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Preparation of 3-Aminonocardicinic Acid and Its Acyl Derivatives

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3-Aminonocardicinic acid (3-ANA, **2**) was prepared by removal of the N-acyl group of nocardicin A (**1**). Reacylation of **2** with some typical side-chain acids gave the corresponding semisynthetic nocardicins **6**.

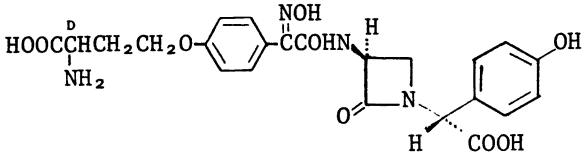
Keywords——3-aminonocardicinic acid; nocardicin; antibacterial activity; structure–activity relationship; the Edman degradation

Nocardicin A (**1**)[1] and its congeners[2] were the first monocyclic β-lactam antibiotics with quite different structures[3] from the classical β-lactam antibiotics, penicillins and cephalosporins. Although nocardicins have a broad antibacterial spectrum, the level of activity is only moderate, especially against gram-positive bacteria.[4] It seemed useful, therefore, to study the structure–activity relationships as a preliminary step toward designing more potent analogues of nocardicins. For the preparation of scuh semisynthetic nocardicins, the removal of the N-acyl group of nocardicin A was required to give 3-aminonocardicinic acid (3-ANA, **2**), the basic nucleus of this family of antibiotics. We previously reported the side-chain cleavage of nocardicin A in a review of the chemistry of nocardicins.[5] This paper is devoted to a full account of the work, also describing the preparation of some new acyl derivatives of 3-ANA.

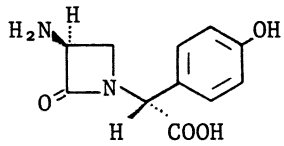
The Edman method is the most common procedure for peptide bond cleavage.[6] We applied this procedure to the preparation of 3-ANA (**2**) from nocardicin A (**1**). For this purpose, the oxime group of **1** was reduced to the corresponding amino group. Catalytic reduction of **1** on 10% Pd–C gave a 1 : 1 mixture of the diastereomeric amino derivatives **3**, one of which was shown to be identical with nocardicin C.[2] This mixture was then treated with phenylisothiocyanate to yield the dithiourea **4** in a ratio reflecting that of the starting amino derivatives **3**. After treatment of **4** with concentrated HCl in AcOH at room temperature followed by dilution with H_2O and adjustment to pH 4 with Amberlite IR-45 under ice-bath cooling, the reaction mixture was concentrated *in vacuo*. The resulting precipitate was collected by filtration and washed with H_2O to afford **2**. Concentration of the mother liquor followed by cooling gave the piperazone **5** as a by-product, which was probably formed from **2** during the reaction and isolation processes, seemingly *via* cyclization of the 3-

amino group with the carboxy group in the 1-substituent, followed by cleavage of the β-lactam ring.[7] Compound **5** was found to be quantitatively obtained by treatment of **2** with concentrated HCl in AcOH. 3-ANA (**2**) was thus highly labile to acids and, therefore, the yield varied widely with slight alterations of the reaction conditions, being at best 40%. Alkaline conditions (*e.g.* K_2CO_3 in aqueous MeOH) were also examined for the Edman degradation of **4**, but gave in poorer yields (*ca.* 25%) of **2**.

