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New α-Pyrone-Containing Metabolites from a Marine-Derived Actinomycete

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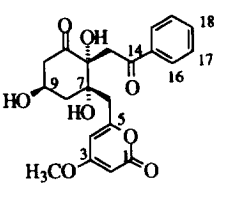
Abstract: Four new α-pyrone-containing metabolites, wailupemycins A-C (**1-3**) and 3-epi-5-deoxyenterocin (**4**), were isolated together with known compounds enterocin (**6**) and 5-deoxyenterocin (**5**) from a *Streptomyces* sp. cultured from shallow water marine sediments. The structures of the new compounds were determined through the interpretation of spectral data. Compound **1** exhibited antimicrobial activity towards the Gram-negative bacterium *Escherichia coli*, while compound **4** inhibited the growth of *Staphylococcus aureus*.

Over the past ten years, marine natural products researchers have expanded the scope of their studies to include not only macroorganisms, such as sponges, ascidians, and soft corals, but marine microorganisms as well.[1,2] Marine bacteria, in particular, have received increased attention as potential sources of biologically active metabolites.[3,4] Although bacteria have been isolated from a variety of marine sources, sediments continue to receive the greatest attention, perhaps because of their similarity to terrestrial soils and because they have been shown to be a good source of Gram-positive organisms.[5] Recent results include the isolation of halobacillin,[6] caprolactins A and B,[7] γ-indomycinone,[8] and the bioxalomycins,[9] which were all produced by sediment-derived Gram-positive bacteria. We would now like to add to this list four new α-pyrone-containing metabolites, wailupemycins A-C (**1-3**) and 3-epi-5-deoxyenterocin (**4**), which were isolated along with known compounds 5-deoxyenterocin (**5**) and enterocin (**6**) from a streptomycete cultured from shallow water marine sediments.



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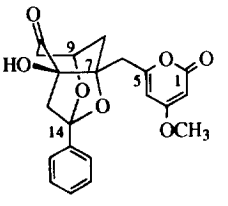

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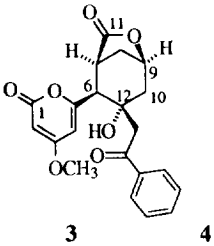

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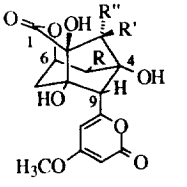


4 R = H; R' = H; R" = COPh
5 R = H; R' = COPh; R" = H
6 R = OH; R' = COPh; R" = H

The actinomycete designated BD-26T(20), isolated from sediments collected at Wailupe beach park on the south-east shore of Oahu, Hawaii, was determined to belong to the genus *Streptomyces* based on morphological and chemotaxomonic analyses. Specifically, the organism produced abundant gray-white aerial mycelium, which transformed into chains of smooth, cylindrical arthrospores, as shown by scanning-electron microscopy. An analysis of the whole-cell hydrolysate revealed the presence of only the LL-isomer of diaminopimelic acid and a fatty acid methyl ester (FAME) analysis showed a pattern consisting primarily of saturated iso and anteiso fatty acids. Isolate BD-26T(20) also demonstrated the ability to metabolize casein, tyrosine, and xanthine, and it produced no characteristic sugars.

For chemical investigation, the organism was fermented in a sea-water based medium,[10] and the whole culture broth was partitioned with ethyl acetate. The crude extract was subjected to a solvent partition sequence yielding fractions containing hexane-, $CHCl_3$-, and aqueous MeOH-soluble materials. Further fractionation of the $CHCl_3$-soluble material using a combination of silica gel and gel permeation column chromatographies and normal phase HPLC over silica yielded four new α-pyrone containing compounds, wailupemycins A-C (**1-3**) and 3-epi-5-deoxyenterocin (**4**), together with the known terrestrial *Streptomyces* metabolites 5-deoxyenterocin (**5**),[11,12] and enterocin (**6**)[13,14] (=vulgamycin[15]).

