Stereocontrolled Synthesis of Andrimid and a Structural Requirement for the Activity

William McWhorter, Andreas Fredenhagen, Koji Nakanishi, and Hajime Komura*

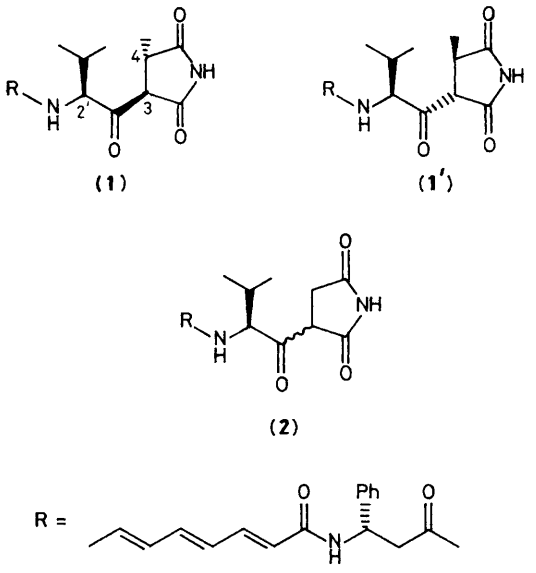
Suntory Institute for Bio-organic Research (SUNBOR), Wakayamadai, Mishimagun, Osaka 618, Japan

Total synthesis of an antibiotic Andrimid was accomplished stereospecifically; it was shown that the chiralities at C-3 and C-4 should be *R* and *S*, respectively, and the presence of the (4*S*)-methyl group is important for the activity.

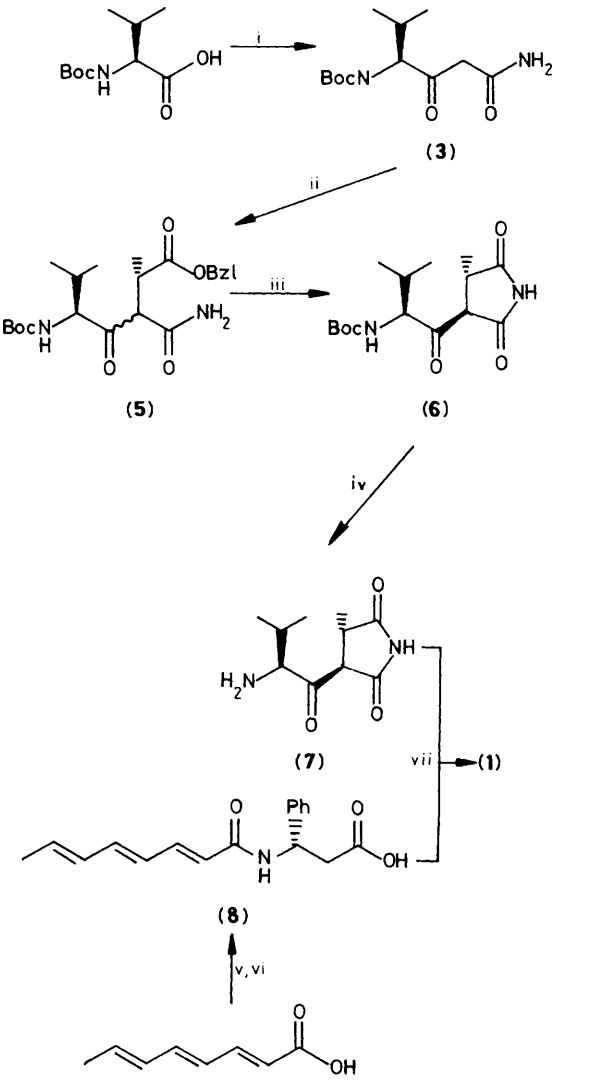
Andrimid,[1] a peptide-like antibiotic isolated from the culture broth of an *Enterobactor sp.*[2] intracellular symbiont of the Brown Planthopper, *Nilaparvata lugens*, exhibits potent activity against *Xanthomonas campestris* pv. *oryzae*, the pathogen causing bacterial blight in rice plants. The antimicrobial spectrum of Andrimid is very specific; among phytopathogens so far tested,[3] 3 *Agrobacterium*, 4 *Corynebacterium*, 9 *Erwinia*, 34 *Pseudomonas*, and 21 *Xanthomonas* bacteria strains, Andrimid shows activity only against the bacterial blight pathogen and a few others such as *X. campestris* pv. *incanae, E. milletiae*, as well as *X. campestris* pv. *oryzacola* which is closely related to the blight pathogen. Structural studies of Andrimid revealed the presence of an unique fragment, the β-ketoamide moiety, which, in part, is common to pepstanone, a pepstatin-like inhibitor of acid(carboxy) peptidases.[4] In order to characterize the mode of action of Andrimid, which can be related to some proteases judging from the structural similarity to pepstanone, a versatile synthetic method for various analogues starting from easily-available materials, such as amino acids in this case, was sought. In addition, stereocontrolled synthesis of both possible diastereoisomers† of Andrimid and thus establishment of the stereochemistry of the chiral centres C-3 and C-4 can be accomplished by use of starting materials with known chiralities. With such constraints on the synthetic strategy, the synthesis of Andrimid was achieved as summarized in Scheme 1 [only for the isomer with correct chirality (**1**) is shown].

Synthesis of the component containing the succinimide ring is the most important synthetic task for the achievement of the total synthesis of (**1**) for the reasons mentioned above.

