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Isolation and Identification of the Icosalides—Cyclic Peptolides with Selective Antibiotic and Cytotoxic Activities

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This article is dedicated to the late Professor K. L. Rinehart, a truly outstanding scientist whose ingenuity and drive paved the way for many interesting discoveries in the field of natural products research. One of us (CP) had the good fortune to be a postdoctoral researcher with Professor Rinehart from 1978–1980.

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Abstract Three cyclic peptolides have been isolated from two different fungal species and their structures determined. Icosalides A1 (**1a**), A2 (**1b**), and B (**1c**) each contain two serine and two leucine amino acid residues and incorporate two fatty acid moieties as part of the central twenty-member ring. **1a** contains L-serine and both D- and L-leucine residues, while **1b** and **1c** contain only L-amino acid residues. Icosalide A1 displays antimicrobial activity against *Streptococcus pyogenes*, *S. pneumoniae* (Felton), and *Enterococcus faecalis*. Icosalides A2 and B are cytotoxic to replicating MDCK cells.

Keywords antibiotic, cyclic depsipeptide, icosalide, fungi, peptolide

Introduction

Cyclic depsipeptide compounds have been identified from marine sponges [1], marine [2] and terrestrial [3] cyanobacteria, Gram-positive [4] and Gram-negative organisms [5], and from both marine [6] and terrestrial fungi [7]. Biological activities are diverse and include antifungal [8, 9] and antibiotic [10, 11] properties as well as calcium channel antagonism [12] and cholesteryl ester transfer protein inhibition [13]. The ring sizes of these oligopeptides are as varied as their biological activities; thirteen-membered rings comprising three peptides [14] and forty-membered rings with twelve peptides [10] bracket a host of ring sizes and components that can include unusual α- and β-amino acids [5, 15, 16] as well as one, two, or three linear and branched alkanoic acid residues ranging in length from three [16] to eighteen [14] and beyond. The icosalides described herein comprise twenty-membered rings formed by amide and ester linkages of two serine residues, two leucine residues, and two hydroxy-alkanoic acid units. These compounds are representative of the peptolide subset of the depsipeptides in that their ester bonds are formed by the incorporation of fatty acids into the ring structure [17] rather than by simple

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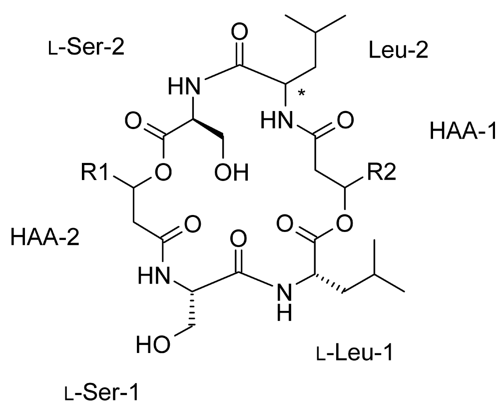

1a : R1= C_7H_{15}; R2 = C_5H_{11}; D-Leu-2
1b : R1= C_5H_{11}; R2 = C_7H_{15}; L-Leu-2
1c : R1= R2 = C_5H_{11}; L-Leu-2

heterodetic linkage of the amino acid carbonyl to a free hydroxyl on an amino acid already incorporated as part of the compound [6, 18].

Results and Discussion

Large-scale antibacterial screening of fungal extracts identified an organism from which we isolated icosalide A1 (**1a**) that manifested activity against *Streptococcus pyogenes* and weaker activity against *S. pneumoniae* (Felton) and *Enterococcus faecalis*. In one of the quirks common in natural products research, we subsequently isolated two related cyclic depsipeptides, icosalide A2 (**1b**) and icosalide B (**1c**), as cytotoxic components of a different fungal extract identified in our anti-influenza screen.

Icosalide A1 (**1a**) was isolated from a MeOH extract of the fungus OSI 59166, an *Aureobasidium* species collected from dead wood in a Peruvian rain forest. HR-FAB-MS of the amorphous solid suggested a molecular formula of $C_{36}H_{64}N_4O_{10}$ [*m/z*, found 713.47100 $(M+H)^+$, calcd 713.47103]. The ^{1}H NMR spectrum obtained in methanol-d_4 manifested four deshielded resonances between 7.6 and 9.0 ppm which disappeared slowly with time, suggesting the presence of four secondary amide protons. Thus, acetone-d_6 was selected as the solvent for the necessary one- and two-dimensional NMR experiments—gradient COSY, TOCSY, gHMQC, and gHMBC. The ^{13}C NMR spectrum of **1a** (Table 1) was in accord with the mass spectrum, showing thirty-six signals—six methyls, sixteen methylenes, eight methines, and six carbonyls—the multiplicities of which were assigned by DEPT.