Enterocin (**6**) was readily identified by comparing its spectral data with those reported in the literature.[13,15] In contrast, because at the time when this investigation took place 5-deoxyenterocin (**5**) had been reported only in the abstracts of two patent applications, without accompanying spectral data,[11,12] independent confirmation of its structure was necessary, especially upon isolation of compound **4** which exhibited spectral characteristics similar to those of **5**. Since that time, complete NMR spectral assignments for 5-deoxyenterocin in DMSO-d_6 have been reported;[16] nevertheless, for the purpose of comparison and because our data was recorded in deuteriochloroform, NMR assignments for both **4** and **5** are included in Table I. EIMS data indicated that both 5-deoxyenterocin (**5**) and metabolite **4** have a molecular formula of $C_{22}H_{20}O_9$, incorporating one less oxygen atom than enterocin ($C_{22}H_{20}O_{10}$), and requiring 13 sites of unsaturation. The NMR spectra of both compounds were qualitatively similar (see Table I), containing signals for a monosubstituted benzene ring and a methoxy-substituted α-pyrone ring; however, the proton chemical shifts for the signals assigned to $H5_{eq}$ (2.19 ppm for **5** vs. 2.95 ppm for **4**) and H9 (4.24 ppm for **5** vs. 3.40 ppm for **4**) showed considerable discrepancy between compounds.

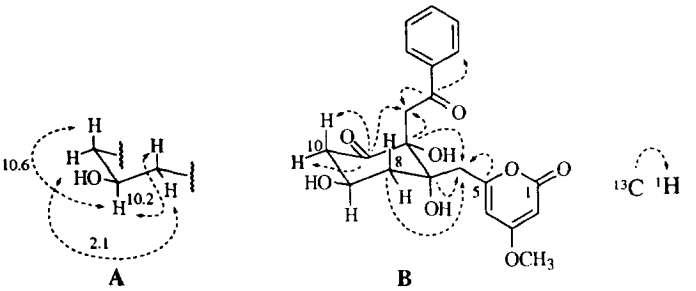
Table I. NMR Data for Compounds 4 and 5.

	4		5	
Atom no.	^{13}C	^1H (mult., *J* (Hz))	^{13}C	^1H (mult., *J* (Hz))
1	172.9		174.6	
2	77.4		77.7	
3	64.9	4.13 (s)	60.0	4.01 (s)
4	75.7		76.7	
5	33.3	H_{eq} 2.95 (br d, 15.4) H_{ax} 2.56 (dd, 15.4, 3.2)	38.3	H_{eq} 2.19 (dd, 15.5, 1.9) H_{ax} 2.86 (dd, 15.5, 4.4)
6	73.6	4.84 (m)	74.9	4.99 (m)
7	36.5	H_{eq} 1.76 (br d, 14.7) H_{ax} 2.48 (dd, 14.7, 3.2)	37.5	H_{eq} 1.86 (dd, 14.7, 2.4) H_{ax} 2.73 (dd, 14.7, 2.9)
8	75.5		76.5	
9	59.3	3.40 (br s)	56.8	4.24 (br s)
10	160.0		160.3	
11	104.6	6.30 (d, 1.8)	105.8	6.31 (d, 1.9)
12	171.1		171.3	
13	88.4	5.49 (d, 1.8)	89.0	5.49 (d, 1.9)
14	164.1		164.4	
15	56.0	3.8 (s)	56.3	3.84 (s)
16	197.7		197.6	
17	137.6		138.6	
18	128.9	7.96 (d, 7.5)	129.1	7.87 (d, 7.5)
19	128.4	7.43 (t, 7.5)	128.6	7.16 (t, 7.5)
20	133.8	7.58 (t, 7.5)	134.6	7.49 (t, 7.5)

All of the ^1H and ^{13}C NMR signals for both **4** and **5** could be assigned using COSY and HMQC experiments, and the carbon frameworks were shown to be identical on the basis of HMBC data. The difference between compounds **4** and **5** was resolved using difference NOE experiments. Irradiation of the H3 signal in the spectrum of 5-deoxyenterocin resulted in an enhancement of the signal assigned to $H5_{eq}$, while irradiation of the

same proton in the spectrum of 3-epi-5-deoxyenterocin resulted in an enhancement of the signal assigned to H9. Therefore, 5-deoxyenterocin (**5**) has the same relative stereochemistry as was determined for enterocin (**6**) and compound **4** can be assigned as the C3 epimer, 3-epi-5-deoxyenterocin.

