Andrimid and Moiramides A–C, Metabolites Produced in Culture by a Marine Isolate of the Bacterium *Pseudomonas fluorescens*: Structure Elucidation and Biosynthesis

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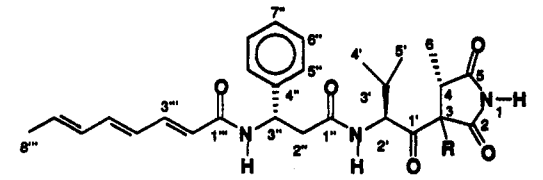
Andrimid (1) and moiramides A–C (2–4) have been isolated from solid agar cultures of a marine isolate of the bacterium *Pseudomonas fluorescens*. The chemical structures of moiramides A–C (2–4) have been elucidated by analysis of spectroscopic data. Andrimid (1) and moiramide B (3) were found to exhibit potent in vitro inhibition of methicillin resistant *Staphylococcus aureus*. Stable isotope incorporation experiments have been used to elucidate the biogenesis of the acylsuccinimide fragment of andrimid (1) that is essential for antimicrobial activity. These experiments demonstrated that the acylsuccinimide fragment is derived from a combination of acetate and amino acid building blocks. It has been proposed that the biosynthesis proceeds through a dipeptide-like intermediate formed from γ-amino-β-keto acids that are in turn formed from valine and glycine homologated with acetate, presumably via malonyl-CoA.

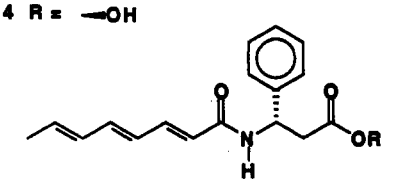
Antibiotic-resistant microorganisms represent an increasingly serious challenge to the successful clinical treatment of human bacterial diseases.[1] A wide variety of approaches to circumventing antibiotic resistance are currently under investigation worldwide.[2] One such approach involves the discovery of new antibiotics belonging to unprecedented chemical structural types that are effective against antibiotic-resistant human pathogens. Microorganisms that inhabit the marine environment represent an underexplored resource with great potential for the discovery of new antibiotics.[3] We have initiated a program to isolate bacteria from marine habitats and screen them for the production of antibiotics that are highly active against clinically relevant antibiotic-resistant human pathogens[2a] such as methicillin resistant *Staphylococcus aureus*, *Enterococcus*, and *Pseudomonas aeruginosa*.[4] As part of this screening program, it was discovered that a marine isolate of the bacterium *Pseudomonas fluorescens*, obtained from tissues of an unidentified tunicate collected at Prince of Wales Island in Moira Sound, Alaska, produced an extract that potently inhibited methicillin resistant *S. aureus*. Bioassay guided fractionation of the extract resulted in the isolation of the known metabolite andrimid (1)[5] and three new related metabolites, moiramides A (2), B (3), and C (4). Andrimid (1) and moiramide B (3) both exhibited potent in vitro antibacterial activity.

Andrimid (1) was first isolated from cultures of an *Enterobacter* sp. that is an intracellular symbiont of the Brown Planthopper, *Nilaparvata lugens*, and it was found to exhibit potent activity against *Xanthomonas campestris* pv. *oryzae*, the pathogen responsible for causing bacterial blight in rice plants.[5a] A stereocontrolled synthesis of andrimid showed that the the acylsuccinimide fragment with a 4*S* methyl substituent is essential for activity against *X. campestris*.[5b] Comparison of the in vitro inhibition of methicillin-resistant *S. aureus* by the metabolites 1–4 isolated from *P. fluorescens* in the present

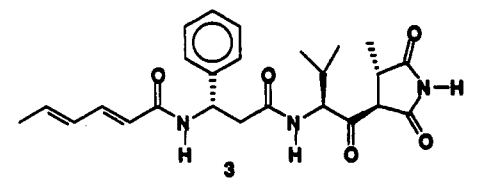


1 R = ···H

4 R = —OH



2 R = H



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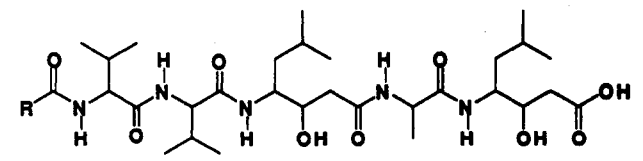

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0022-3263/94/1959-2058$04.50/0

