ANTIBIOTICS

Rearranged and Unrearranged Angucyclinones from Indonesian *Streptomyces* spp.

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Received: March 15, 2008 / Accepted: July 8, 2008

Abstract Two Indonesian *Streptomyces* strains, ICBB8309 and ICBB8415, were investigated for their ability to produce antibiotic compounds. In addition to the known antibiotics actiphenol, naramycin B, and sabaramycin, six new angucyclinones were identified. The isolation, structure elucidation and biological activities for the six new compounds are presented. The angucyclinones 7-deoxo-6-deoxy-7-hydroxy-8-*O*-methylrabelomycin, 1-deoxo-1-hydroxy-8-*O*-methylrabelomycin, and the angucycline 7-deoxo-7-hydroxy-1-*O*-α-rhamnosyl-8-*O*-methyltetrangulol have common angular backbones, while angucyclinone C, limamycin A, and limamycin B appear to be rearranged angucyclinones.

Keywords *Streptomyces* metabolites, angucyclinone, polyketide, antibiotics, MTT assay

Introduction

In the course of screening for new antibiotics from Indonesian Black Water Ecosystem microorganisms [1], two *Streptomyces* isolates were found to produce a variety of related bioactive metabolites. The strain ICBB8309 was isolated from a peat soil sample collected from the Black Water river Sungai Kala, Garung Village, Pulang Pisau and ICBB8415 was isolated from a soil sample from the Black Water river Pangkoh Lima, Malibu Village, Pulang Pisau. The pH of the water in this ecosystem is 5.0. Further investigation of the extracts from these strains revealed known antibiotics, as well as several new angucyclinone polyketides.

The *Streptomyces* isolate ICBB8309 was cultured at 28°C for 10 days in yeast, malt, and glucose (YMG) medium. The yellow crude extract obtained from the culture broth was separated on silica gel and Sephadex LH-20, and final purification was done by preparative HPLC which delivered the known antibiotics actiphenol [2] and naramycin B [3] and the previously described angucyclinones (−)-6-deoxy-8-*O*-methylrabelomycin (**1a**) [4], 8-*O*-methylrabelomycin (**1b**) [4], SM-196B (**1c**) [5], and tetrangulol methyl ether (**2a**) [6]. These known compounds were identified by substructure, MS and NMR data searches in the natural products database, Antibase [7]. In addition, the new angucyclinones 7-deoxo-6-deoxy-7-hydroxy-8-*O*-methylrabelomycin (**1d**) and angucyclinone C (**3**) were discovered (Fig. 1), as well as 4-methoxy-3*H*-isobenzofuran-1-one [8], which is described here for the first time from a natural source. In the same manner, the crude extract of the isolate ICBB8415 grown in YMG medium delivered in addition to the known metabolites found in ICBB8309, the polyene sabaramycin A [9]

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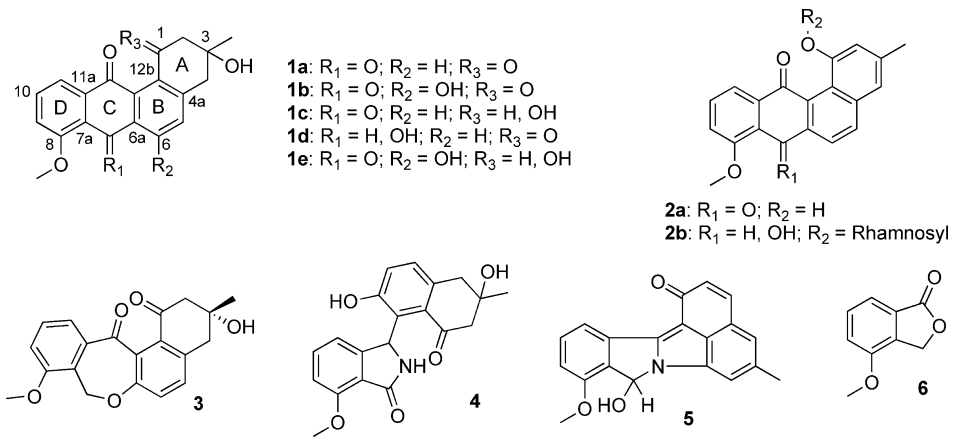

Fig. 1 Structures of angucyclinones and a related compound from *Streptomyces* strains ICBB8309 and ICBB8415.

1a=(−)-6-deoxy-8-*O*-methylrabelomycin; **1b**=8-*O*-methylrabelomycin; **1c**=SM-196B; **1d**=7-deoxo-6-deoxy-7-hydroxy-8-*O*-methylrabelomycin; **1e**=1-deoxo-1-hydroxy-8-*O*-methylrabelomycin; **2a**=tetrangulol methyl ether; **2b**=7-deoxo-7-hydroxy-1-*O*-α-rhamnosyl-8-*O*-methyltetrangulol; **3**=angucyclinone C; **4**=limamycin A; **5**=limamycin B; **6**=4-methoxy-3*H*-isobenzofuran-1-one.

and the novel metabolites 1-deoxo-1-hydroxy-8-*O*-methylrabelomycin (**1e**), 7-deoxo-7-hydroxy-1-*O*-α-rhamnosyl-8-*O*-methyltetrangulol (**2b**), limamycin A (**4**), and limamycin B (**5**) (Fig. 1).