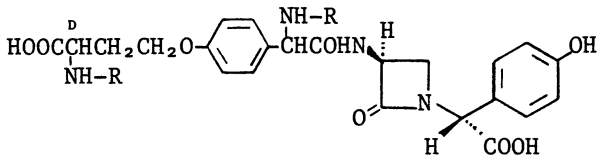
For the preparation of semisynthetic nocardicins, **2** was reacylated with typical side-chain acids. Compounds **6a** and **6b** were prepared by acylation with phenoxyacetyl chloride and phenylacetyl chloride, respectively, in aqueous acetone in the presence of $NaHCO_3$. Compounds **6c** and **6d** were obtained by the acid chloride method. The acids used for **6c** and **6d**, in which the oxime function was protected by the dichloroacetyl group, were converted on treatment with PCl_5 into the corresponding acid chlorides which were immediately reacted with a solution of the trimethylsilyl ester of **2** in CH_2Cl_2. The oxime protecting group was then removed by treatment with aqueous $NaHCO_3$. The same procedure was used for the preparation of **6e** and **6f** starting from 2-(2-trifluoroacetamido-1,3-thiazol-4-yl)-2-methoxyiminoacetic acid and 2-(4-hydroxyphenyl) glyoxylic acid, respectively. The N-protecting group of the former was removed by treatment with aqueous AcONa. Compounds **6g** and **6h** were prepared by the active ester procedure using *N*-hydroxysuccinimide. Thus, the *N*-*tert*-butoxycarbonyl derivatives of *p*-hydroxyphenylglycine and phenylglycine were converted to the active esters, which were reacted with **2** in aqueous acetone in the presence of $NaHCO_3$. Deprotection of the products by treatment with trifluoroacetic acid gave **6g** and **6h**.



1



2



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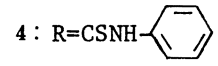

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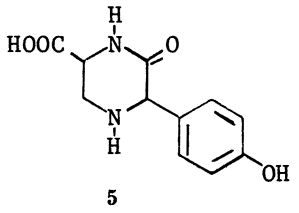


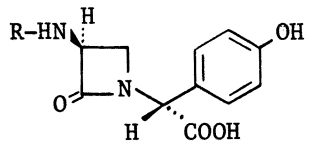
5

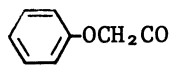
The antibacterial activity of the derivatives described above was determined by the agar dilution method. The data are summarized in Table I. All the derivatives were found to be less active than the parent nocardicin A. In particular, the compounds lacking oxime and keto groups (**6a**, **b**, **g** and **h**) were considerably less active or devoid of activity. The oxime and keto derivatives (**6c**, **d**, **e** and **f**) were active against *Pseudomonas aeruginosa*, *Escherichia coli*, and *ES*-114, indicating that these functions are desirable for biological activity. However, even these compounds showed reduced activity as compared to nocardicin A. This indicates that the D-homoserine side-chain in nocardicin A is also important for antibacterial activity.

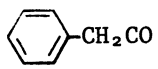
TABLE I. MIC's of Nocardicins[a)]

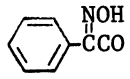
Compound	Organism					
	Pseudomonas aeruginosa 10490	*Escherichia coli* NIHJ, JC-2	*Escherichia coli* 114[b)]	*Proteus vulgaris*	*Staphylococcus aureus*	*Bacillus subtilis*
6a	0.375	>3	3	>3	3	0.75
6b	>10	>10	>10	>10	>10	2.5
6c	0.6	10	0.6	2.5	0.6	2.5
6d	0.1	3.2	0.4	10	2.5	—
6e	2.5	1.25	0.6	10	—	—
6f	2.5	5	1.25	2.5	>10	>10
6g	0.1	>3.2	>3.2	>10	>10	1.25
6h	1.25	>5	>5	>5	>5	>5
Nocardicin A	0.025	0.8	0.0063	0.25	—	0.1

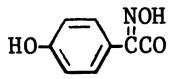
a) Agar dilution method (mg/ml). *b*) A mutant strain of *E. coli* NIHJ: sensitive to β-lactam antibiotics.