Table 1. NMR Data for Andrimid (1) and the Moiramides A–C (2–4). All Spectra Were Recorded in DMSO-d_6 at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Coupling Constants, Listed in Parentheses, Are in Hz

	andrimid (1)		moiramide A (2)		moiramide B (3)		moiramide C (4)	
carbon no.	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
1-NH	11.35, bs				11.33, bs		11.41, bs	
2		173.6				173.7		174.8
3	3.92, d (5.5)	57.8			3.91, d(5.5)	57.8		86.8
4	2.91, dq (7.3, 5.5)	38.9			2.90, dq (7.3, 5.5)	38.9	2.89, q (7.1)	48.6
5		179.9				180.0		176.6
6	1.07, d (7.3)	14.5			1.07, d (7.3)	14.5	0.71, d (7.1)	7.7
1′		203.3				203.3		208.6
2′	4.63, dd (8.4, 5.4)	63.0			4.61, dd (8.4, 5.4)	63.0	5.18, dd (9.4, 3.4)	58.0
3′	2.29 m	28.1			2.28, m	28.1	2.50, m	27.4
4′	0.74, d (6.7)	17.2			0.74, d (6.7)	17.2	0.61, d (6.8)	15.9
5′	0.80, d (6.7)	19.3			0.79, d (6.7)	19.4	0.81, d (6.8)	19.7
2′-NH	8.09, d (8.4)				8.08, d (8.4)		7.83, d (9.4)	
1″		169.8		171.6		169.8		169.5
2″	2.65, dd (14.3, 6.1)	41.9	2.68, m	40.9	2.63, dd (14.3, 6.0)	41.9	2.66, d (7.4)	41.8
2″	2.76, dd (14.3, 8.5)				2.76, dd (14.3, 8.6)			
3″	5.28, m	49.8	5.25, dd (15.3, 8.3)	49.5	5.27, m	49.8	5.25, m	49.9
4″		142.8		142.6		142.8		142.7
5″	7.3	126.4	7.30, d (4.1)	126.4	7.3	126.4	7.3	126.3
6″	7.3	128.2	7.30, d (4.1)	128.2	7.3	128.2	7.3	128.0
7″	7.20, m	126.8	7.22, m	126.9, m	7.20, m	126.8	7.19, m	126.7
8″	7.3	128.2	7.30, d (4.1)	128.2	7.3	128.2	7.3	128.0
9″	7.3	126.4	7.30, d (4.1)	126.4	7.3	126.4	7.3	126.3
3″-NH	8.40, d (8.5)		8.48, d (8.3)		8.37, d (8.4)		8.33, d (8.5)	
1‴		164.2		164.2		164.4		164.1
2‴	6.01, d (15.1)	124.2	6.00, d (15.0)	124.1	5.92, d (15.1)	122.9	5.99, d (15.1)	124.2
3‴	7.00, dd (15.1, 11.3)	139.4	7.00, dd (15.0, 11.3)	139.4	6.59, dd (15.1, 10.8)	139.4	6.98, dd (15.1, 11.4)	139.2
4‴	6.25, dd (14.8, 11.3)	128.0	6.25, dd (14.8, 11.3)	128.0	6.19, dd (15.0, 10.8)	129.9	6.24, dd (15.0, 11.4)	128.0
5‴	6.53, dd (14.8, 10.8)	138.8	6.53, dd (14.8, 10.7)	138.9	6.06, m	136.6	6.53, dd (15.0, 10.9)	138.8
6‴	6.17, ddd (15.2, 10.8, 1)	131.4	6.18, ddd (15.0, 10.7, 1.5)	131.4	1.78, d (6.5)	18.2	6.18, ddd (15.0, 10.9, 1.5)	131.4
7‴	5.89, m	133.3	5.89, m	133.3			5.90, m	133.2
8‴	1.78, d (6.5)	18.2	1.76, d (6.6)	18.2			1.76, d (6.4)	18.2
CO_2H			12.25, bs					
3-OH							6.99, s	

study has confirmed that the acylsuccinimide fragment is required for antimicrobial activity. The biogenetic origin of the acylsuccinimide unit in andrimid (1) and moiramides B (3) and C (4) was not immediately obvious at the outset. Therefore, as part of the investigation of *P. fluorescens* metabolism, stable isotope labeling experiments were conducted in order to probe the biosynthesis of the acylsuccinimide fragment. The results have revealed a biogenetic pathway involving an interesting combination of acetate and amino acid building blocks that proceeds through a putative dipeptide-like intermediate generated from γ-amino-β-keto acids.