The gCOSY and gTOCSY NMR experiments allowed the assignment of six separate proton spin systems, and the chemical shifts of several ^{1}H and ^{13}C resonances were characteristic of amino acids. In four cases, the first resonance in the spin-system was found to be a heteroatom-bearing methine (δ 4.68, 4.60, 4.49, 4.21 ppm). Gradient HMQC experiments indicated that the heteroatom in each case was nitrogen since each proton was directly attached to a carbon with a chemical shift between 52 and 56 ppm. Gradient HMBC experiments showed by two-bond proton–carbon correlations that each of these methine groups was also adjacent to a carbonyl carbon (δ 173.4, 173.8, 171.1, 173.0 ppm, respectively), suggesting the presence of four amino acids. Analysis of the NMR data for each spin system allowed the identification of two serine and two leucine residues.

In the two remaining proton spin systems, the first resonance in each sequence was found to be a methylene adjacent to a carbonyl; appropriate proton and carbon chemical shifts (δ_H 2.2~2.8 ppm and δ_C 41 ppm) and two-bond correlations (gHMBC) between the methylene protons and carbonyl carbons (δ_C 169 ppm) were observed. The protons β to each carbonyl were oxygenated methines (δ_H 5.0 ppm and δ_C 73 ppm). Both spin systems then wandered down a saturated alkyl chain of methylene groups before terminating in a methyl group. Based on this, the two groups were tentatively identified as 3-hydroxyoctanoic and 3-hydroxydecanoic acid residues.

The linkage order of the four amino acid and two 3-hydroxycarboxylic acid residues was determined primarily by analysis of the gHMBC data (Table 1). Two-bond proton–carbon correlations were observed between the four amide protons and the carbonyl carbon of the adjacent amide or ester, and three-bond correlations were observed between three of the four amino acid α-protons and the corresponding *N*- or *O*-carbonyl carbon. Three-bond correlations were also observed between β protons on the carboxylic acid units and the ester carbonyls of the attached amino acid residues. These data allowed the tentative assignment of the amino/carboxylic acid sequence shown for **1a**.

To confirm the sequencing, to unequivocally identify the hydroxyalkanoic acids, and to aid the stereochemical assignment of **1a**, a sample was treated with 0.4 N KOH. This reaction yielded two products formed by hydrolysis of the two ester bonds (Figure 1). Analysis of the 1- and 2-D NMR data (see Experimental) in a manner similar to that of **1a** showed that the two structures were Ser/Leu/3-hydroxyoctanoate (**2**) and Leu/Ser/3-hydroxydecanoate (**3**). HR-FAB-MS further supported these structures: **2** [*m/z*, found 361.23385 $(M+H)^+$, calcd for $C_{17}H_{33}N_2O_6$

Table 1 ^1H and ^{13}C NMR data of icosalide A1 (**1a**) in acetone-d_6

Position	^{13}C (ppm)[a]	mult[b]	^1H (ppm, int, m, J in Hz)[c]	HMBC ^{13}C (ppm)
Serine-1				
C=O	173.4	s		
α	55.5	d	4.68 (1H, m)	173.4, 169.1, 63.4
β	63.4	t	3.73 (1H, dd, 11.0, 5.1)	173.4, 55.5
			3.66 (1H, dd, 11.0, 7.0)	173.4, 55.5
NH			7.37 (1H, d, 8.6)	169.1, 55.5
Leucine-1				
C=O	173.0	s		
α	53.8	d	4.21 (1H, m)	173.0, 40.0, 25.6
β	40.0	t	1.72 (2H, m)	173.0, 53.8, 25.6, 23.0, 22.3
γ	25.6	d	1.73 (1H, m)	40.0
γ-CH_3	23.0	q	0.96 (3H, d, 6.5)	40.0, 25.6, 22.3
γ-CH_3	22.3	q	1.02 (3H, d, 6.0)	40.0, 25.6, 23.0
NH			8.38 (1H, d, 4.4)	173.4, 53.8, 40.0
3-Hydroxyoctanoic acid moiety				
C=O	168.7	s		
2	41.4	t	2.77 (1H, dd, 14.0, 3.8)	168.7, 73.2, 32.7
			2.29 (1H, dd, 14.0, 3.4)	168.7, 52.8, 73.2
3	73.2	d	4.99 (1H, m)	173.0, 168.7, 41.4, 32.7, 26.0
4	32.7	t	1.63 (2H, m)	73.2, 41.4, 32.5, 26.0
5	26.0	t	1.40 (2H, m)	73.2, 32.7, 23.4
6	32.5	t	1.28 (2H, m)	
7	23.4	t	1.27 (2H, m)	
8	14.5	q	0.87 (3H, t, 6.8)	32.5, 23.4
Leucine-2				
C=O	173.8	s		
α	52.8	d	4.60 (1H, ddd, 12.1, 9.3, 3.4)	173.8, 168.7, 42.0, 25.3
β	42.0	t	1.79 (1H, ddd, 13.3, 13.0, 3.4)	173.8, 52.8, 25.3, 24.3, 21.1
			1.54 (1H, m)	52.8, 25.3, 24.3, 21.1
γ	25.3	d	2.00 (1H, m)	52.8, 42.0, 24.3, 21.1
γ-CH_3	24.3	q	0.98 (3H, d, 6.0)	42.0, 25.3, 21.1
γ-CH_3	21.1	q	0.94 (3H, d, 6.5)	42.0, 25.3
NH			7.97 (1H, d, 9.3)	168.7, 52.8, 42.0
Serine-2				
C=O	171.1	s		
α	55.1	d	4.49 (1H, ddd, 9.0, 2.7, 2.4)	173.8, 171.1
β	64.1	t	4.14 (1H, dd, 10.3, 2.4)	171.1
			3.84 (1H, dd, 10.4, 2.7)	171.1, 55.1
NH			7.59 (1H, d, 9.0)	173.8, 64.1, 55.1
3-Hydroxydecanoic acid moiety				
C=O	169.1	s		
2	41.5	t	2.63 (1H, dd, 13.7, 3.9)	169.1, 72.6, 33.0
			2.25 (1H, dd, 13.7, 3.5)	169.1, 72.6, 33.0
3	72.6	d	5.06 (1H, m)	171.1, 169.1, 41.5, 33.0, 26.7
4	33.0	t	1.54 (2H, m)	72.6, 41.5, 30.3, 26.7
5	26.7	t	1.26 (2H, m)	
6	30.3	t	1.28 (2H, m)	
7	30.1	t	1.28 (2H, m)	
8	32.2	t	1.28 (2H, m)	
9	23.2	t	1.32 (2H, m)	32.2
10	14.3	q	0.89 (3H, t, 6.4)	32.2, 23.2