The EIMS of wailupemycin A (**1**) showed its highest mass ion at *m/z* 384, with HRMS supporting a molecular formula of $C_{21}H_{20}O_7$; however, the presence of an MH^+ ion at *m/z* 403 in the FABMS suggested that the correct formula is $C_{21}H_{22}O_8$, with the EIMS ion resulting from dehydration. The NMR data (Table II) confirmed the presence of the monosubstituted benzene and the α-pyrone ring, while the ^{13}C NMR data identified two additional ketone carbonyls. Altogether, these functional groups account for 10 of the required 11 sites of unsaturation. Because no additional sp^2 hybridized carbons were apparent in the ^{13}C NMR spectrum, wailupemycin A must contain one additional ring. The ^1H NMR spectrum included signals for one carbinol methine (4.30 ppm) and 4 methylenes, two of which were isolated as indicated by their AB/AX spin systems. Analysis of the ^1H-^1H coupling constants indicated that the remaining two methylenes (H8 and H10) are each adjacent to the methine to form a 5 proton spin system, as in substructure **A**. Observation of the large diaxial couplings between H9 and both $H8_{ax}$ and $H10_{ax}$, together with the 2.2 Hz W-coupling between $H8_{eq}$ and $H10_{eq}$, require the hydroxyl group to lie in the equatorial position, as shown below (**A**). The HMBC experiment (see Table III) then allowed assembly of the molecule, as shown in structure **B**.



A **B**

The relative stereochemistry of wailupemycin A (**1**) was determined using difference NOE techniques. Dipolar coupling was observed between the H8 signal at δ 2.00, the H10 signal at δ 2.84, and the H13 signal at δ 3.44 indicating that all three of these groups are axial. Irradiation of the H6 signal at δ 2.76 resulted in an enhancement of the signal assigned to $H8_{ax}$ (2.00 ppm) placing the pyrone-containing side chain in the equatorial position.

The absolute stereochemistry of wailupemycin A (**1**) was investigated using the modified Mosher's method.[17] Treatment of **1** with both (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride in pyridine yielded the (*S*)- and (*R*)-MTPA esters, respectively. Subtraction of the ^1H NMR chemical shifts observed for the (*R*)-MTPA ester from those of the (*S*)-ester resulted in the δΔ values shown in Figure 1. Based on these results, the absolute stereochemistry is 7*S*, 9*R*, 12*R*, as shown.

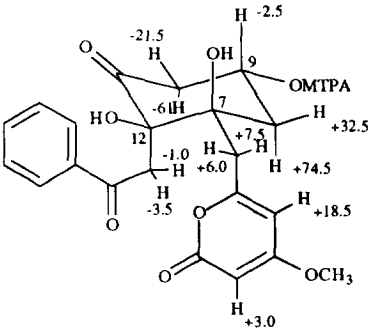


Figure 1. δΔ values (Hz) generated from subtracting δ_S-δ_R of the MTPA esters of **1**.

HREIMS indicated that wailupemycin B (**2**) had a molecular formula of $C_{21}H_{20}O_7$, differing from that of wailupemycin A by the absence of a molecule of H_2O, increasing the number of unsaturations to 12. The NMR spectra (see Table II) were similar to those of compound **1**, except for the absence of one signal assigned to a ketone carbonyl and the addition of one new carbon signal at δ 105.9. The HMBC data (see Table III) indicated that both wailupemycins A and B have the same carbon

Table II. NMR Data for Wailupemycins A-C (1-3).