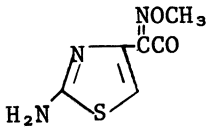


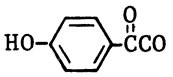
6 a: R = 

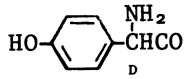
6 b: R = 

6 c: R = 

6 d: R = 

6 e: R = 

6 f: R = 

6 g: R = 

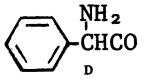
6 h: R = 

Fig. 1

Experimental

Melting points were measured on a Thomas–Hoover apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded using a Hitachi 260-10 spectrophotometer and JEOL PS-100 spectrometer, respectively. Optical rotations were measured on a JASCO automatic polarimeter.

Minimum inhibitory concentrations (MIC's) of the nocardicin analogues were determined by the agar dilution method. One loopful of an overnight culture of each test organism in Tripticase broth (about 10^8 viable cells/ml) was streaked on heart infusion agar containing graded concentrations of the drugs and was incubated at 37 °C for 18 h.

Diastereomeric Nocardicin C (3)——A mixture of nocardicin A (**1**) sodium salt (100.0 g) and 10% Pd–C (30.0 g) was shaken with hydrogen under atmospheric pressure until the absorption of hydrogen ceased. The catalyst was filtered off, and the filtrate was adjusted to pH 3 with 10% HCl under ice-cooling, decolorized with activated charcoal, and diluted with acetone (1.9 l). A crystalline solid separated out, and was collected by filtration and washed with acetone to give **3** (78.2 g, 84.8%): mp 196—200 °C (dec.). IR (Nujol): 3400, 3300, 1735 (β-lactam C=O), 1610 cm^{-1}. ^{1}H-NMR ($D_2O+NaHCO_3$) δ: 2.52 (2H, m, CCH_2C), 3.05 and 3.07 (1H, two dd, J=3, 5 Hz, β-lactam 4β-H), 3.66—3.93 (2H, m, β-lactam ring 4α-H and NCHCOO), 4.12 and 4.16 (2H, two t, J=6 Hz, CCH_2O), 5.13 (1H, m, β-lactam 3α-H), 6.86—7.40 (8H, m, ArH). *Anal.* Calcd for $C_{23}H_{26}N_4O_8 \cdot 1/2H_2O$: C, 55.75; H, 5.49; N, 11.31. Found: C, 55.53; H, 5.39; N, 11.28.

Diastereomeric Bis(phenylthioureido)nocardicin C (4)——Phenylisothiocyanate (47.8 g) was added to a solution of 50% aqueous pyridine (1.1 l) and diastereomeric nocardicin C (**3**, 57.8 g) over a period of 5 min at room temperature. The reaction mixture was stirred for 1 h, during which time the pH was maintained at 9 by adding $NaHCO_3$. The reaction mixture was cooled to room temperature and extracted with Et_2O. The aqueous layer was cooled to approximately 10 °C and adjusted to pH 2 with 20% HCl to precipitate a solid, which was collected by filtration and washed with H_2O to give **4** (69.7 g, 78.1%): mp 144—145 °C (dec.). IR (Nujol): 3300—3200, 1735 (β-lactam C=O) cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 2.26 (2H, m, CCH_2C), 2.98 and 3.10 (1H, two dd, J=3, 5 Hz, 4β-H), 3.78 and 3.80 (1H, two t, J=5 Hz, 4α-H), 4.05 (2H, t, J=6 Hz, CCH_2C), 4.33 (1H, m, NCHCOO), 5.32 and 5.34 (1H, two s, ArCHCO), 5.45 (1H, s, ArCHCOO), 6.62—7.45 (18H, m, ArH). *Anal.* Calcd for $C_{37}H_{36}N_6O_8S_2 \cdot H_2O$: C, 57.35; H, 4.94; N, 10.85. Found: C, 57.33; H, 5.11; N, 10.63.