[a] 100 MHz, acetone-d_6 ref. 29.9 ppm. [b] Determined by DEPT. [c] 400 MHz, acetone-d_6 ref. 2.05 ppm.

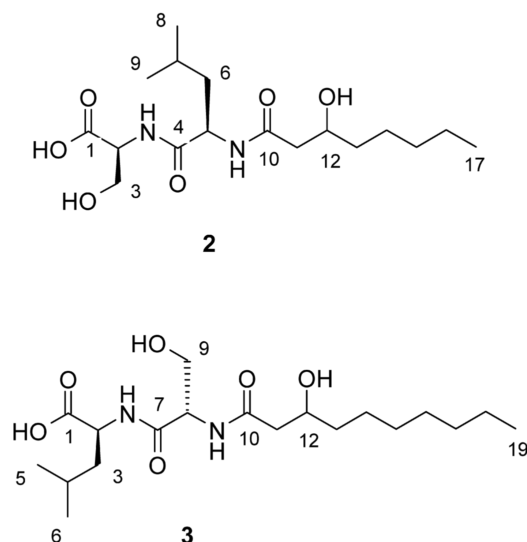

Fig. 1 Alkaline hydrolysis products of **1a**.

361.23385] gave fragments at *m/z* 256 [M−serine]$^+$, 228 [M−serine−CO]$^+$, and 106 [serine+H]$^+$; **3** [*m/z*, found 389.26498 (M+H)$^+$, calcd for $C_{19}H_{37}N_2O_6$ 389.26497] gave fragments at *m/z* 258 [M−leucine]$^+$, 230 [M−leucine−CO]$^+$, and 132 [leucine+H]$^+$.

Acidic hydrolyses of the two products from base hydrolysis, **2** and **3**, provided the constituent amino acids. The former were compared with standards *via* Marfey analysis [19, 20]. A portion of each hydrolysate mixture was derivatized with 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA), and the derivative mixtures were compared by C_{18} HPLC to authentic amino acids (also derivatized with FDAA) using both external- and internal-standard methodology. While **3** contained L-serine (6.05 minutes) and L-leucine (19.0 minutes), **2** contained L-serine and D-leucine (21.2 minutes). Total acid hydrolysis of **1a** and subsequent Marfey analysis of the hydrolysis products [20] provided similar results for the amino acids. The hydrolysis of **1a** also allowed the isolation of two mixtures of fatty acids—3-hydroxyoctanoic and 3-hydroxydecanoic acids and their respective dehydration products *trans*-2-octenoic and *trans*-2-decenoic acids. The stereochemistries of the hydroxyacids were not determined.

Icosalides A2 (**1b**) and B (**1c**) were isolated from an extract of the fungus OSI 74159 (a sterile, nondescript species isolated from dead wood in a Ghanian rain forest) that inhibited replication of the influenza virus in confluent MDCK cells. Analysis of the ^{1}H and ^{13}C NMR data in conjunction with the HR-FAB-MS molecular ions of **1b** [$C_{36}H_{65}N_4O_{10}$ *m/z* 713.4685 (M+H)$^+$] and **1c** [$C_{34}H_{61}N_4O_{10}$ *m/z* 685.4361 (M+H)$^+$] suggested that the former contained two more methylene units on the alkanoic acid chain than did the latter. Tentative gross structures of **1b** and **1c** (the major component) were determined by 1- and 2-D NMR spectroscopy (**1c**, Table 2) in a fashion similar to that described for **1a**. Chiral TLC analysis of the total acid hydrolysates [21, 22] of **1b** and **1c** (6 N HCl, 110°C) showed that, in contrast to **1a**, all amino acids in these two compounds had the L-configuration.