Results and Discussion

Compound **1d** was obtained as a yellow oil. The ^{1}H- and ^{13}C-NMR spectra exhibited 16 proton and 20 carbon resonances, including two carbonyls at δ=198.5 and 186.4 instead of three as in the spectrum of **1a**. The HSQC spectrum indicated the presence of one *C*- and one *O*-methyl, two methylenes, one *O*-substituted methine, and five aromatic methines. The remaining ^{13}C-NMR resonances included one quaternary sp^3, two carbonyls and seven quaternary sp^2 carbon atoms. The HREI-MS for the molecular ion at *m/z* 338.1143 along with the NMR data corresponded to a molecular formula of $C_{20}H_{18}O_5$ suggesting the presence of two exchangeable protons. The proton NMR spectrum of **1d** is very similar with that of **1a** with a 1,2,3-trisubstituted aromatic system, isolated *ortho*-coupled aromatic protons, and a methoxy group at δ=3.99 which exhibited a correlation in the HMBC spectrum to the carbon at δ=157.3 (C-8) (Fig. 2). Two methylene groups, one as a coupled pair of doublets at δ=3.04 and 2.76 (J=16.0 Hz) and one as a singlet at δ=3.04, along with a methyl singlet at δ=1.36 were visible and were correlated to the quaternary carbon at C-3 (δ=70.5) in the HMBC spectrum. Furthermore, a methine singlet appears at δ=5.88 that was absent in **1a** suggesting the reduction of one carbonyl group in **1a**. This methine correlates to the carbon at δ=61.6 in the HSQC spectrum. The position of the new secondary alcohol was defined by the correlation of H-6 to the methine carbon at δ=61.6 indicating that the carbonyl at C-7 in **1a** was reduced in **1d** providing the new angucyclinone, 7-deoxo-6-deoxy-7-hydroxy-8-*O*-methylrabelomycin (**1d**). Compound **1d**, although chemically stable, was readily oxidized to 6-deoxy-8-*O*-methylrabelomycin (**1a**) using pyridinium chlorochromate (PCC).

Compound **1e** was obtained as a yellow oil from a preparative HPLC separation. The ^{1}H-NMR spectrum is very similar to that of **1b**, with the major difference between the spectra being the presence in **1e** of a broad apparent quartet (δ=5.40) and doublet (δ=4.92; exchangeable proton) integrating for one proton each suggesting that one of the carbonyls in **1b** has been reduced. The ^{1}H,^{1}H-COSY spectrum showed a coupling between the methine at δ=5.40 and the doublet of doublet methylene protons (H-2) indicating that the C-1 carbonyl has been reduced. The carbon spectrum supported this conclusion on the basis of only two carbonyl groups at δ=188.6 and 187.3 in **1e** and the presence of an additional methine carbon at δ=65.0. The reduction of the carbonyl at C-1 was confirmed by the HMBC correlation of the H-2 protons to C-1 at δ=65.0 (Fig. 2). The name 1-deoxo-1-hydroxy-8-*O*-methylrabelomycin is proposed for **1e**. Although the compound was found to be stable in solution, it appears to be readily oxidized to **1b** during measurement of the mass spectrum.

High resolution MS data of the yellow compound **2b** suggested a molecular formula of $C_{26}H_{26}O_8$. The ^{1}H-NMR spectrum was similar to that of **2a**, but there was a new

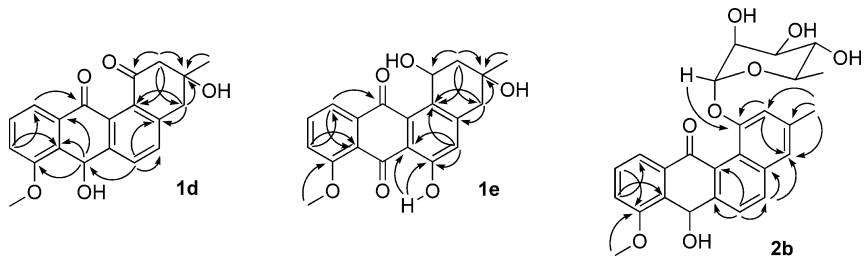

Fig. 2 Selected HMBC correlations for compounds **1d**, **1e**, and **2b**.

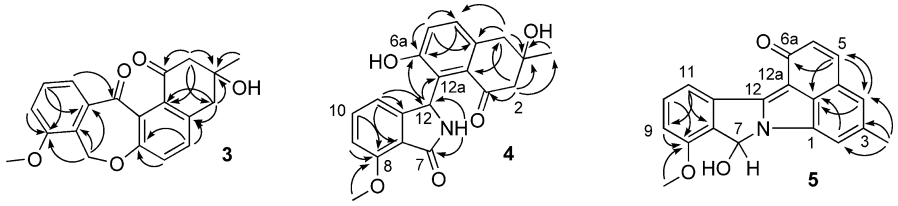

Fig. 3 Selected HMBC correlations for compounds **3**, **4**, and **5** (the numbering for compounds **4** and **5** retains the numbering from the putative angucyclinone precursors).

singlet at δ=7.00, five new coupled protons ranging from δ=5.78 to 3.65, and a methyl doublet at δ=1.33. Coupling data in the ^{1}H,^{1}H-COSY spectrum indicated the presence of a sugar residue in **2b** and the anomeric proton at δ=5.78 had a small coupling (J=1.9 Hz) which indicated an α-linkage to the aglycone. The sugar was identified as a rhamnose on the basis of the coupling constants and comparison with literature data [10, 11].