† It was shown that the C-3 and C-4 substituents are *trans* to each other by a nuclear Overhauser enhancement (n.O.e.) experiment in ^{1}H n.m.r. spectroscopy (Ref. 1). Therefore, only two possibilities remained; (3*S*,4*R*)-isomer(**1′**) which was proposed previously based on a preliminary circular dichroism (c.d.) experiment, and the diastereoisomeric (3*R*,4*S*)-isomer (**1**).



However, because of the stereochemically fragile nature of the acylsuccinimide fragment under basic conditions,‡§ the construction of this particular component required us to solve some problems: i, how to extend the carbon chain with proper substituents with correct chiralities starting from protected L-valine; ii, how and when to close the imide ring. The first problem was solved by a two-step homologation of the chain by mimicking an expected biosynthetic pathway;¶ addition of a protected acetamide C_2-unit to activated Boc-L-valine to form a β-ketoamide (**3**),** then the addition of the (2*S*)-propionyl unit. However, at the second alkylation step with benzyl 2-bromopropionate(**4**),[9] which was derived from L-alanine with retention of stereochemistry[10] in 84% enantiomeric excess (48% yield), a column chromatographically separable diastereoisomeric product was also formed in the 3:1 ratio.†† The second point, ring closure of (**5**) to (**6**) was



Scheme 1. *Reagents and conditions*: i, Im_2CO/tetrahydrofuran (THF), room temp., then added to *N*,*O*-$(TMS)_2$-Acetamide anion/THF, (TMS = tetramethylsilane) −78 °C, 44%; ii, Benzyl (2*S*)-bromopropionate (**4**),[9] KH/dimethylformamide (DMF), room temp., 16 h, 45%; iii, 20% aq. NaOH, $Bu^n_4NHSO_4/CH_2Cl_2$, −20 °C, 5 min 96%; iv, Trifluoroacetic acid (TFA)/CH_2Cl_2, room temp., 30 min, quant; v, EtOCOCl/Et_3N, then D-β-Phenylalanine Me ester/CH_2Cl_2; vi, 0.5 M LiOH aq-MeOH (1:3), 0 °C, 80% over two steps; vii, Diphenylphosphoryl azide (DPPA), Et_3N/DMF, 46%.

achieved under mild conditions without further activation of the amide acceptor carboxylate counter part. Low temperature (−20 °C) and short reaction period (<5 min) are critical to avoid racemization at both the C-4 and C-2′ centres.§ Synthesis of Andrimid was completed by the condensation[11] of the deprotected succinimide moiety (**7**) with octatrienoyl-D-β-phenylalanine (**8**) which can be prepared from octatrienic acid[12] and D-β-phenylalanine.[13]

Spectroscopic comparison of the natural and two synthetic materials, (**1**) and its C-3/C-4 epimer (**1′**), particularly by ^1H n.m.r. signal of the C-2′H's, δ 4.74 (*J* 5.0 Hz, d) and 4.41 (*J* 6.2 Hz, d) in MeOH-[$^2H_4$], respectively, clearly distinguished that the structure of Andrimid should be (**1**) (*i.e.*, 3*R*,4*S*), but not (**1′**) which was proposed previously.[1] It should also be noted that the antimicrobial activity of the di-epimer

‡ Acylsuccinimides have rather low p*K*a's; for example, 3-acetylsuccinimide: 7.5 (Ref. 5), and Andrimid: 6.8 (Ref. 1).

§ In fact, complete deuteria exchange on the methyne protons took place at the C-4 and C-2′ centres when the cyclization reaction of (**5**) was performed in 1 M NaOD in D_2O at r.t. for 40 h.

¶ In the biosynthesis of statin, which is a component of pepstatins, L-leucine residue is homologated with malonyl-CoA to give a C_3-homologue which undergoes decarboxylation to end up with C_2-homologue statone residue by the aid of a multi-enzyme system. Reduction of statone to statin takes place at the following stage (Ref. 6).

** The epimerization of the product (**3**) should be much less than 10% based on the result obtained by the reaction of Boc-L-isoleucine, which is structurally very close to Boc-L-valine; when the corresponding product, β-ketoamide with isoleucine residue was examined by 360 MHz n.m.r. spectroscopy, only one component was observed. However, when the same reaction was carried out with Boc-L-threonine benzyl ether, a *ca.* 2:1 mixture of diastereoisomers were determined on a 500 MHz ^1H n.m.r. spectrum. For similar homologation of amino acids, see Refs. 7 and 8.

†† Formation of a rather higher amount of the diastereoisomer could be due to the partial racemization of the unreacted alkyl bromide (**4**) by the action of the bromide ions released during the S_N2 alkylation reaction, which in the case of secondary bromide is quite slow compared to the reaction with primary alkyl bromide. In fact, in the case of ethyl bromoacetate, a primary bromide, the reaction was faster and gave 75% yield of the corresponding alkylated product with 16% recovery of the β-ketoamide.

Table 1. Effect of 4-methyl group for the expression of antibacterial activity against *X. campestris* pv. *oryzae*.

	Activity (MIC in μg/ml)		
	compounds		
Tester strains	(**1**)	(**1′**)	(**2**)
X. campestris pv. *oryzae*			
SAM 0589	0.195	25	25
SAM 0590	0.391	>100	50

(**1′**) and de-methylated compound (**2**) are as low as 1/100 or less than that of (**1**) (Table 1). The extreme importance of the presence of the (4*S*)-methyl group which is equivalent to that of D-alanine, is thus suggested. It is, however, not clear yet at this stage whether this fact is related to the affinity of the compound to the target macromolecule(s) or is related to the permeability through the membrane. Further structure-activity relationship studies and investigations into the mechanism of the biological activity of Andrimid are in progress.

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