Icosalide A1 (**1a**) was assayed for antimicrobial activity against the following panel of organisms: *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *S. aureus* (ATCC 33591), *S. haemolyticus* (ATCC 29970), methicillin-resistant *S. epidermidis* (clinical isolate), *Streptococcus pneumoniae* (mouse infectivity strain Felton), penicillin-resistant *S. pneumoniae* (ATCC 35088), *S. pyogenes* (ATCC 12384), *Enterococcus faecalis* (ATCC 29212), vancomycin-resistant *E. faecium* (ATCC 51559), *Escherichia coli* (ATCC 35218), and *Candida albicans* (ATCC 90028), showing only weak activity against *Enterococcus faecalis* and *Streptococcus pneumoniae* (Felton) but significantly inhibiting *S. pyogenes* (MIC 8∼16 μg/ml). Icosalides A2 (**1b**) and B (**1c**), having been isolated from an extract identified in our antiviral screening program, were not assayed as pure compounds against the microbial panel. However, neither pure **1b** nor **1c** inhibited the conformational change of viral hemagglutinin A, and both compounds were cytotoxic in replicating MDCK cells (CC_{50} 5 μg/ml and 9 μg/ml, respectively); the more potent activity of **1b** is consistent with the observation that longer alkyl chain lengths increase the potency of these types of compounds [23].

The icosalides are new members of the peptolide class of cyclic depsipeptides. Although their activities are modest, they do exhibit some interesting structural features. To our knowledge, they are only the second twenty-membered ring depsipeptide [6] and the first such peptolide. Many peptolides include only a single fatty acid in their ring systems, but like the tetrapeptidic sporidesmolides [24], the icosalides incorporate two such residues—in the present case, these are either closely related (**1a** and **1b**) or identical (**1c**) 3-hydroxy-alkanoic acids. Only the 3-hydroxy-decanoic acid has previously been reported in a peptolide [25, 26], although several hydroxy-octanoic acid congeners have been identified in this class [1, 2, 10, 13]. Finally, the inclusion of only a single D-leucine in **1a** is an interesting deviation from the predomination of L-amino acids in all three molecules [27].

Table 2 ^{1}H and ^{13}C NMR data of icosalide B (**1c**) in DMSO-d_6

Position	^{13}C (ppm)[a]	mult[b]	^{1}H (ppm, int, m, J in Hz)[c]	HMBC ^{13}C (ppm)
Serine-1				
C=O	170.3	s		
α	54.4	d	4.33 (1H, br dt, 8.4, 3.6)	172.8, 170.3, 62.5
β	62.5	t	3.91 (1H, m)	
			3.66 (1H, ddd, 11, 4.8, 3.6)	172.8, 54.4
NH			7.45 (1H, d, 8.4)	172.8, 62.5, 54.4
OH			5.78 (1H, br t, 3.6)	62.5, 54.4
Leucine-1				
C=O	172.8	s		
α	51.6	d	4.38 (1H, ddd, 9.0, 9.0, 3.0)	172.8, 167.9, 40.8, 23.7
β	40.8	t	1.58 (1H, m); 1.36 (1H, m)	172.8, 23.7, 20.2
γ	23.7	d	1.85 (1H, m)	40.8, 23.3, 20.2
γ-CH_3	23.3	q	0.85 (3H, d, 6.6)	40.8, 23.7, 20.2
γ-CH_3	20.2	q	0.83 (3H, d, 6.6)	40.8, 23.7, 23.3
NH			7.99 (1H, d, 9.0)	167.9, 51.6, 40.8
3-Hydroxyoctanoic acid-1				
C=O	167.9	s		
2	40.7	t	2.59 (1H, dd, 13, 3.6)	167.9, 71.7, 31.4
			2.25 (1H, dd, 13, 3.6)	167.9, 71.7, 31.4
3	71.7	d	4.87 (1H, ddd, 8.4, 8.4, 4.2)	171.9, 167.9, 40.7, 30.2, 24.3
4	31.4	t	1.51 (2H, m)	71.7, 40.7, 30.2
5	24.3	t	1.24 (2H, m)	
6	30.2	t	1.22 (1H, m); 1.18 (1H, m)	
7	22.4	t	1.20 (1H, m); 1.15 (1H, m)	
8	14.0	q	0.84 (3H, t, 6.6)	30.2, 22.4
Leucine-2				
C=O	171.9	s		
α	52.6	d	3.94 (1H, m)	171.9, 38.5, 24.2
β	38.5	t	1.58 (2H, m)	171.9, 52.6
γ	24.2	d	1.58 (1H, m)	38.5
γ-CH_3	21.7	q	0.93 (3H, d, 6.0)	38.5, 24.2, 22.6
γ-CH_3	22.6	q	0.88 (3H, d, 6.0)	38.5, 24.2, 21.7
NH			9.02 (1H, d, 4.2)	171.9, 52.6, 38.5
Serine-2				
C=O	171.9	s		
α	54.9	d	4.48 (1H, ddd, 8.4, 8.4, 4.2)	171.9, 168.1, 61.9
β	61.9	t	3.56 (1H, ddd, 11, 8.4, 4.2)	171.9, 54.9
			3.44 (1H, ddd, 11, 8.4, 5.4)	171.9, 54.9
NH			7.29 (1H, d, 8.4)	171.9, 168.1, 61.9, 54.9
OH			4.83 (1H, t, 5.4)	61.9, 54.9
3-Hydroxyoctanoic acid-2				
C=O	168.1	s		
2	40.6	t	2.48 (1H, dd, 13, 3.6)	168.1, 71.4, 31.7
			2.17 (1H, dd, 13, 3.6)	168.1, 71.4, 31.7
3	71.4	d	4.93 (1H, br ddd, 9.0, 7.8, 4.2)	170.3, 168.1, 40.6, 31.7, 24.8
4	31.7	t	1.44 (1H, m); 1.36 (1H, m)	71.4, 30.9, 24.8
5	24.8	t	1.19 (1H, m); 1.10 (1H, m)	71.4, 30.9
6	30.9	t	1.24 (1H, m); 1.12 (1H, m)	
7	21.9	t	1.23 (2H, m)	
8	14.1	q	0.82 (3H, t, 7.2)	30.9, 21.9