Isolation and Structure Elucidation. The major metabolite obtained from cultures of *P. fluorescens* was identified as andrimid (1) by comparing its MS and NMR data (Table 1) with the literature values.[5a] Moiramide A (2), one of the three minor metabolites, was obtained as an amorphous white solid that gave a parent ion in the EIHRMS at m/z 285.1366 appropriate for a molecular formula of $C_{17}H_{19}NO_3$ (ΔM 0.1 mmu). The IR spectrum of 2 contained absorption bands that could be assigned to carboxylic acid and amide functional groups. Inspection of the ^{1}H and ^{13}C NMR data obtained for moiramide A (Table 1) showed that it contained only the octatrienoyl and β-phenylalanine fragments found in andrimid (1). COSY, HMQC, and HMBC experiments routinely confirmed the proposed constitution for 2. The features of the CD spectrum obtained from moiramide A (2) (see supplementary material), including a strong negative Cotton effect at 244.8 nm ([θ] −11 230) and a weaker negative Cotton effect at 292.6 nm ([θ] −6003), were attributed to the presence of aromatic and trienoyl chromophores adjacent to the lone chiral center at C3″ in the β-phenylalanine residue. The CD spectrum of andrimid (1), which differs from that of moiramide A (2) in the long wavelength region (>260 nm) presumably due to the presence of three additional chrial centers at C2′, C3, and C4 in the acylsuccinimide fragment of 1, nevertheless shows a Cotton effect at 240.2 nm which has the same sign and virtually identical intensity ([θ] −11 480) as the Cotton effect at 244.8 nm in the CD spectrum of 2, indicating that the β-phenylalanine residue has the same configuration (*S*)[5b] in both metabolites as shown.

Moiramide B (3) was obtained as an amorphous white solid that gave a parent ion in the EIHRMS at m/z 453.2262 corresponding to a molecular formula of $C_{25}H_{31}N_3O_5$ (ΔM −0.1 mmu). Comparison of the ^{1}H and ^{13}C NMR data obtained for moiramide B (Table 1) with the data for andrimid revealed that identical β-phenylalanine and acylsuccinimide fragments were present in both molecules and that they differed only in the fatty acyl fragment. COSY and MS data showed that moiramide B (3) contained a hexadienoyl fragment in place of the octatrienoyl fragment present in andrimid (1). A combination of coupling constant analysis (Table 1) and NOE experiments verified that the $\Delta^{2''',3'''}$ and $\Delta^{4''',5'''}$ olefins in moiramide B (3) both had the *E* configuration. The nearly identical ^{1}H and ^{13}C NMR chemical shifts observed for the acyl succinimide and β-phenylalanine portions of andrimid (1) and moiramide B (3) indicated that the two molecules had identical relative configurations in these fragments as shown. A strong negative Cotton effect observed at 237.6 nm ([θ] −24 140) in the CD spectrum of 3 (supplementary material) was once again assigned to the presence of an (*S*)-β-phenylalanine residue. The increase in intensity of this Cotton effect in 3 was attributed to a shift to shorter wavelength in the UV λ_{max} of the

Table 2. Antimicrobial Activities of Andrimid (1) and Moiramide B (3). Values Listed Are "Minimum Inhibitory Concentrations" (MIC) in μg/mL

test organism	andrimid (1)	moiramide B (3)
S. aureus	4	2
S. aureus (methicillin resistant)	2	0.5
S. aureus (oxacillin resistant)	8	1
S. aureus (oxacillin, gentamicin, ciprofloxacin resistant)	2	0.5
E. faecium (vancomycin resistant)	32	4
Escherichia coli (permeability mutant)	2	0.25
E. coli	64	16
P. aeruginosa	>128	>128
Candida albicans	>128	>128

hexadienoyl fragment of **3** relative to λ_{max} of the octatrienoyl fragment in **1** and **2**.

Moiramide C (**4**), the final minor metabolite isolated from the *P. fluorescens* cultures, gave a parent ion in the EIHRMS at *m/z* 495.2378 corresponding to a molecular formula of $C_{27}H_{33}N_3O_6$ (ΔM +0.9 mmu). The molecular formula of moiramide C (**4**) differed from that of andrimid (**1**) simply by the addition of one oxygen atom. Inspection of the ^1H and ^{13}C NMR data for moiramide C (**4**) (Table 1) revealed that it contained the octatrienoyl and β-phenylalanine fragments present in andrimid (**1**) but that it differed from andrimid in the acylsuccinimide fragment. The most apparent differences in the ^1H NMR spectra of moiramide C (**4**) and andrimid (**1**) were (i) the absence of a resonance that could be assigned to H3 in moiramide C, (ii) the simplification of the resonance assigned to H4 in moiramide C to a quartet, and (iii) the appearance of a sharp singlet at δ 6.99 (1H), assigned to an OH proton, in the spectrum of moiramide C. These features of the ^1H NMR spectrum of moiramide C (**4**) were all consistent with the presence of a hydroxyl functionality at C3. Further support for the C3 hydroxyl substituent in **4** came from the observation of a resonance at δ 86.8 (C3) in the ^{13}C NMR spectrum that was correlated in the HMBC spectrum to the NH1 proton at δ 11.41 and to the 3-OH resonance at δ 6.99. An additional HMBC correlation between the 3-OH proton resonance at δ 6.99 and a carbonyl resonance at δ 208.6, assigned to C1′, was also consistent with a 3-OH substituent. Irradiation of the H4 resonance (δ 2.89) in moiramide C (**4**) induced a NOE in the 3-OH resonance at δ 6.99, indicating that the 3-OH and 4-methyl substituents on the succinimide ring were trans. A negative Cotton effect at 237.8 nm ([θ] −12 360) in the CD spectrum of moiramide C (**4**) (supplementary material) indicated the presence of a (*S*)-β-phenylalanine residue. We have assumed that the C4 configuration in moiramide C (**4**) is identical to the C4 configuration in andrimid, resulting in the stereochemistry shown for **4**.