The ^{13}C-NMR spectrum showed 26 carbons, which includes the six carbons for the rhamnose, but revealed only one downfield signal (δ=185.0) instead of two as in **2a**, suggesting a reduction of one carbonyl, which was supported by the presence of a sp^3 methine carbon at δ=66.9. A ^{1}H,^{1}H-COSY correlation between a broad singlet at δ=7.00 and H-6 suggests that the C-7 carbonyl was reduced. The position of the *O*-sugar was determined from the interpretation of the HMBC spectrum, which exhibited a small cross peak of the anomeric proton (δ=5.78) to the C-1 carbon at δ=156.7 (Fig. 2). The name 7-deoxo-7-hydroxy-1-*O*-α-rhamnosyl-8-*O*-methyltetrangulol is proposed for compound **2b**.

Compound **3** was obtained as a yellow oil. EIMS and HRCI-MS presented the molecular formula for **3** as $C_{20}H_{18}O_5$, which was supported by the NMR data. The ^{1}H-NMR spectrum shows similarity with those of **1a** and **1b** with the three consecutive aromatic protons, a methoxy resonance, the methyl singlet and two AB resonances attributed to two methylene groups at δ 3.08 and δ 2.62. In addition, the ^{1}H-NMR spectrum indicates a new two-proton singlet at δ 5.08, while the methine singlet which appears in **1d** at δ 5.58 (H-7) is absent in **3**. The ^{13}C-NMR spectrum indicated 20 carbon resonances including two carbonyl carbons as in **1d** and **1e**. The DEPT experiment established the multiplicities of the carbon resonances as five sp^2 methines, one methoxy, one methyl, and three methylene carbons, and the remaining eight carbon signals represent quaternary carbons (seven sp^2 and one sp^3). The major difference between the ^{13}C spectra of **1d** and **3** is the replacement of the methine at δ 61.6 in **1d** by an oxygen-bearing methylene at δ 55.0 in compound **3**.

Interpretation of the HMBC spectrum indicated that both methylene protons at δ=3.08 and 2.62 had correlations to the carbon at δ=70.9 (C-3), the quaternary carbon at δ=130.5 (C-12b) and the methyl carbon (δ=27.8). In addition, the methyl proton and the H-4 protons correlated to the carbon at δ=133.6 (C-4a) and important couplings were observed between the proton at δ=7.31 (H-5) and the methylene carbon at δ=42.0 (C-4) and the quaternary carbon at δ=153.1 (C-6a), providing the A and B rings seen in compounds **1a** and **1d** (Fig. 3). A key difference, however, is the ^{13}C-NMR shift of C-6a (δ=153.1), which indicates C-6a is connected to an oxygen, rather than a carbon.

The second structure element of **3** was determined based on the similarity of the aromatic regions of the NMR spectra with those of **1a** and **1b** (a 1,2,3-trisubstituted benzene ring bearing a methoxy group). The remaining carbonyl group at δ=201.1 and the methylene at δ=55.0

are required to connect the substructures. The important HMBC correlation between the protons at δ 5.08 and the carbon at δ=158.2 (C-8), the ^{13}C chemical shift of C-6a, as well as the lack of a correlation between H-6 and the carbonyl at δ=201.1, support structure **3**. The structure represented by **3** was previously reported as angucyclinone C in a review by Rohr *et al.* [12], but no data was available to support the structure. Therefore, we report these data here for the first time. Angucyclinone C (**3**) is a ring-expanded angucyclinone that has the same tetracyclic ring structure as emycin D, an angucyclinone from the mutant strain *Streptomyces cellulosae* ssp. *griseoincarnatus* 1114-2 [13]. It may arise from the oxidative cleavage of 6-deoxy-8-*O*-methylrabelomycin (**1a**) followed by enzymatic reduction at C-7. A similar mechanistic hypothesis has been previously described by Rohr *et al.* [13] for the formation of emycin D through an enzymatic Baeyer-Villiger oxidation of ochromycinone. Because (−)-6-deoxy-8-*O*-methylrabelomycin (**1a**) has the *R* configuration at C-3 [14], and **3** likely derives from **1a**, the *R* configuration is also proposed for C-3 of **3**.

Compound **4** was isolated as a white powder. The molecular formula, $C_{20}H_{19}NO_5$, was suggested from the HRESI-MS spectrum. The ^{13}C-NMR spectrum included carbons at δ=201.4 and 169.7, which indicated the presence of a ketone and an amide, acid or ester, respectively. The presence of two methylene groups as AB quartets at δ=2.97 and 2.78 and the methyl singlet (δ=1.27) in the ^{1}H-NMR spectrum reflected a similar aliphatic region to that observed in **3**, suggesting an identical A-ring. The sp^2 region of **4** indicated two exchangeable protons at δ=9.17 and 8.16 in addition to four doublets and a triplet, which represent the 1,2,3 trisubstituted aromatic system and the isolated *ortho*-coupled protons that are found in compounds **1a**, **1c**, **1d**, **2a**, **2b** and **3**. Finally, a broad methine singlet appeared at δ=6.84.

Interpretation of the HMBC spectrum confirmed an A-ring identical to that in **3**, as well as establishing that it was fused to an aromatic ring bearing *ortho*-coupled protons. Important correlations included the proton at δ=7.08 (H-5) to the carbons at δ=155.9 (C-6a), 133.0 (C-12b), and 124.5 (C-12a), the proton at δ=6.77 (H-6) to the carbons at δ=133.7 (C-4a) and 124.5 (C-12a), and the protons at δ=2.97 (H-4) to the carbons at δ=133.7 and 130.4 (C-5) (Fig. 3). The HMBC correlations provided A and B rings nearly identical to those seen in **3**, including the oxygen-bearing C-6a.