	1		2		3	
Atom no.	^{13}C	^{1}H (mult., *J* (Hz))	^{13}C	^{1}H (mult., *J* (Hz))	^{13}C	^{1}H (mult., *J* (Hz))
1	164.7		164.4		163.5	
2	88.3	5.47 (d, 2.2)	88.1	5.43 (d, 2.5)	88.8	5.33 (d, 2.2)
3	170.9		171.0		170.2	
4	103.5	5.95 (d, 2.2)	103.2	6.02 (d, 2.5)	103.1	6.40 (d, 2.2)
5	160.3		160.4		162.4	
6	40.5	H_a 2.76 (d, 14.2) H_b 2.95 (d, 14.2)	38.7	H_a 2.86 (d, 14.9) H_b 3.03 (d, 14.9)	54.1	3.22 (d, 2.0)
7	77.7		85.3		40.0	2.93 (m)
8	40.6	H_{ax} 2.00 (dd, 14.4, 10.2) H_{eq} 2.50 (ddd, 14.4, 5.3, 2.2)	36.0	H_a 1.64 (d, 13.3) H_b 2.41 (ddd, 13.3, 5.0, 2.5)	38.6	H_{ax} 2.17 (d, 12.0) H_{eq} 2.60 (m)
9	66.4	4.30 (m)	69.0	4.76 (m)	76.7	4.90 (m)
10	46.2	H_{ax} 2.84 (dd, 12.8 10.6) H_{eq} 3.09 (ddd, 12.8, 5.3, 2.2)	43.7	H_a 2.72 (dd, 18.6, 5.3) H_b 3.15 (br d, 18.6)	43.0	H_{ax} 2.09 (d, 15.1) H_{eq} 2.60 (m)
11	206.4		208.9		176.8	
12	81.0		85.0		75.6	
13	42.6	H_a 3.44 (d, 15.7) H_b 3.89 (d, 15.7)	54.6	H_a 2.56 (d, 13.6) H_b 2.76 (d, 13.6)	43.4	H_a 3.20 (d, 17.2) H_b 3.30 (d, 17.2)
14	197.2		105.9		201.1	
15	136.4		138.4		136.3	
16	128.8	7.97 (d, 7.5)	125.0	7.59 (d, 7.5)	128.4	7.86 (d, 8.0)
17	128.7	7.50 (t, 7.5)	128.3	7.40 (t, 7.5)	128.8	7.47 (t, 8.0)
18	134.0	7.62 (t, 7.5)	128.9	7.37 (t, 7.5)	134.0	7.59 (t, 8.0)
21	55.9	3.80 (s)	55.8	3.80 (s)	55.6	4.60 (s)

skeleton. The difference between these compounds could be determined from the long range correlations observed from the signal at 105.9 to the carbinol proton H9, the methylene protons H13, and the ortho-aromatic protons H16, thus indicating that the secondary and one of the tertiary alcohols in wailupemycin A had reacted with the benzylic ketone to form an acetal at C14, as in structure **2**. Interestingly, conversion of wailupemycin A (**1**) to wailupemycin B (**2**) would require epimerization of the stereocenter at C7. Formal interconversion between **1** and **2** is possible through a retro-aldol/aldol type mechanism, involving an intermediate with a carbonyl at C7. Unfortunately, the limited amount of sample precluded our attempt to perform the appropriate experiment to test this possibility. Our efforts to hydrolyze the acetal under a variety of acidic conditions either resulted in no reaction or in decomposition. Apparently, the relative stereochemistry of wailupemycin B favors formation of the acetal, while the relative stereochemistry of wailupemycin A favors the free ketone.

Table III. HMBC Data Showing Long-Range ^{13}C->^{1}H Correlations.

Carbon No.	1	2	3
1		H2	
2	H4	H4	H4
3	H2, H4, H21	H2, H4, H21	H2, H4, H21
4	H2, H6	H2, H6	H2, H6
5	H4, H6	H4, H6	H4, H6
6		H4	H7, H8, H13, C12-OH
7	H6, $H8_{eq}$	H6, $H8_b$, H9, $H13_b$, C12-OH	H6, H8, H9
8	H6, H10	H6, H9, H10	H6
9	H8, H10	$H10_b$, $H8_b$	H7, H8
10	$H8_{ax}$	H8, H9	H8, H13, C12-OH
11	H10, H13B	H9, H10, H13, C12-OH	H6, H7, $H8_{ax}$, H9
12	H8, H10, H13	H13	H6, H10, H13, C12-OH
13			H6, H10
14	H13, H16	H9, H13, H16	H13, H16
15	H17	H13B, H18	H17
16	H17, H18	H17	H18
17	H16, H18	H16	H16
18			H16