Table 2 lists the in vitro antimicrobial activities of andrimid (**1**) and the moiramides A–C (**2**–**4**). Only andrimid (**1**) and moiramide B (**3**) are active, which reinforces the observation by McWhorter et al. that there is a rather strict requirement in the structure of the acylsuccinimide fragment for antimicrobial activity. Its total absence, as in moiramide A (**2**), or the presence of 3(*R*)-hydroxyl substitution, as in moiramide C (**4**), both result in total loss of *S. aureus* activity. Andrimid (**1**) was found to have only weak in vivo antimicrobial activity against methicillin resistant *S. aureus* in mice ($ED_{50} \geq 16$ mg/kg).

Biosynthetic Studies. A search of the literature failed to uncover any clear experimental precedent for the biogenetic origin of the 3-acyl-4-methylsuccinimide fragment found to be essential for the antimicrobial activity of andrimid (**1**) and moiramide B (**3**). McWhorter et al. based their stereocontrolled synthesis of the acylsuccinimide fragment of andrimid (**1**) in part on the biogenetic proposal that C1′–C5′ originated from valine which was homologated with acetate via malonyl-CoA as a source of C2 and C3.[5b] A precedent for the homologation of amino acids with acetate is found in the biosynthetic studies of 4-amino-3-hydroxy-6-methylheptanoic acid, which is a component of the pepstatins (i.e., **5**).[6] The McWhorter et al. proposal, even though it did not provide any insight into the origin of C4, C5, and C6 or the succinimide nitrogen atom, did provide a logical starting point in the investigation of the biosynthesis of the acylsuccinimide fragment of andrimid (**1**).

To test the McWhorter et al. proposal, we fed solid agar cultures of *P. fluorescens* [1-^{13}C]valine, [1-^{13}C]acetate, and [1,2-$^{13}C_2$]acetate in independent experiments (Table 3). ^{13}C NMR analysis of the andrimid (**1**) formed in the [1-^{13}C]valine feeding experiment showed efficient incorporation (22.4% specific incorporation) of the carboxyl carbon of valine into the C1′ position of andrimid as predicted by the proposal. Similarly, the acetate feeding experiments provided clear evidence for the intact incorporation of an acetate unit at C2 and C3 (17.0 and 16.2 % specific incorporation). Interestingly, the acetate feeding experiments also provided the unexpected observation that Me6 was effectively labeled by C2 of acetate (17.0% specific incorporation) but that the succinimide ring carbons at C4 and C5 were not effectively labeled by acetate.

The unexpected labeling of Me6 by C2 of acetate allowed us to formulate the proposal shown in Scheme 1 for the biogenesis of the acylsuccinimide ring. In this proposal, a valine unit is homologated with acetate via malonyl-CoA to give a new γ-amino-β-keto acid. This new amino acid then forms a peptide bond with a molecule of glycine, which is in turn homologated with acetate via malonyl-CoA. A condensation reaction followed by decarboxylation with the simultaneous loss of hydroxyl generates the five-membered heterocyclic ring containing an exocyclic double bond at C4. Reduction of the $\Delta^{4,6}$ olefin and adjustment of the oxidation state of C5 leads to andrimid (**1**).