[a] 100 MHz, DMSO-d_6 ref. 39.5 ppm. [b] Determined by DEPT. [c] 600 MHz, DMSO-d_6 ref. 2.49 ppm.

Experimental

General

Specific optical rotations were obtained on a Jasco digital polarimeter. IR spectra were recorded with a Jasco 420 FTIR spectrophotometer. For **1a**, ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra and 2-D COSY, TOCSY, HMQC, and HMBC NMR contour plots were recorded in d_6-acetone at room temperature (20°C) on a Varian Mercury NMR spectrometer using 5 mm Varian inverse detection pulsed field gradient or broad band probes tuned to the nucleus of interest; acetone-d_6 solvent signals were used as references (δ_H 2.05 ppm, δ_C 29.9 ppm). For **1b** and **1c**, ^{1}H NMR data (600 MHz) were recorded on a Bruker AMX-600; DMSO-d_6 was used as solvent (δ_H 2.49 ppm, δ_C 39.5 ppm). Low- and high-resolution FAB-MS data were collected using a Finnegan MAT-90 spectrometer (matrix/standard: **1a** glycerol/PEG, **1b** and **1c** 3-nitrobenzyl alcohol/PEG). Low-resolution ESI-MS data were collected using a Micromass Platform-II spectrometer. HPLC was conducted using either a Gilson system equipped with a Gilson 170 diode-array detector and a Gilson 215 liquid handler or a Rainin SD-1 system equipped with a Rainin Dynamax UV-1 single-wavelength detector (λ=225 nm) and a Gilson 215 liquid handler.

Producing Microorganisms and Fermentation

OSI 59166 is an *Aureobasidium* sp. isolated from dead ground wood in a tropical rain forest in Jenaro Herrara, Dpto. Loreto, Prov. Requena, Peru. OSI 74159 is a sterile, brown, microscopically non-descript organism with little aerial hyphae and was isolated from dead wood in the central region of Kakum National rain forest, Jukwa-Abrafo, Ghana. Voucher specimens of both strains are preserved at Mycosynthetix, Inc. (Durham, NC). Both were cultured and fermented using a reported procedure standard in the MYCOsearch laboratories [28] except that the scale-up media were modified: OSI 59166 was fermented in 500 ml Nunc plates on solid medium containing 2% casein, 2% dextrose, 1% yeast extract, and 1.8% agar; OSI 74159 was fermented with shaking in 2-liter Fernbach flasks in liquid medium (500 ml) containing 0.5% mannitol and 2% soy grits.

Isolation of Icosalide A1 (1a)

Crude MeOH extract of 1.5 liters of the total freeze-dried fermentation was dissolved in 50% aq MeOH (300 ml) and partitioned sequentially with hexanes (2×250 ml) and $CHCl_3$ (2×300 ml). Antibiotic activity was observed in only the aq MeOH fraction, and this material was further separated by C_{18} preparative chromatography with a step-gradient elution (H_2O, 20% MeOH - H_2O, 50% MeOH - H_2O, and MeOH). Bacterial inhibition was concentrated primarily in the final fraction (650 mg). This portion was chromatographed by C_8 HPLC (9.4×250 mm, 30% MeCN - H_2O to 100% MeCN solvent gradient modified with 0.06% TFA, 2 hours elution) to give **1a** (95 mg) as a colorless, amorphous solid: $[\alpha]_D^{20}$ +40° (*c* 0.480, MeOH); UV λ_{max} 210 nm (55% MeOH - H_2O); HR-FAB-MS *m/z* 713.47100 $C_{36}H_{65}N_4O_{10}$ $(M+H)^+$; ^{1}H and ^{13}C NMR see Table 1; yield ~63 mg/liter.