The second part of the structure contained the methoxy substituted aromatic system (D ring), a carbonyl at δ=169.7, a methine carbon at δ=52.8, and an exchangeable proton at δ 8.16. The nitrogen indicated by the MS data also must be included in this substructure. The HMBC correlations of the triplet at δ=7.35 (H-10) and the doublet at δ=6.67 (H-11) to the methine carbon at δ=52.8 (C-12) place the methine adjacent to H-11. The HMBC spectrum also displayed correlations of the proton at δ=8.16 to both the carbonyl at δ=169.7 and to the methine at δ=52.8. Because the two oxygens available for this substructure have already been incorporated (methoxy, carbonyl), the exchangeable proton must be connected to the lone nitrogen. This positions the nitrogen between the carbonyl and the methine at δ=52.8 (C-12), whose chemical shift is consistent with a bond to nitrogen. Finally, an HMBC correlation from δ=6.91 (H-9) to the carbon at δ=169.7 (C-7) places the carbonyl adjacent to the methoxy group. The dihydroisoindolone substructure is supported by comparison of the ^{13}C-NMR shifts with those for the model compound 2,3-dihydro-7-methoxy-1*H*-isoindol-1-one [15].

Weak HMBC correlations from H-12 (δ=6.84) to C-12a (δ=124.5) and C-6a (δ=155.9) connect the two substructures, providing structure **4**, which has been named limamycin A. It is likely derived from a rearrangement of (−)-6-deoxy-8-*O*-methylrabelomycin (**1a**), but the origin of the nitrogen is unknown. The structure of limamycin A has the same carbon skeleton as emycin E isolated from *Streptomyces cellulosae* ssp. *griseoincarnatus* 1114-2 [13].

Compound **5** was obtained as a yellow oil from preparative HPLC. The proton NMR spectrum of **5** shows some similarities to that of **2b** with the methoxy group at δ=3.95, the aromatic methyl singlet at δ=2.55 and a downfield methine proton at δ=6.88. The aromatic protons were sorted into three spin systems from the ^{1}H,^{1}H-COSY spectrum: a 1,2,3-trisubstituted aromatic system, two doublets of adjacent protons and two aromatic protons in a *meta* relationship. The carbon NMR spectrum indicated 20 carbon signals including only one carbonyl at δ=179.6, one sp^2 oxygenated carbon at δ=155.9, and a methine carbon at δ=81.4 which is downfield compared to the C-7 methine of the reduced angucyclinones **1d** and **2b**, suggesting that this methine is in a different environment. The molecular weight of 317 Daltons was deduced from the (+)-ESI MS and the high resolution measurement of that peak indicated the formula $C_{20}H_{15}NO_3$, which was consistent with the NMR data. This formula requires 14 unsaturations, which is one or two unsaturations higher than angucyclinones **1a**~**e**. Interpretation of the HMBC spectrum indicated correlations of the aromatic methyl to the carbons at δ=133.8 (C-3), 123.5 (C-4) and 113.9 (C-2). In addition, the proton at δ=7.79 (H-5) displayed cross peaks to the carbons at δ=123.5 (C-4), 128.2 (C-12b), and to the carbonyl at δ=179.6, and the *meta* coupled protons

H-2 and H-4 had correlations to δ=128.2 (C-12b), which helped to construct a methyl-substituted naphthalenone substructure. An additional substructure was confirmed as a 1,2,3-trisubstituted aromatic system with a methoxy substitutent due to the correlations of the H-10, H-9, and methoxy protons to the carbon at δ=155.9 (C-8).

A search in AntiBase with these fragments yielded HKI 0231A and B, which were isolated from *Streptomyces* sp. HKI 0231 [16]. These compounds, however, possess three or two methoxy groups, respectively, instead of one as in **5**. Compound **5** was determined to be the *O*-desmethyl analog of HKI 0231B and has been named limamycin B. Limamycin B may be the biosynthetic precursor to HKI 0231B, although it should be noted that **5** is optically active, whereas HKI 0231B is not.

Limamycin B (**5**) was previously reported as an intermediate in the synthesis of HKI 0231B [17], but this is the first report of **5** from a natural source. Biosynthetically it may be derived from **4** by cyclization followed by dehydration and rearrangement, along with reduction at C-7 of **4** (which may occur prior to cyclization).

Compound **6** was isolated as a white powder. The proton NMR spectrum exhibited an apparent triplet at δ=7.57 (J=7.7 Hz) and two doublets at δ=7.45 (J=7.7 Hz) and 7.32 (J=7.7 Hz) which were attributed to a 1,2,3-trisubstituted aromatic system. The aliphatic region indicated only two singlets, due to methylene and methoxy protons at δ=5.34 and 3.96, respectively. Based on the MW=165 and the presence of a carbonyl carbon in the ^{13}C-NMR spectrum, the structure must contain another ring in addition to the aromatic ring. Combining the partial structures led to the two known phthalides, 4-methoxy-3*H*-isobenzofuran-1-one and 7-methoxy-3*H*-isobenzofuran-1-one. Comparison of ^{1}H-NMR data in $CDCl_3$ with values in the literature suggested that **6** was 4-methoxy-3*H*-isobenzofuran-1-one [8]. An HMBC spectrum which showed correlations from H-3 to C-1, C-3a, C-4, and C-6a confirmed this assignment. Although compound **6** has been prepared synthetically [8, 18], this is the first report as a natural product. The structural similarity of **6** to the D-rings of the angucyclinones isolated from ICBB 8309 suggests that this compound arises from oxidative cleavage of one of them, such as angucyclinone C (**3**).