Wailupemycin C (**3**) was shown by MS to be isomeric with wailupemycin B (**2**), having a molecular formula of $C_{21}H_{20}O_7$. The IR spectrum displayed absorptions at 3441, 1769, and 1694 cm^{-1}, consistent with the presence of hydroxyls, an α-pyrone or γ-lactone, and unsaturated ketone functionalities, respectively. In addition to the characteristic signals, the ^{13}C NMR spectrum (see Table II) showed a signal assigned to an additional ester carbonyl (176.8 ppm), a ketone carbonyl (201.1 ppm), an oxygenated quaternary carbon (76.7 ppm), 3 methines (75.6, 54.1, and 40.0 ppm), and 3 methylene carbons (43.4, 43.0, and 38.6 ppm). The ^{1}H NMR spectrum (Table II) included signals for three methylene groups (H8, H10, and H21), three methines (H6, H7, and H9), and an exchangeable proton. The COSY spectrum indicated that the methines and two of the three methylenes could be combined into a single spin system containing carbons C6-C10. The HMBC data (see Table III) allowed the remainder of the molecule to be assembled. Important correlations for assigning the bicyclic ring system included those from carbons C6, C10, and C12 to the exchangeable proton C11-OH, allowing the formation of a 6-membered ring, and from lactone carbonyl C11 to protons H6, H7, H8, and H9. The attachment of the α-pyrone ring to C6 was established based on a correlation from C5 to H6 and the position of the benzophenone moiety was determined through the observation of correlations from ketone carbonyl C14 to methylene protons H13 and to the ortho protons (H16) of the phenyl ring.

The relative stereochemistry of wailupemycin C (**3**) was established using difference NOE experiments. Enhancements observed for the signals assigned to $H8_{ax}$ and $H10_{ax}$ upon irradiation of H6 indicated that these

protons are all in axial positions and that the α-pyrone ring lies in an equatorial orientation. Also, irradiation of the H13 signal at δ 3.20 resulted in an enhanced signal for $H10_{eq}$, but not for $H10_{ax}$, suggesting that the tertiary hydroxyl group is equatorial and the acetophenone group is axial.

The α-pyrone moiety is commonly observed in many antibiotics and toxins. Examples of simple α-pyrone containing metabolites include nectriapyrone, isolated from terrestrial fungus *Gyrostroma missouriense*,[18] nectriapyrones A and B, isolated from an unidentified marine fungus separated from the sponge *Jaspis johnstoni*,[19] and tetra-acetic acid lactone, isolated from *Penicillium stipitatum*.[20] A more complex example includes citreoviridin, a potent α-pyrone-containing mycotoxin produced by a variety of strains of *Penicillium* fungi.[21,22] Additional citreoviridin-related compounds have been isolated from the fungus *Aspergillus terreus*.[23] Of special interest is the recent isolation of enterocin and 5-deoxyenterocin, along with the 5-behenate and 5-arachidate esters of enterocin, from a marine ascidian of the genus *Didemnum*.[16] The occurrence of the same compounds in organisms as disparate as prokaryotes and chordates emphasizes a question of significant current interest. That is, whether symbiotic or associated microorganisms play a role in the production of the metabolites isolated from marine invertebrates.

Enterocin (**6**) was reported to be bacteriostatic against Gram-positive and Gram-negative bacteria, including *Escherichia coli* and species within the genera *Proteus*, *Sarcina*, *Staphylococcus* sp., and *Corynebacterium*, at the high concentration of 4 mg/mL.[24] In addition, when enterocin was applied postemergence on cotton, rice, and wheat, it controlled weeds such as *Ipomia* without crop damage.[25] 5-Deoxyenterocin (**5**) was reported in a patent abstract as an antibiotic which inhibited *Sarcina lutea*, *Staphylococcus aureus*, *Klebsiella pnuemoniae*, and *Vibrio percolans* at a concentration of 0.5 mg/mL.[11]

Compounds **1-4** were tested for antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli in vitro* using the paper disk diffusion method.[26] Compound **4** exhibited activity against only *S. aureus* (18 mm zone of inhibition at 1 mg/6 mm disk), while compound **1** was inhibitory to only *E. coli* (15 mm zone at 0.1 mg/6 mm disk). Compounds **2** and **3** were inactive against all three test organisms at 0.1 mm/disk. Further determination of minimum inhibitory concentrations (MIC's) was not possible due to the limited amount of compounds available.

EXPERIMENTAL SECTION

General. All NMR experiments were performed on a GE Omega 500 instrument at 500 MHz and 125 MHz operating frequencies for ^1H and ^{13}C experiments, respectively. Chemical shifts are referenced to solvent peaks: 7.24 ppm (residual $CHCl_3$) and 77.0 ppm for $CDCl_3$. IR spectra were obtained using a Perkin-Elmer 1600 FTIR and mass spectral data were obtained on a VG-70SE mass spectrometer operating in the EI and FAB modes. The UV spectral data were recorded on a Milton Roy spectronic 3000 diode array spectrophotometer.