Alkaline Hydrolysis of 1a

1a (25 mg) was dissolved in 0.4 N KOH and left to stand at RT for 4 hours. 1 N HCl was added dropwise to neutralize the reaction mixture. Concentration *in vacuo* followed by C_8 HPLC (9.4×250 mm, isocratic at 0.05% TFA/30% MeCN - H_2O for 5 minutes then 30% MeCN - H_2O to 100% MeCN solvent gradient modified with 0.05% TFA, 30 minutes elution) yielded **2** (Rt 12 minutes, 10.7 mg) and **3** (Rt 19 minutes, 10.5 mg) as colorless oils. **2**: ^{1}H NMR (400 MHz, acetone-d_6) δ 4.52 (1H, m, H-2), 4.50 (1H, m, H-5), 3.96 (1H, m, H-12), 3.92 (1H, dd, $J_{3a,3b}$=11.2 Hz, J=4.2 Hz, H-3a), 3.83 (1H, dd, $J_{3b,3a}$=11.2 Hz, $J_{3b,11}$= 3.7 Hz, H-3b), 2.44 (1H, dd, $J_{11a,11b}$=13.6 Hz, $J_{11a,3b}$=3.7 Hz, H-11a), 2.31 (1H, dd, $J_{11b,11a}$=13.6 Hz, J=8.7 Hz, H-11b), 1.75 (1H, m, H-7), 1.75 (1H, m, H-6), 1.60 (1H, m, H-6), 1.49 (1H, m, H-14), 1.48 (2H, m, H_2-13), 1.33 (2H, m, H_2-15), 1.32 (1H, m, H-14), 1.32 (2H, m, H_2-16), 0.95 (3H, d, J=6.3 Hz, H_3-8), 0.92 (3H, d, J=6.3 Hz, H_3-9), 0.88 (3H, t, J=6.8 Hz, H_3-17); ^{13}C NMR (100 MHz, acetone-d_6) δ 173.3 (C-10), 173.0 (C-4), 171.2 (C-1), 69.7 (C-12), 62.8 (C-3), 55.3 (C-2), 52.7 (C-5), 44.2 (C-11), 41.3 (C-6), 38.1 (C-13), 32.6 (C-15), 26.0 (C-7), 25.5 (C-14), 23.6 (C-8), 23.4 (C-16), 21.8 (C-9), 14.4 (C-17); FAB-MS *m/z* 383 $(M+Na)^+$, 361 $(M+H)^+$, 343 $(M-H_2O+H)^+$, 256 $(M-Ser)^+$, 228 $(M-Ser-CO)^+$, 106 $(Ser+H)^+$; HR-FAB-MS *m/z* found 361.23385 $(M+H)^+$, calcd for $C_{17}H_{33}N_2O_6$ 361.23385. **3**: ^{1}H NMR (400 MHz, acetone-d_6) δ 4.50 (1H, m, H-8), 4.49 (1H, m, H-2), 3.93 (1H, m, H-12), 3.84 (1H, dd, $J_{9a,9b}$=10.9 Hz, J=4.9 Hz, H-9a), 3.70 (1H, dd, $J_{9b,9a}$=10.9 Hz, J=5.4 Hz, H-9b), 2.48 (1H, dd, $J_{11a,11b}$=14.5 Hz, J=3.7 Hz, H-11a), 2.29 (1H, dd, $J_{11b,11a}$=14.5 Hz, J=8.3 Hz, H-11b), 1.76 (1H, m, H-4), 1.65 (2H, m, H_2-3), 1.47 (2H, m, H_2-13), 1.47 (2H, m, H_2-14), 1.30 (2H, m, H_2-15), 1.30 (2H, m, H_2-16), 1.30 (2H, m, H_2-17), 1.29 (2H, m, H_2-18), 0.94 (3H, d, J=6.6 Hz, H_3-5), 0.92 (3H, d, J=6.6 Hz, H_3-6), 0.88 (3H, t, J=7.0 Hz, H_3-19); ^{13}C NMR (100 MHz, acetone-d_6) δ 174.1 (C-1), 172.9 (C-10), 171.2 (C-7), 69.0 (C-12), 63.1 (C-9), 55.7 (C-8), 51.5 (C-2), 43.8 (C-11), 41.3 (C-3), 37.9 (C-13),

32.7 (C-17), 30.4 (C-15), 30.1 (C-16), 26.5 (C-14), 25.5 (C-4), 23.4 (C-18), 23.4 (C-5), 21.9 (C-6), 14.4 (C-19); FAB-MS *m/z* 411 $(M+Na)^+$, 389 $(M+H)^+$, 258 $(M-Leu)^+$, 230 $(M-Leu-CO)^+$, 219 $(LeuSer+H)^+$, 132 $(Leu+H)^+$; HR-FAB-MS *m/z* found 389.26498 $(M+H)^+$, calcd for $C_{19}H_{37}N_2O_6$ 389.26497.