Biological Activities

Although the new compounds did not display the dominant antibiotic activity of these strains, which is attributed to actiphenol, naramycin B, and sabaramycin, they were all tested for antibiotic activity. Compounds **1d**, **3**, **4** and **5** were tested at a concentration of 50 μg/disk, while compounds **1e** and **2b** were tested at 60 and 45 μg/disk, respectively, against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis*, *Mucor miehei* and *Candida albicans* in disk diffusion assays. Compound **1d** exhibited weak activity against *S. aureus* (8.0 mm), *P. aeruginosa* (11 mm) and *Mucor miehei* (8.0 mm); compound **1e** had weak activity against *S. aureus* (10 mm) and *Mycobacterium smegmatis* (8.0 mm), and compound **3** inhibited *S. aureus* (8.0 mm) and *Mucor miehei* (8.0 mm). For comparison, tetramycin at 50 μg/disk as a positive control against *Mucor miehei* provided a 17 mm inhibition zone. No antibiotic activity was observed for the other metabolites. Compounds **1d** and **3** also exhibited moderate antiproliferative activity against PC3-cells (androgen independent prostate cancer cells) as determined by MTT assays [19]. They were tested at two concentrations (10 and 100 μM) and cell viability was measured at 24 hours and 48 hours. Neither compound significantly affected cell viability at 24 hours at either concentration, but both compounds resulted in a cell viability of 50% at 48 hours at each concentration.

Experimental

General

NMR spectra were measured on a Bruker Unity 300 MHz spectrometer. ESI-MS were recorded on a ThermoFinnigan LCQ Advantage system. HRESI mass spectra were recorded on a Waters/Micromass LCT spectrometer. HREI-MS and HRCI-MS were measured on a JEOL HMS-600H MS route magnetic sector instrument. IR spectra were recorded on a Nicolet IR100 FT-IR spectrophotometer. UV-VIS spectra were recorded on a Beckmann DU 640 B spectrophotometer. Optical rotations were measured on a Jasco P1010 polarimeter (10 and 100 mm cells were used). Preparative HPLC was performed using an RP C18 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). Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Identification of the Producing Strains

Details of the isolation of bacterial strains and general procedures for the 16S rDNA analysis were as previously described [1]. The cultures of *Streptomyces* strains ICBB8309 and ICBB8415 were deposited at the 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 sequences of *Streptomyces* strains ICBB8309 (Genbank

EU490287) and ICBB8415 (Genbank EU490288) were found to be 99.8% identical over a 1394 bp overlap region. Both were found to have >99.4% identity (~1400 bp overlap) with the 16S rRNA of *Streptomyces* sp. 98-62 (Genbank DQ450946) and >99.2% identity (~1400 bp overlap) with the 16S rRNA of *Streptomyces gelaticus* strain NRRL B-2928 (Genbank DQ026636).

Fermentation and Isolation

The strain *Streptomyces* sp. ICBB8309 was cultured in 20 1-liter Erlenmeyer flasks each containing 250 ml of YMG medium at 28°C for 10 days: 4.0 g glucose, 4.0 g yeast, 10 g malt and 1-liter tap water. The pH was adjusted to 7.8 with 2 N NaOH before sterilization. The yellow culture broth was filtered over Celite and the mycelial cake was extracted sequentially with EtOAc and MeOH. The aqueous filtrate was passed through Diaion HP-20 resin, which was then washed with deionized water and the desired compounds were eluted with MeOH. The organic phases from the mycelium and resin extracts were evaporated and the residual water was extracted with EtOAc. Because both extracts contained the same constituents based on TLC, they were mixed and concentrated to a dark yellow oil. The extract was chromatographed on silica gel with a CH_2Cl_2/MeOH gradient and nine fractions were obtained. Fraction 1 contained only fatty acids and was not further separated. Preparative TLC (PTLC; CH_2Cl_2) of fraction 2 followed by preparative HPLC with a gradient from 20% MeOH/H_2O to 100% MeOH delivered tetrangulol methyl ether (**2a**, 5.0 mg) and 4-methoxy-3H-isobenzofuran-1-one (**6**, 3.0 mg). Trituration of fraction 3 in MeOH delivered the colorless solid, actiphenol (70 mg). The purification of fraction 4 on Sephadex LH-20 (50% MeOH/CH_2Cl_2) gave three sub-fractions, 4I, 4II and 4III. Sub-fraction 4I contained naramycin B (100 mg). PTLC of fraction 4II in 6.0% MeOH/CH_2Cl_2 and Sephadex LH-20 (MeOH) chromatography delivered 6-deoxy-8-*O*-methylrabelomycin (**1a**, 3.0 mg) and 8-*O*-methylrabelomycin (**1b**, 4.0 mg). Preparative HPLC of fraction 4III using a gradient from 20% MeOH/H_2O to 100% MeOH yielded SM-196-B (**1c**, 7.0 mg). Fraction 6 was chromatographed on Sephadex LH-20 (MeOH), resulting in two sub-fractions. The purification of 6I on HPLC gave 7-deoxo-6-deoxy-7-hydroxy-8-*O*-methylrabelomycin (**1d**, 8.0 mg). The purification of fraction 8 on Sephadex LH-20 (MeOH) and preparative HPLC delivered angucyclinone C (**3**, 500 mg) as a major metabolite. It is important to note that during all the chromatographic separations, the flasks as well as test tubes were covered with aluminum foil to prevent light degradation of the metabolites, which was observed during screening on a 1-liter scale.