Culture Conditions. The bacterium, designated BD-26T(20), was obtained from a shallow water sediment sample collected from Wailupe beach park, on the south-east shore of the island of Oahu, Hawaii. The sample was diluted with 2 mL of sterile artificial sea water and shaken well. The supernatant (50 μL) was plated on the marine agar 2216 (Difco) with a glass spreader and inoculated at 23-28 °C. A pure colony was selected and grown in 20 two-liter flasks each containing 500 mL of marine broth 2216 (Difco) supplemented with 1% aqueous starch for 3 days at 23-25 °C on orbital shakers at 230 rpm.

Taxonomic Analysis. Whole-cell sugars were determined using the procedure of Lechevalier[27] and analysis of the isomers of diaminopimelic acid (DAP) contained in the cell wall was performed as described by Becker *et al.*[28] Casein, tyrosine, and xanthine media were prepared according to Staneck and Roberts.[29]

Extraction and Isolation. The liquid culture was extracted with ethyl acetate yielding 854 mg of organic soluble material. The crude organic extract was dissolved in 90% aqueous methanol (100 mL) and the resulting solution was partitioned with hexane (2 x 100 mL). The aqueous methanol layer was further diluted with water to give a 65% aqueous methanol solution which was extracted with chloroform (2 x 150 mL). The chloroform fraction (411 mg) was subjected to silica gel flash chromatography, employing a step-wise chloroform to

methanol gradient to a pure fraction of enterocin (**6**, 43 mg), and two mixed fractions which gave ^{1}H NMR spectra similar to that of **6**.

Further purification of the mixed fractions over Sephadex LH-20 (110 x 2 cm) using $CHCl_3$/MeOH (1:1) as eluent followed by repetitive normal-phase chromatography [Rainin Dynamax 60-A Si, 25 cm x 10 mm, $CHCl_3$/MeOH (98:2), 2.30 mL/min] yielded 5-deoxyenterocin (**5**, 19 mg), and three new compounds wailupemycins A (**1**, 4 mg), B (**2**, 7 mg) and C (**3**, 3 mg).

Investigation of a second batch of BD-26T(20) gave 3.9 g of crude extract (from 40 liters of the fermentation broth). The crude extract was subjected to the solvent partition scheme to give hexane, chloroform and methanol fractions. The chloroform fraction was further purified using normal phase flash chromatography, Sephadex LH-20, and repetitive normal phase HPLC (all of the solvent systems and conditions are same as mentioned above) to give enterocin (**6**, 460 mg), 5-deoxyenterocin (**5**, 685 mg), 3-epi-5-deoxyenterocin (**4**, 27 mg), and wailupemycin A (**1**, 7 mg).

Wailupemycin A (1): $[\alpha]_D$ +30.0° (0.4 mg/mL, MeOH), UV (MeOH) λ_{max} 208 (ε 18,600), 251 (ε 13,540), 272 nm (ε 12,250); IR (film) υ 3445, 1716, 1697, 1646, 1558, 1456, 1417, 1250, 1146, 1036, 755 cm^{-1}; ^{1}H NMR see Table II; ^{13}C NMR see Table II; EIMS *m/z* (rel int) 384 (0.4), 366 (2), 348 (11), 288 (10), 211 (10), 185 (40), 125 (37), 105 (100), 77 (54); HREIMS 384.1193 ($C_{21}H_{20}O_7$, Δ 1.5 mmu).

Wailupemycin B (2): $[\alpha]_D$ +77.7° (0.7 mg/mL, MeOH), UV (MeOH) λ_{max} 208 (ε 17,660), 279 (ε 6,830); IR (film) υ 3446, 2925, 1716, 1699, 1646, 1559, 1457, 1417, 1338, 1248, 1118, 1020, 943, 758 cm^{-1}; ^{1}H NMR see Table II; ^{13}C NMR see Table II; EIMS *m/z* (rel int) 384 (15), 356 (12), 209 (29), 140 (64), 125 (47), 105 (100), 77 (59); HREIMS 384.1200 ($C_{21}H_{20}O_7$, Δ -0.9 mmu).