Acid Hydrolysis of Peptides 1a, 2, and 3 [20]

Separate solutions of **1a** (4.5 mg), **2** (2 mg) and **3** (2 mg) in 5 N HCl (5 ml) were heated at 105°C for 12 hours. The hydrolysates were cooled to RT and extracted with EtOAc. The aqueous portions were concentrated *in vacuo* and subjected to Marfey analysis to determine the configurations of the constituent amino acids (see below). The organic extract from hydrolysis of **1a** was chromatographed by C_{18} HPLC (Zorbax XDB 4.6×250 mm, 5 μm, isocratic at 0.05% TFA/30% MeCN-H_2O for 10 minutes then 30% MeCN-H_2O to 100% MeCN solvent gradient modified with 0.05% TFA, 1 ml/minute, 65 minutes elution) to provide clean peaks at 30 minutes and 46 minutes. The former was found by ^{1}H and ^{13}C NMR to contain a mixture of 3-hydroxyoctanoic and 3-hydroxydecanoic acids, while the latter contained a mixture of the *trans*-2-octenoic and *trans*-2-decenoic acids resulting from dehydration of the 3-hydroxyalkanoic acids.

Marfey Analysis of Amino Acids from Acid Hydrolysis of Peptides 1a, 2, 3 [19, 20]

Small portions of each of the aqueous layers from the acid hydrolyses of **1a**, **2**, and **3** were added to separate solutions of 0.1% FDAA in acetone (100 μl) and 0.1 N $NaHCO_3$ (200 μl) and heated at 80°C for 3 minutes. After cooling to RT, the reaction mixtures were neutralized with 0.2 N HCl (100 μl), diluted with MeCN/H_2O/TFA (50 : 50 : 0.05, 200 μl), analyzed by C_{18} HPLC (Zorbax XDB 4.6×250 mm, 5 μm, isocratic at 0.05% TFA/30% MeCN-H_2O for 5 minutes then 30% MeCN-H_2O to 80% MeCN-H_2O solvent gradient modified with 0.05% TFA, 1 ml/minute, 25 minutes elution). Commercial samples of D- and L-leucine and D- and L-serine (Aldrich) were similarly derivatized for use as standards. Analysis clearly showed the presence of the D-leucine derivative (21.2 minutes) in **2** and of the L-leucine derivative (19.0 minutes) in **3**. The serine derivative peaks were not as well resolved, but spiking of the hydrolysis samples with the D-serine derivative (6.20 minutes) showed a clear doubling of the peak attributed to the L-serine derivative (6.05 minutes) present in both **2** and **3**.

Isolation and Spectral Data of Icosalides A2 (1b) and B (1c)