The strain ICBB8415 was cultivated on a 5-liter scale in YMG medium for 5 days followed by a standard work up. During all separations the columns were wrapped with foil to avoid photochromism and slow decomposition. The yellow crude extract was dissolved in a mixture of 50% MeOH/CH_2Cl_2 and a yellow solid powder remained, which was identified as sabaramycin A (6.0 mg). The soluble material was separated over silica gel using a MeOH/CH_2Cl_2 gradient to give five fractions. Purification of fraction 2 delivered naramycin B (25 mg), 8-*O*-methylrabelomycin (**1b**, 2.0 mg), 1-deoxo-1-hydroxy-8-*O*-methylrabelomycin (**1e**, 4.0 mg) and limamycin B (**5**, 3.0 mg). Trituration of fraction 3 with MeOH delivered the solid actiphenol and purification of the mother liquor yielded angucyclinone C (**3**, 15 mg). Fraction 4 was purified on HPLC using a gradient from 20% MeOH/H_2O to 100% MeOH to yield 6-deoxy-8-*O*-methyl rabelomycin (**1a**, 16 mg), 7-deoxo-7-hydroxy-1-*O*-α-rhamnosyl-8-*O*-methyltetrangulol (**2b**, 4.0 mg) and limamycin A (**4**, 15 mg).

7-Deoxo-6-deoxy-7-hydroxy-8-*O*-methylrabelomycin (**1d**)

Yellow oil; $[\alpha]_D^{23}=+53°$ (*c* 0.078, MeOH); UV (MeOH): λ (log ε)=331 (3.71), 278 (4.10), 290 (4.09), 236 (4.50) nm; IR (neat): ν=3419, 2959, 2909, 2847, 1687, 1658, 1590, 1466, 1302, 1259, 1120, 1068, 1021, 820, 746 cm^{-1}; ^{1}H-NMR (300 MHz, MeOH-d_4,): δ=7.70 (d, *J*=8.0 Hz, 1H, H-5), 7.56~7.49 (m, 2H, H-10, H-11), 7.42 (d, *J*=8.0 Hz, 1H, H-6), 7.27 (dd, *J*=7.9, 1.4 Hz, 1H, H-9), 5.88 (s, 1H, H-7), 3.99 (s, 3H, OCH_3), 3.04 (d, *J*=16.0 Hz, 1H, H-2a), 3.04 (s, 2H, H-4), 2.76 (d, *J*=16.0 Hz, 1H, H-2b), 1.36 (s, 3H, 3-CH_3); ^{13}C-NMR (75.5 Hz, MeOH-d_4): δ=198.5 (C-1), 186.4 (C-12), 157.3 (C-8), 142.6 (C-4a), 142.4 (C-11a), 135.4 (C-12a), 134.6 (C-6a), 134.0 (C-12b), 133.1 (C-5), 132.9 (C-10), 129.6 (C-6), 129.4 (C-7a), 118.0 (C-11), 114.8 (C-9), 70.5 (C-3), 61.6 (C-7), 55.1 (OCH_3), 53.0 (C-2), 43.1 (C-4), 28.5 (3-CH_3); EI-MS (70 eV) *m/z* (%)=338 ($[M]^+$, 100), 318, 278, 188, 171, 126, 84; HREI-MS: 338.1143 (338.1154 calcd. for $C_{20}H_{18}O_5$).

1-Deoxo-1-hydroxy-8-*O*-methylrabelomycin (**1e**)

Yellow oil; $[\alpha]_D^{23}=+6.1°$ (*c* 0.11, MeOH); UV (MeOH): λ (log ε)=416 (4.33), 251 (4.58), 227 (4.60) nm. IR (neat): ν=3419, 2959, 2909, 2847, 1687, 1658, 1590, 1466, 1302, 1259, 1120, 1068, 1021, 820, 746 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ=13.28 (s, 1H, 6-OH), 7.91 (d, *J*=8.4 Hz, 1H, H-11), 7.76 (d, *J*=8.4 Hz, 1H, H-10), 7.37 (d, *J*=8.4 Hz, 1H, H-9), 7.10 (s, 1H, H-5), 5.40 (brq, *J*=5.5 Hz, 1H, H-1), 4.92 (d, *J*=4.3 Hz, 1H, 1-OH), 4.09 (s, 3H, OCH_3), 3.00 (AB, *J*=17.0 Hz, 2H, H-4), 2.33 (brdd, *J*=14.1, 5.9 Hz, 1H, H-2a), 2.17 (brdd, *J*=14.1, 5.5 Hz, 1H,

H-2b), 1.52 (s, 3H, 3-CH_3); ^{13}C-NMR (75.5 Hz, $CDCl_3$): δ=188.6 (C-7), 187.3 (C-12), 161.8 (C-6), 160.3 (C-8), 146.3 (C-4a), 136.9 (C-11a), 135.8 (C-10), 133.7 (C-12b), 133.1 (C-12a), 125.9 (C-5), 120.4 (C-11), 120.0 (C-7a), 117.9 (C-9), 117.2 (C-6a), 69.1 (C-3), 65.0 (C-1), 56.7 (OCH_3), 45.6 (C-4), 44.2 (C-2), 29.5 (3-CH_3); EI-MS (70 eV) *m/z* (%)=354 $[M]^+$, 336; HREI-MS: 354.1064 (354.1103 calcd. for $C_{20}H_{18}O_6$).