To test the proposal shown in Scheme 1, solid agar cultures of *P. fluorescens* were fed [1,2-$^{13}C_2$]glycine and [1,2-$^{13}C_2$,^{15}N]glycine in separate experiments (Table 3). The ^{13}C NMR spectrum of the andrimid (**1**) formed in the [1,2-$^{13}C_2$]glycine feeding experiment contained significantly enhanced resonances for both C4 (29.3% specific incorporation) and C5 (30.0% specific incorporation). In this spectrum, the C4 resonance appeared as a doublet (J = 45.8 Hz), arising from one bond $^{13}C/^{13}C$ coupling in doubly labeled molecules, flanking a central singlet arising from the natural abundance ^{13}C in unlabeled molecules. The C5 resonance also appeared as a doublet (J = 45.8 Hz) flanking a central singlet; however, the C5 central singlet was substantially enhanced (12.5% specific incorporation) above natural abundance, indicating the presence of some molecules singly labeled at C5. Analysis of the ^{13}C NMR spectrum of the andrimid (**1**) formed in the [1,2-$^{13}C_2$,^{15}N]glycine feeding experiment showed that the succinimide nitrogen in andrimid also comes directly from glycine. In this experiment, the andrimid (**1**) C4 resonance

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Table 3. Stable Isotope Incorporation Results As Determined by ^{13}C NMR Analysis of Andrimid (1) (DMSO-d_6, 125 MHz)

carbon no.	δ ^{13}C	[1-^{13}C]-valine	[1-^{13}C]-acetate	[1,2-$^{13}C_2$]lactate			[1,2-$^{13}C_2$]glycine		[1,2-^{13}C,^{15}N]glycine				
				doublets[a]	singlets[a]	$^1J_{C,C}$ (Hz)	doublets[a]	singlets[a]	doublet of doublets[a]	doublets[a]	$^1J_{C,C}$ (Hz)	$^1J_{C,N}$ (Hz)	$^2J_{C,N}$ (Hz)
2	173.6	0.0	18.4	17.0	0.9	42.8	1.2	0.1					
3	57.8	–0.1	–0.2	16.2	1.1	42.8	0.3	0.7					
4	38.9	0.0	1.1		0.3		29.3	1.9	10.2		45.8		5.7
5	179.9	0.0	–0.1		–0.1		30.0	12.5	9.7	2.3	45.8	12.4	
6	14.5	0.0	0.0		17.0		1.1	1.0					
1′	203.3	22.4	0.1		–0.1			0.1					
2′	63.0	0.0	0.1		–0.1			0.1					
3′	28.1	0.0	0.0		–0.1			0.1					
4′	17.2	0.0	0.0		0.0			0.0					
5′	19.3	0.1	0.1		0.0			0.1					
1″	169.8	0.0	0.2		0.1			0.0					
2″	41.9	0.1	0.1		0.0			0.1					
3″	49.8	0.0	0.0		0.1			0.0					
4″	142.8	0.1	0.1		–0.1			0.0					
5″	126.4	0.0	–0.1		0.0			0.1					
6″	128.2	0.1	0.1		0.1			0.1					
7″	126.8	0.1	–0.1		0.0			0.0					
8″	128.2	0.1	0.1		0.1			0.1					
9″	126.4	0.0	–0.1		0.0			0.1					
1‴	164.2	0.1	21.7	24.9	1.7	64.4	1.3	2.5					
2‴	124.2	0.1	0.1	20.2	1.9	64.4	1.1	1.7					
3‴	139.4	0.1	17.7	17.7	1.9	56.3	1.1	2.5					
4‴	128.0	0.0	–0.3	14.5	2.1	56.3	0.9	1.6					
5‴	138.8	0.0	18.7	18.3	1.5	55.7	1.0	2.3					
6‴	131.4	0.1	–0.2	16.8	2.1	55.7	1.2	2.1					
7‴	133.3	0.1	17.2	19.0	1.6	43.0	1.0	2.5					
8‴	18.2	0.1	–0.1	18.9	1.1	43.0	1.4	2.1					

[a] Numbers listed are specific incorporations = percent enrichments above natural abundance = 1.1% × (combined integrated peak area of the multiplet components in the enriched signal minus integrated peak area of natural abundance singlet)/(integrated peak area of natural abundance singlet).[10]

appeared as a dd (J = 45.8 and 5.7 Hz) (Figure 1), arising from the one-bond $^{13}C/^{13}C$ and two-bond $^{13}C/^{15}N$ coupling, flanking a central singlet arising from the natural abundance ^{13}C signal from unlabeled molecules. The C5 resonance appeared as a dd (J = 45.8 and 12.4 Hz) (Figure 1), arising from the one-bond $^{13}C/^{13}C$ and one-bond $^{13}C/^{15}N$ coupling in triply labeled molecules, flanking a doublet (J = 12.4 Hz), arising from one-bond $^{13}C/^{15}N$ coupling in doubly labeled (^{13}C and ^{15}N) molecules, and a singlet arising from natural abundance ^{13}C in unlabeled molecules. These results clearly demonstrate the intact incorporation of a glycine residue into the succinimide ring of andrimid (1). In addition, they provide evidence for a pathway leading to incorporation of only the nitrogen atom and the C2 carbon of glycine into positions 1 and 5 of the succinimide ring.