Wailupemycin C (3): $[\alpha]_D$ +16.0° (1.0 mg/mL, MeOH); UV (MeOH) λ_{max} 207, 239, 281 nm; IR (film) υ 3441, 2928, 1769, 1694, 1644, 1567, 1538, 1455, 1415, 1352, 1246, 1135, 1079, 956, 802, 754 cm^{-1}; ^{1}H NMR see Table II; ^{13}C NMR see Table II; EIMS *m/z* (rel int) 384 (2), 120 (20), 105 (100), 77 (74); HREIMS 384.1212 ($C_{21}H_{20}O_7$, Δ -0.3 mmu).

3-epi-5-Deoxyenterocin (4): $[\alpha]_D$ -22.9° (1 mg/mL), UV (MeOH) λ_{max} 207 (ε 21,500), 239 (ε 9,980), 281 nm (ε 10,750); IR (film) υ 3407, 2942, 1684, 1640, 1562, 1456, 1409, 1336, 1290, 1252, 1165, 1070, 989, 907, 815, 757 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR see Table I; EIMS *m/z* (rel int) 428 (5), 323 (12), 235 (22), 185 (22), 167 (26), 140 (24), 125 (37), 105 (100), 77 (40); HREIMS 428.1113 ($C_{22}H_{20}O_9$, Δ -0.6 mmu).

5-Deoxyenterocin (5): $[\alpha]_D$ -38.8° (47 mg/mL), UV (MeOH) λ_{max} 248 (ε 13,800), 282 (ε 15,800) nm; IR (film) υ 3438, 1748, 1687, 1639, 1559, 1455, 1403, 1370, 1336, 1251, 1223, 1147, 1081, 1000, 749 cm^{-1}; ^{1}H NMR see Table I; ^{13}C NMR see Table I; EIMS *m/z* (rel int) 428 (2), 348 (15), 243 (20), 235 (13), 185 (31), 167 (16), 140 (24), 125 (42), 105 (100), 77 (66); HREIMS 428.1127 ($C_{22}H_{20}O_9$, Δ -2.0 mmu).

Esterification of wailupemycin A (1) (R)- and (S)- MTPA esters: To a solution of wailupemycin A (1 mg, 2.4 μmol) in d_5-pyridine (250 μL) was added (*R*)-MTPA chloride (10 μL, 54.3 μmol). After 10 min at room temperature, the solvent was evaporated and the resulting residue was subjected to normal phase HPLC over silica gel, affording the pure (*S*)-MTPA ester. ^{1}H NMR (CD_2Cl_2) δ 7.93 (dd, 2H, *J* = 8.5,1 Hz), 7.61 (m, 1H), 7.45 (m, 7H), 5.97 (d, 1H, *J* = 2 Hz), 5.47 (d, 1H, *J* = 2 Hz), 5.45 (m, 1H), 3.99 (d, 1H, *J* = 16 Hz), 3.81 (s, 3H), 3.53 (s, 3H), 3.36 (d, 1H, *J* = 16 Hz), 3.12 (ddd, 1H, *J* = 12.0, 5.5, 2.5 Hz), 2.97 (d, 1H, *J* = 14 Hz), 2.89 (t, 1H, *J* = 12.0), 2.75 (d, 1H, *J* = 14 Hz), 2.57 (ddd, 1H, *J* = 14, 5.5, 2.5 Hz), 2.20 (dd, 1H, *J* = 14, 10.5 Hz).

The preparation of (*R*)-MTPA ester followed the same procedure as the preparation of (S)-MTPA ester. ^{1}H NMR (CD_2Cl_2) δ 7.96 (dd, 2H, *J* = 8.5, 1 Hz), 7.60 (m, 1H), 7.48 (m, 7H), 5.93 (d, 1H, *J* = 2.5 Hz), 5.46 (d, 1H, *J* = 2.5 Hz), 5.45 (m, 1H), 3.96 (d, 1H, *J* = 15.5 Hz), 3.81 (s, 3H), 3.52 (s, 3H), 3.36 (d, 1H, *J* = 15.5 Hz), 3.17 (ddd, 1H, *J* = 12.5, 5.5, 2 Hz), 3.04 (t, 1H, *J* = 11.0 Hz), 2.95 (d, 1H, *J* = 14 Hz), 2.74 (d, 1H, *J* = 14 Hz), 2.50 (ddd, 1H, *J* = 14, 5.5, 2 Hz), 2.05 (dd, 1H, *J* = 14, 8.5 Hz).

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