7-Deoxo-7-hydroxy-1-*O*-α-rhamnosyl-8-*O*-methyltetrangulol (**2b**)

Yellow oil; $[\alpha]_D^{20}=-83°$ (*c* 0.01; MeOH); UV (MeOH): λ (log ε)=380 (sh), 275 (3.72), 231 (4.03) nm; IR (neat): ν=3342, 2968, 2941, 2837, 1768, 1674, 1583, 1492, 1458, 1439, 1304, 1267, 1235, 1209, 1101, 1029, 957, 921, 880, 823 cm^{-1}; ^{1}H-NMR (300 MHz, Acetone-d_6): δ=8.13 (d, *J*=8.6 Hz, 1H, H-6), 7.87 (d, *J*=8.6 Hz, 1H, H-5), 7.63 (t, *J*=8.0 Hz, 1H, H-10), 7.44 (s, 1H, 2-H), 7.43 (s, 1H, H-4), 7.36 (d, *J*=8.0 Hz, 1H, H-11), 7.16 (d, *J*=8.0 Hz, 1H, H-9), 7.00 (s, 1H, H-7), 5.78 (d, *J*=1.9 Hz, 1H, H-1′), 4.67 (m, 1H, H-2′), 4.26 (dd, *J*=9.0, 3.6 Hz, 1H, H-3′), 3.90 (s, 3H, OCH_3), 3.88 (m, 1H, H-5′), 3.65 (t, *J*=9.0 Hz, 1H, H-4′), 2.53 (s, 1H, 3-CH_3), 1.33 (d, *J*=6.2 Hz, 3H, H-6′); ^{13}C-NMR (75.5 Hz, Acetone-d_6): δ=185.0 (C-12), 161.2 (C-8), 156.7 (C-1), 147.5 (C-11a), 140.0 (C-12a), 139.9 (C-3), 139.3 (C-6a), 135.3 (C-10), 133.3 (C-4a), 129.7 (C-5), 124.8 (C-6), 123.6 (C-11), 123.2 (C-4), 122.0 (C-7a), 114.4 (C-2), 113.0 (C-9), 108.2 (C-12b), 101.6 (C-1′), 74.2 (C-4′), 73.7 (C-3′), 72.0 (C-2′), 71.3 (C-5′), 66.9 (C-7), 56.7 (OCH_3), 22.2 (CH_3-3), 18.7 (C-6′); ESI-MS *m/z* (%)=467 ($[M+H]^+$, 33), 489 ($[M+Na]^+$, 100), 955 ($[2M+Na]^+$, 95); HRESI-MS: 467.1702 (467.1706 calcd. for $C_{26}H_{27}O_8$).

Angucyclinone C (**3**)

Yellow oil; $[\alpha]_D^{30}=-13°$ (*c* 0.12; MeOH); UV (MeOH): λ (log ε)=324 (3.20), 256 (3.72), 226 (4.12) nm; IR (neat): ν=3342, 2968, 2941, 2837, 1768, 1674, 1583, 1492, 1458, 1439, 1304, 1267, 1235, 1209, 1101, 1029, 957, 921, 880, 823 cm^{-1}; ^{1}H-NMR (300 MHz, MeOH-d_4): δ=7.31 (d, *J*=8.4 Hz, 1H, H-5), 7.25~7.14 (m, 3H, 6, 9, H-10), 6.92 (brdd, *J*=6.7, 1.5 Hz, 1H, H-11), 5.08 (s, 2H, H-7), 3.90 (s, 3H, OCH_3), 3.08 (AB, *J*=16.6 Hz, 2H, H-4), 2.62 (AB, *J*=16.6 Hz, 2H, H-2), 1.35 (s, 3H, 3-CH_3); ^{13}C-NMR (75.5 Hz, MeOH-d_4): δ=201.1 (C-12), 197.8 (C-1), 158.2 (C-8), 153.1 (C-6a), 139.1 (C-11a), 133.6 (C-4a), 131.4 (C-5), 130.5 (C-12b), 129.1 (C-7a), 128.1 (C-10), 127.6 (C-12a), 122.7 (C-6), 121.7 (C-11), 114.7 (C-9), 70.9 (C-3), 55.3 (OCH_3), 55.0 (C-7), 51.4 (C-2), 42.0 (C-4), 27.8 (3-CH_3); EI-MS (70 eV) *m/z* (%)=338 ($[M]^+$, 28), 320 (100), 291 (261), 277 (12), 253 (13), 160 (10); HRCI-MS: 338.1153 (338.1154 calcd. for $C_{20}H_{18}O_5$).