Klein and Sagers have shown that the carboxyl group of [1-^{14}C]glycine can be efficiently exchanged with bicarbonate by cells and isolated enzyme preparations of *Peptococcus glycinophilus*.[7] A similar glycine carboxyl exchange with bicarbonate from the culture medium of *P. fluorescens* would account for the observed incorporation of only the nitrogen and the C2 carbon of glycine into some fraction of the molecules of andrimid (1) generated in the glycine feeding experiments. It was also observed that the [1,2-^{13}C]glycine feeding experiment (Table 3) resulted in a low level of incorporation into the same sites that were efficiently labeled in the acetate feeding experiments described above. This low-level incorporation pattern can be explained by the conversion of glycine to acetate by *P. fluorescens*.[8]

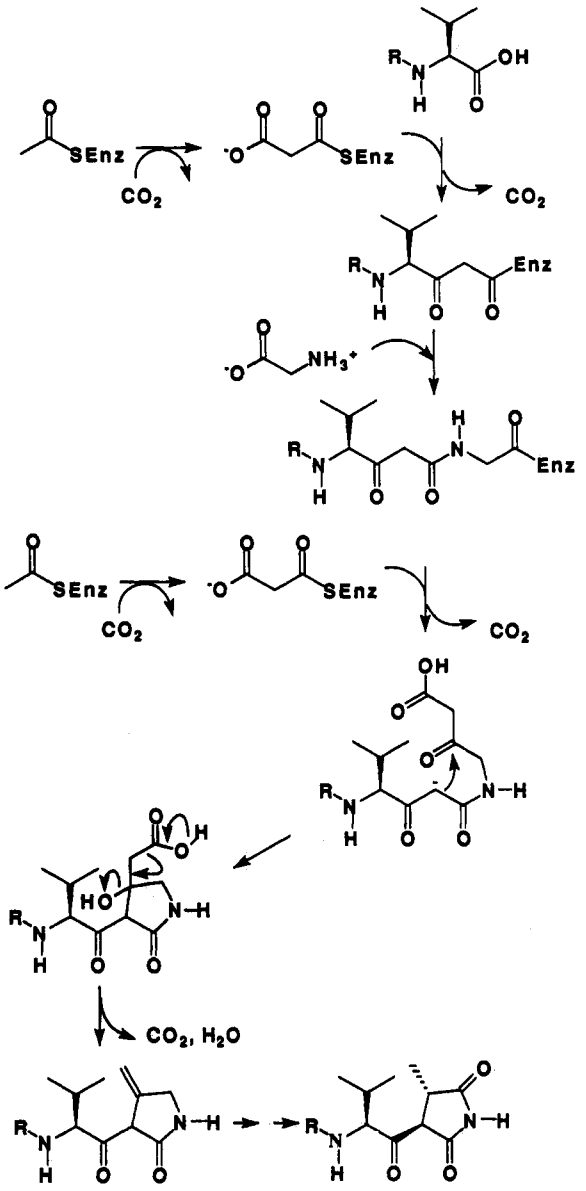
In summary, stable isotope incorporation experiments have shown that the acylsuccinimide fragment of andrimid (1) is formed from valine, glycine, and acetate units. Scheme 1 presents a proposed pathway for the incorporation of the precursors into andrimid (1) that is consistent with the labeling results. The proposal envisages the formation of a dipeptide-like intermediate formed from γ-amino-β-keto acids that are in turn formed from valine and glycine homologated with acetate, presumably via malonyl-CoA as in the biosynthesis of pepstatin (i.e., **5**).[6] Neither of the γ-amino-β-keto acid building blocks, 4-amino-3-keto-5-methylhexanoic acid or 4-amino-3-ketobutanoic acid, have been reported from nature.[9] Furthermore, the steps involved in converting the putative dipeptide-like intermediate to the succinimide substructure are to the best of our knowledge thus far unique to the biosynthesis of andrimid (1). The coproduction of moiramide A (2) and andrimid (1) by cultures of *P. fluorescens* poses the question of the timing of assembly of the various subunits of andrimid. One possibility, as shown in Scheme 1, is that moiramide A (2) represents an early intermediate in the biosynthetic pathway (i.e., *R* in Scheme 1) onto which the dipeptide-like intermediate required for succinimide formation is built up in a linear fashion by analogy with polyketide biosynthesis. Other possibilities involve convergent biosynthesis or linear buildup starting from the 4-amino-3-ketobutanoic acid terminus. Experiments to address the question of the sequence of subunit assembly are currently underway.