3-Aminonocardicinic Acid (3-ANA, 2)——Method 1: Diastereomeric bis(phenylthioureido)nocardicin C (**4**, 30.0 g) was dissolved in AcOH (80 ml). The solution was then cooled to 10 °C and concentrated HCl (6.4 ml) was added in one portion with vigorous stirring. Stirring was continued for 30 min at the same temperature, then the reaction mixture was poured into ice water (300 ml) and extracted with AcOEt. The aqueous layer was stirred under ice-cooling and Amberlite IR-45 (about 200 ml) was added to adjust the pH to 2.4—4.4. After the resin had been removed by filtration, the filtrate was evaporated under high vacuum at 35—40 °C. The crystalline solid was washed with acetone to give **2** (3.77 g, 40.0%): mp 198—200 °C (dec.). $[\alpha]_D^{25}$ −252° (c=1.1, 0.1 N $NaHCO_3$). IR (Nujol): 1763 (β-lactam C=O), 1742 cm^{-1}. ^{1}H-NMR (D_2O+NaOD) δ: 2.89 (1H, dd, J=3, 5 Hz, 4β-H), 3.79 (1H, t, J=5 Hz, 4α-H), 4.22 (1H, dd, J=3, 5 Hz, 3α-H), 5.26 (1H, s, ArCHCOO), 6.91 (2H, d, J=8 Hz, ArH), 7.23 (2H, d, J=8 Hz, ArH). *Anal.* Calcd for $C_{11}H_{12}N_2O_4$: C, 55.93; H, 5.12; N, 11.86. Found: C, 56.05; H, 5.01; N, 11.58.

Method 2: Diastereomeric bis(phenylthioureido)nocardicin C (**4**, 1.44 g) was suspended in H_2O (10 ml) and K_2CO_3 (0.56 g) was added. The reaction mixture was stirred at 30 °C for 24 h. After the precipitate had been removed by filtration, the filtrate was adjusted to pH 2 with 5% HCl and extracted with AcOEt. The aqueous layer was adjusted to pH 4 with $NaHCO_3$. The mixture was evaporated and the residue was subjected to column chromatography on activated charcoal with H_2O. The eluate was evaporated and the resulting residue was washed with acetone to give **2** (0.133 g, 25.7%).

The Piperazone 5——A solution of 3-ANA (**2**, 0.1 g) in AcOH (3 ml) was cooled to 10 °C, and concentrated HCl (0.2 ml) was added. The reaction mixture was allowed to stand overnight. The solvent was evaporated off and the residue was dissolved in hot H_2O (20 ml). The aqueous solution was cooled at 5 °C for 2 h, during which time crystals separated out; they were collected and washed with cold H_2O and acetone to give **5** as colorless needles (0.087 g, 87.0%): mp 206—209 °C. IR (Nujol): 3475, 3295, 1670 (amide C=O), 1630 cm^{-1}. ^{1}H-NMR (D_2O) δ: 3.57 (1H, dd, J=4, 12 Hz, 6-H), 3.71 (1H, dd, J=4, 12 Hz, 6-H), 4.34 (1H, br t, J=4 Hz, 5-H), 5.19 (1H, s, 2-H), 6.96 (2H, d, J=8 Hz, ArH), 7.36 (2H, d, J=8 Hz, ArH). *Anal.* Calcd for $C_{11}H_{12}N_2O_4$: C, 55.93; H, 5.12; N, 11.86. Found: C, 55.79; H, 5.31; N, 11.74.

3-Phenoxyacetamidonocardicinic Acid (6a)——A solution of phenoxyacetyl chloride (0.89 g) in acetone (10 ml) was added dropwise at −5 °C to a solution of 3-ANA (**2**, 0.94 g) and $NaHCO_3$ (0.74 g) in 50% aqueous acetone (20 ml). The reaction mixture was stirred at the same temperature for 2 h and then the acetone was evaporated off. The remaining aqueous layer was washed with Et_2O, adjusted to pH 2 with 10% aqueous HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried over $MgSO_4$ and evaporated to give a residue, which was crystallized from a mixture of AcOEt and Et_2O to give **6a** as colorless needles (0.78 g, 40.0%): mp 138—140 °C. IR (Nujol): 1745 (β-lactam C=O), 1690, 1660 cm^{-1}. ^{1}H-NMR ($D_2O+NaHCO_3$) δ: 2.99 (1H, dd, J=3, 5 Hz, 4β-H), 3.79 (1H, t, J=5 Hz, 4α-H), 4.56 (2H, s, $PhOCH_2$), 5.02 (1H, dd, J=3, 5 Hz, 3α-H), 5.35 (1H, s, ArCHCOO), 6.86—7.39 (9H, m, ArH). *Anal.* Calcd for $C_{19}H_{18}N_2O_6$: C, 61.61; H, 4.90; N, 7.56. Found: C, 61.69; H, 4.77; N, 7.40.