Limamycin A (**4**)

White powder; $[\alpha]_D^{20}=+14°$ (*c* 0.25; MeOH); UV (MeOH): λ (log ε)=329 (3.66), 291 (3.80), 258 (3.82), 237 (3.87), 212 (3.87) nm; IR (neat): ν=3342, 2968, 2941, 2837, 1768, 1674, 1583, 1492, 1458, 1439, 1304, 1267, 1235, 1209, 1101, 1029, 957, 921, 880, 823 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 9.17 (s, 1H, 6a-OH), 8.16 (s, 1H, NH), 7.35 (t, *J*=8.0 Hz, 1H, H-10), 7.08 (d, *J*=8.2 Hz, 1H, H-5), 6.91 (d, *J*=8.2 Hz, 1H, H-9), 6.84 (brs, 1H, H-12), 6.77 (brd, *J*=8.2 Hz, 1H, H-6), 6.67 (d, *J*=8.2 Hz, 1H, H-11), 4.85 (s, 1H, 3-OH), 3.83 (s, 3H, OCH_3), 2.97 (AB, *J*=16.3 Hz, 2H, H-4), 2.78 (AB, *J*=15.6 Hz, 2H, H-5), 1.27 (s, 3H, 3-CH_3); ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ=201.4 (C-1), 169.7 (C-7), 157.0 (C-8), 155.9 (C-6a), 151.6 (C-11a), 133.7 (C-4a), 133.0 (C-12b), 132.9 (C-10), 130.4 (C-5), 124.5 (C-12a), 121.4 (C-6), 120.9 (C-7a), 115.0 (C-11), 110.2 (C-9), 70.5 (C-3), 55.8 (OCH_3), 54.9 (C-2), 52.8 (C-12), 44.1 (C-4), 29.9 (3-CH_3); ESI-MS *m/z* (%)=354 ($[M+H]^+$, 100), 729 ($[2M+Na]^+$, 30); HRESI-MS: 376.1187 (376.1185 calcd. for $C_{20}H_{19}NO_5Na$).

Limamycin B (**5**)

Yellow oil; $[\alpha]_D^{21}=+84°$ (*c* 0.01, MeOH); UV (MeOH): λ (log ε)=463 (3.26), 436 (3.31), 380 (sh), 366 (3.22), 287 (3.31), 215 (3.93) nm; IR (neat): ν=3339, 2925, 2853, 1672, 1674, 1586, 1558, 1488, 1457, 1377, 1268, 1080, 1024, 855, 756 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ=8.00 (d, *J*=7.9 Hz, 1H, H-11), 7.79 (d, *J*=9.6 Hz, 1H, H-5), 7.64 (t, *J*=7.9 Hz, 1H, H-10), 7.61 (s, 1H, H-2), 7.45 (d, *J*=9.1 Hz, 1H, 7-OH), 7.45 (s, 1H, H-4), 7.26 (d, *J*=7.9 Hz, 1H, H-9), 6.88 (d, *J*=9.1 Hz, 1H, H-7), 6.57 (d, *J*=9.6 Hz, 1H, H-6), 3.95 (s, 3H, OCH_3), 2.55 (s, 3H, CH_3); ^{13}C-NMR (DMSO-d_6, 75.5 MHz): δ=179.6 (C-6a), 155.9 (C-8), 147.5 (C-12), 137.5 (C-5), 133.8 (C-3), 132.6 (C-7a), 132.2 (C-10), 132.0 (C-6), 131.1 (C-1), 130.5 (C-11a), 128.2 (C-12b), 123.6 (C-12a), 123.5 (C-4), 116.2 (C-11), 114.3 (C-9), 113.9 (C-2), 108.0 (C-4a), 81.4 (C-7), 55.7 (OCH_3), 21.7 (3-CH_3) (the ^{13}C-NMR shifts all differ by ~9 ppm from those previously published [17] due to an inadvertent error in referencing the spectrum; personal communication from A. Scopton and T.R. Kelly); ESI-MS *m/z* (%)=318 ($[M+H]^+$, 100), 340 ($[M+Na]^+$, 100), 657 ($[2M+Na]^+$, 37); HREI-MS: 317.1047 (317.1052 calcd. for $C_{20}H_{15}NO_3$).

4-Methoxy-3*H*-isobenzofuran-1-one (**6**)

White powder; ^{1}H-NMR (300 MHz, MeOH-d_4): δ=7.57 (t, *J*=7.7 Hz, 1H, H-6), 7.45 (d, *J*=7.7 Hz, 1H, H-7), 7.32 (d, *J*=7.7 Hz, 1H, H-5), 5.34 (s, 2H, H-2), 3.96 (s, 3H, OCH_3); ^{13}C-NMR (75.5 MHz, MeOH-d_4): δ=171.2 (C-1), 154.2 (C-4), 137.8 (C-3a), 127.4 (C-7a), 116.3 (C-7), 115.1 (C-

5), 68.2 (C-3), 54.9 (OCH_3); ESI-MS *m/z* (%)=165 ($[M+H]^+$, 100), 187 ($[M+Na]^+$, 85).

Biological Assays

The disk diffusion assay [20] was used to test antibiotic susceptibility. Each test organism was grown at 37°C for 24 hours in 3 ml of medium (tryptic soy broth for *S. aureus*; YMG medium for *E. coli*, *P. aeruginosa*, and *My. smegmatis*; potato dextrose broth for *M. miehei*; and Sabourand broth for *C. albicans*). The 24 hours cultures (150 μl) were mixed with agar medium (15 ml total) and poured into Petri dishes. MeOH was used to dissolve each pure compound and 20 μl of the compound solution (representing 45~60 μg) was applied to a 6.0 mm paper disk (Whatman). The disk was allowed to dry for an hour before it was placed on the plate. The plates were incubated at 37°C for bacteria and 30°C for fungi and the diameter of the inhibition zone (mm) of each active compound was measured after 24 hours. Disks treated with 20 μl MeOH were used as negative controls. The MTT cell viability assay was performed as previously described [21].

Acknowledgements We thank J. Morré for high resolution mass spectra (supported by a grant from NIEHS-ES00210) and Dr. Emily Ho for conducting the MTT assays. This work was supported by research funds from the College of Pharmacy, Oregon State University.

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