Experimental Section

Isolation of Andrimid and the Moiramides. Strain 91QQ48 was isolated from an unidentified tunicate collected from Prince

(7) Klein, S. M.; Sagers, R. D. *J. Biol. Chem.* **1967**, *242*, 297.
(8) Sagers, R. D.; Gunsalus, I. C. *J. Bacteriol.* **1961**, *81*, 541.
(9) 4-Amino-3-ketobutanoic acid is closely related to carnitine (3-hydroxy-4-(trimethylamino)butanoate) which is widely distributed in nature. 4-Amino-3-keto-5-methylhexanoic acid is related to the 4-amino-3-hydroxy-5-methylhexanoic acid residue found in nordidemnin B; see: Jouin, P.; Poncet, J.; Dufour, M.; Pantaloni, A.; Castro, B. *J. Org. Chem.* **1989**, *54*, 617.
(10) Grue-Sorensen, G.; Spenser, I. D. *J. Am. Chem. Soc.* **1993**, *115*, 2052.

Scheme 1



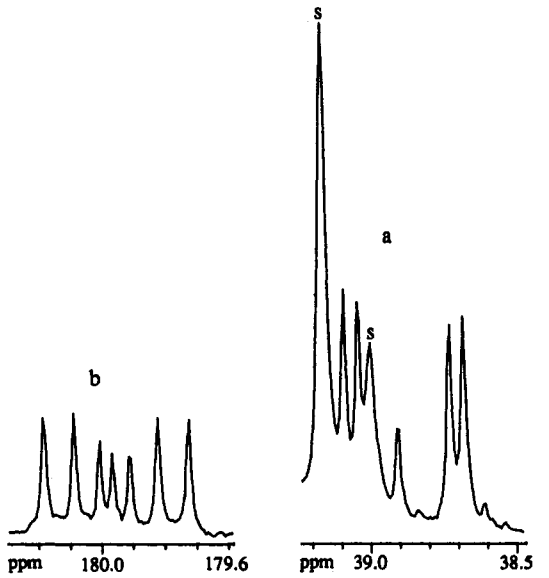


Figure 1. Expansions of the C4 and C5 resonances from the proton noise decoupled ^{13}C NMR spectrum (125 MHz, DMSO-d_6) of andrimid (1) formed in the [1,2-$^{13}C_2$,^{15}N]glycine incorporation experiment. (a) The C4 resonance. Peaks labeled s are from the DMSO-d_6 solvent. (b) The C5 resonance.

of Wales Island off the coast of Alaska. The tunicate was sectioned and inoculated directly to the surface of a variety of media. Strain 91QQ48 was recovered from starch agar. The organism was identified as *Pseudomonas fluorescens* by using standard classical biochemical identification tests and cellular fatty acid analysis (MIDI System, Newark, DE).

Preliminary experiments showed that liquid shake cultures of *P. fluorescens* (91QQ48) failed to produce extracts with any antibacterial activity but that cultures grown on solid media gave extremely active extracts. Therefore, *P. fluorescens* was grown in moderate scale as lawns on solid media. Forty six aluminum trays (23 × 33 × 5 cm) each containing 500 mL of tryptic soy broth medium, 1% agar, and 1% sodium chloride were each inoculated with 2 mL of an actively growing liquid culture of *P. fluorescens*. The cultures were incubated for 3 days at room temperature after which time the cells were gently scraped off the surface of the solid media. The solid media from which the cells had been removed was sectioned into 3- × 3- x 1.5-cm chunks and immersed ethyl acetate for 24 h. The ethyl acetate was decanted, dried with sodium sulfate, and concentrated in vacuo to give a dark red oil. The ethyl acetate extraction of the agar chunks was repeated three times to give 12.4 g of the crude oil.

The crude oil was processed in a batchwise manner beginning with reversed-phase silica flash chromatography (eluent: 65:35 methanol/0.025 M phosphate buffer, pH 6.86). The chromatographic fractions were pooled into four groups on the basis of their TLC behavior and antibacterial activities. Each of the four pooled fractions was concentrated in vacuo to remove the methanol, and the aqueous residue was exhaustively extracted with ethyl acetate. The ethyl acetate soluble portion from the fastest eluting pooled fraction (6.4 g) was chromatographed on Sephadex LH-20 (eluent: ethyl acetate/methanol/water (8:2:1)), and again the fractions were pooled on the basis of their TLC behavior and antibacterial activity. Further purification of the pooled antibacterial fractions from the Sephadex LH-20 chromatography by reversed-phase (C_{18}) HPLC (eluent: methanol/0.025 M phosphate buffer, pH 6.86; detection at 254 nm) followed by ethyl acetate extraction of the HPLC fractions gave pure samples of andrimid (1) (1 g), moiramide A (2) (25 mg), and moiramide B (3) (27 mg) as well as a partially purified sample of moiramide C (4). Final purification of the impure moiramide C (4) via normal-phase silica gel HPLC (eluent: ethyl acetate/hexane (7:3)) gave 1.5 mg of pure compound.