Crude MeOH extract of 60 ml of the total freeze-dried fermentation was dissolved in 50% aq MeOH and partitioned sequentially with hexanes and $CHCl_3$. The $CHCl_3$ fraction (33 mg) was concentrated and subjected to semi-preparative reversed-phase HPLC (Alltech HS Hyperprep 100 BDS C_{18}, 10×250 mm, 2 ml/minute, isocratic at 10% MeCN-H_2O for 10 minutes then increasing to 100% MeCN over 50 minutes and eluting isocratically for an additional 10 minutes) to afford **1c** (Rt 55 minutes, 2.8 mg) and **1b** (Rt 62 minutes, 0.9 mg) as colorless oils. **1b**: IR (CH_2Cl_2) 2961, 2930, 2857, 1739, 1681, 1661, 1533, 1466 cm^{-1}; HR-FAB-MS *m/z* 713.4685 $(M+H)^+$, calcd for $C_{36}H_{65}N_4O_{10}$ 713.4701; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.04 (1H, d, J=4.2 Hz, *leu-2* NH), 7.99 (1H, d, J=9.0 Hz, *leu-1* NH), 7.45 (1H, d, J=8.4 Hz, *ser-1* NH), 7.28 (1H, d, J=8.4 Hz, *ser-2* NH), 5.79 (1H, br s, *ser-1* OH), 4.93 (1H, br ddd, J=9.0, 7.8, 3.6 Hz, *HOA-3*), 4.88 (1H, br ddd, J=8.4, 8.4, 4.2 Hz, *HDA-3*), 4.80 (1H, br s, *ser-2* OH), 4.48 (1H, ddd, J=8.4, 8.4, 4.8 Hz, *ser-2*α), 4.38 (1H, ddd, J=13, 10, 3.0 Hz, *leu-1*α), 4.33 (1H, br dt, J=8.4, 3.0 Hz, *ser-1*α), 3.93 (1H, m, *leu-2*α), 3.91 (1H, br t, J=11 Hz, *ser-1*β1), 3.65 (1H, br dd, J=11, 3.0 Hz, *ser-1*β2), 3.56 (1H, dd, J=11, 4.2 Hz, *ser-2*β1), 3.43 (1H, br dd, J=11, 8.4 Hz, *ser-2*β2), 2.59 (1H, dd, J=13, 3.6 Hz, *HDA-2a*), 2.48 (1H, dd, J=13, 3.6 Hz, *HOA-2a*), 2.24 (1H, dd, J=13, 3.6 Hz, *HDA-2b*), 2.18 (1H, dd, J=13, 3.6 Hz, *HOA-2b*), 1.85 (1H, m, *leu-1*γ), 1.59 (1H, m, *leu-1*β1), 1.58 (2H, m, *leu-2*β), 1.58 (1H, m, *leu-2*γ), 1.53 (1H, m, *HDA-4a*), 1.44 (1H, m, *HOA-4a*), 1.40 (1H, m, *HDA-4b*), 1.36 (1H, m, *HOA-4b*), 1.36 (1H, m, *leu-1*β2), 1.26 (2H, m, *HDA-9*), 1.25 (2H, m, *HDA-5*), 1.24 (1H, m, *HOA-6a*), 1.23 (2H, m, *HOA-7*), 1.22 (4H, m, *HDA-6*, *HDA-8*), 1.21 (2H, m, *HDA-7*), 1.18 (1H, m, *HOA-5a*), 1.12 (1H, m, *HOA-6b*), 1.10 (1H, m, *HOA-5b*), 0.93 (3H, d, J=6.0 Hz, *leu-2*γ-Me-1), 0.88 (3H, d, J=6.0 Hz, *leu-2*γ-Me-2), 0.85 (3H, d, J=6.0 Hz, *leu-1*γ-Me-1), 0.84 (3H, t, J=6.0 Hz, *HDA-10*), 0.83 (3H, d, J=6.0 Hz, *leu-1*γ-Me-2), 0.81 (3H, t, J=7.8 Hz, *HOA-8*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8 (s, *ser-1* C=O), 172.8 (s, *leu-1* C=O), 171.9 (s, *leu-2* C=O), 168.1 (s, *ser-2* C=O), 168.1 (s, *HOA* C=O), 167.9 (s, *HDA* C=O), 71.5 (d, *HDA* C-3), 71.4 (d, *HOA* C-3), 62.2 (t, *ser-1*β), 61.6 (t, *ser-2*β), 54.5 (d, *ser-2*α), 54.0 (d, *ser-1*α), 52.2 (d, *leu-2*α), 51.5 (d, *leu-1*α), 40.3 (t, *HOA-2*), 40.3 (t, *HDA-2*), 40.2 (t, *leu-1*β), 38.4 (t, *leu-2*β), 31.5 (t, *HOA* C-4), 30.9 (t, *HOA* C-6), 30.8 (t, *HDA* C-4), 30.7 (t, *HDA* C-6), 30.7 (t, *HDA* C-8), 28.1 (t, *HDA* C-7), 28.0 (t, *HDA* C-9), 24.7 (t, *HOA* C-5), 24.5 (t, *HDA* C-5), 24.1 (d, *leu-2*γ), 23.6 (d, *leu-1*γ), 23.4 (q, *leu-1*γ-Me-1), 22.2 (q, *leu-2*γ-Me-1), 21.9 (t, *HOA* C-7), 21.4 (q, *leu-2*γ-Me-2),

20.2 (q, *leu-1γ*-Me-2), 13.8 (q, *HDA* C-10), 13.5 (q, *HOA* C-8); yield ~15 mg/liter. **1c**: IR (CH_2Cl_2) 2961, 2930, 2873, 1741, 1682, 1660, 1526, 1465 cm^{-1}; HR-FAB-MS *m/z* 685.4361 $(M+H)^+$, calcd for $C_{34}H_{61}N_4O_{10}$ 685.4388; ^1H and ^{13}C NMR see Table 2; yield ~47 mg/liter.

Acid Hydrolysis and Chiral TLC of 1b and 1c [21, 22]

A sample of each compound (0.3 mg) was individually sealed in a hydrolysis tube with 6 N HCl (0.5 ml) and heated at 110°C for 24 hours. The solution was then evaporated under N_2 (g) to afford an oily residue. Small samples of each hydrolysate were compared to standard D,L- and L-amino acids by chiral TLC (Machery-Nagel CHIRALPLATE, developing solvent 4 : 1 : 1 BuOH - H_2O - AcOH). The serine and leucine in each hydrolysate co-chromatographed with the L-standards (Rf 0.35 and 0.75, respectively), and each was clearly resolved from the D-standard (Rf 0.31 and 0.69, respectively).

Antibacterial Assays for 1a

Antibacterial assays for the determination of the MIC values for **1a** were performed according to the National Committee for Clinical Laboratory Standards' "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically" [29]. Vancomycin, ciprofloxacin, and amphotericin B were used as positive-control compounds.

Cytotoxicity Assay for 1b and 1c

MDCK cells (5×10^4 per well) were seeded into 96-well cell culture plates and treated with different concentrations of test sample for 72 hours at 37°C either before or after reaching confluency. To determine cell viability, 25 μl of 1 mg/ml XTT solution containing 25 μM phenazine methosulfate was added to each well, and the plates were incubated for 3 hours at 37°C before the OD at 450 nm was read.

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