Andrimid (1): isolated as an amorphous white solid; CD (methanol) $[\theta]_{215.8}$ 4944, $[\theta]_{240.2}$ −11 480, $[\theta]_{274.8}$ −567, $[\theta]_{309.0}$ −6096; UV (methanol), λ_{max} (ϵ) 292.1 (35759) nm; IR (film), ν_{max} 3282, 1712, 1650, 1644, 1538, 1531 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 1; HREIMS $C_{27}H_{33}N_3O_5$ (M^+) m/z 479.2416 (ΔM −0.4 mmu); LREIMS m/z (formula, relative intensity) 479 ($C_{27}H_{33}N_3O_5$), 358 ($C_{19}H_{24}N_3O_4$, 17), 268 ($C_7H_{18}NO_2$, 81),146 (C_9H_8NO, 74), 121 (C_8H_9O, 100).

Moiramide A (2): isolated as an amorphous white solid; CD (methanol) $[\theta]_{212.4}$ 13 080, $[\theta]_{244.8}$ −11 230, $[\theta]_{292.6}$ −6003; UV (methanol), λ_{max} (ϵ) 293.8 (27421) nm; IR (film), ν_{max} 3281, 1698, 1648 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 1; HREIMS $C_{17}H_{19}NO_3$ (M^+) m/z: 285.1366 (ΔM +0.1 mmu); LREIMS m/z (formula, relative intensity) 285 ($C_{17}H_{19}NO_3$, 36), 164 ($C_9H_{10}NO_2$, 100), 121 (C_8H_9O, 68).

Moiramide B (3): isolated as an amorphous white solid; CD (methanol) $[\theta]_{214.0}$ 6302, $[\theta]_{237.6}$ −24 140, $[\theta]_{289.0}$ 570, $[\theta]_{311.4}$ −3187; UV (methanol), λ_{max} (ϵ) 255.9 (29040) nm; IR (film), ν_{max} 3272, 1725 (sh), 1712, 1658, 1633, 1538 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 1; HREIMS $C_{25}H_{31}N_3O_5$ (M^+) m/z 453.2262 (ΔM −0.1 mmu); LREIMS m/z (formula, relative intensity) 453 ($C_{25}H_{31}N_3O_5$, 5), 358 ($C_{19}H_{24}N_3O_4$, 14), 242 ($C_{15}H_{16}NO_2$, 69), 200 ($C_{13}H_{14}NO$, 39), 146 (C_9H_8NO, 63), 95 (C_6H_7O, 100).

Moiramide C (4): isolated as an amorphous white solid; CD (methanol) $[\theta]_{218.0}$ −2075, $[\theta]_{237.8}$ −12 360, $[\theta]_{303.2}$ 4971; UV (methanol), λ_{max} (ϵ) 289.2 (29 771) nm; IR (film), ν_{max} 3247, 1729 (sh), 1717, 1636, 1608, 1541 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 1; HREIMS

$C_{27}H_{33}N_3O_6$ (M^+) m/z 495.2378 (ΔM +0.9 mmu); LREIMS m/z (formula, relative intensity) 495 ($C_{27}H_{33}N_3O_6$, 0.6), 146 (C_9H_8NO, 91), 121 (C_8H_9O, 39).

Stable Isotope Feeding Experiments. The labeled precursors [1-^{13}C, 99%]sodium acetate (1 g), [1,2-$^{13}C_2$, 99%]sodium acetate (0.5 g), [1-^{13}C, 99%]-DL-valine (0.5 g), [1,2-$^{13}C_2$, 99%]-glycine (0.25 g), and [1,2-$^{13}C_2$,98%;^{15}N, 98%]glycine (0.1 g) were fed in separate experiments. All of the isotopically enriched precursors were obtained from Cambridge Isotope Laboratories, Andover, MA. In a typical experiment, the labeled precursor was dissolved in water and filter sterilized before being added to the culture medium (4 × 500 mL of tryptic soy broth, 1% agar, 1% sodium chloride) just before the media was poured into the aluminum trays as described above. The trays were each inoculated with 2 mL of an actively growing liquid culture of *P. fluorescens* and incubated for 3 days at room temperature. Andrimid (1) was isolated according to the procedure outlined above, and the incorporation of precursors was detected by ^{13}C NMR analysis (see Table 3 for results).

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Supplementary Material Available: ^{1}H and ^{13}C NMR and CD spectra for andrimid (1) and moiramides A–C (2–4) (9 pages). This material is contained in libraries on microfiche, immediately follows this artile in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.