3-Phenylacetamidonocardicinic Acid (6b)——This compound was prepared from 3-ANA (0.94 g) and phenylacetyl chloride (0.80 g) in a manner similar to that used for **6a**, (0.97 g, 69.0%): mp 165—167 °C. $[\alpha]_D^{25}$ −206° (c=1.0, MeOH). IR (Nujol): 1740 (β-lactam C=O), 1695, 1640 cm^{-1}. ^{1}H-NMR ($D_2O+NaHCO_3$) δ: 2.85 (1H, dd, J=3, 5 Hz, 4β-H), 3.45 (2H, s, $ArCH_2CO$), 3.64 (1H, t, J=5 Hz, 4α-H), 5.26 (1H, s, ArCHCOO), 6.82 (2H, d, J=8 Hz, ArH), 7.07 (2H, d, J=8 Hz, ArH), 7.20 (5H, m, ArH). *Anal.* Calcd for $C_{19}H_{18}N_2O_5 \cdot 1/4H_2O$: C, 63.59; H, 5.19; N,

7.80. Found: C, 63.70; H, 5.07; N, 7.82.

3-(2-Phenyl-2-hydroxyiminoacetamido)nocardicinic Acid (6c)——PCl_5 (0.25 g) was added to a suspension of 2-phenyl-2-(2,2-dichloroacetoxyimino) acetic acid (0.355 g) in benzene (7 ml) at 0—5 °C, and the mixture was stirred for 1 h. The solvent was evaporated off and benzene (7 ml) was added to the residue and evaporated. After this operation had been repeated three times, the residue was dissolved in CH_2Cl_2 (10 ml). 3-ANA (0.236 g) was suspended in CH_2Cl_2 (20 ml), then *N,N*-bis(trimethylsilyl)acetamide (0.87 g) was added and the mixture was stirred at ambient temperature. The resulting solution was added to the solution obtained above under cooling at 0—5 °C, and the mixture was stirred for 1 h. The reaction mixture was washed with H_2O and evaporated to give an oily residue, to which AcOEt and 5% aqueous $NaHCO_3$ were added. The aqueous layer was separated, adjusted to pH 1—2 with 10% HCl and then extracted with AcOEt. The extract was washed with H_2O, dried over $MgSO_4$ and evaporated to give an oily residue. The oil was triturated with a small amount of $CHCl_3$ to give **6c** as crystals (0.095 g, 27.0%): mp 197—199 °C (dec.). IR (Nujol): 1730 (β-lactam C=O), 1660 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.09 (1H, dd, J=3, 5 Hz, 4β-H), 3.78 (1H, t, J=5 Hz, 4α-H), 5.01 (1H, m, 3α-H), 5.12 (1H, s, ArCHCOO), 7.28—7.52 (10H, m, ArH). *Anal.* Calcd for $C_{19}H_{17}N_3O_6 \cdot 1/2H_2O$: C, 58.16; H, 4.62; N, 10.71. Found: C, 58.38; H, 4.57; N, 10.69.

3-[2-(4-Hydroxyphenyl)-2-(hydroxyimino)acetamido]nocardicinic Acid (6d)——This compound was prepared from 3-ANA (0.47 g) and 2-(4-hydroxyphenyl)-2-(2,2-dichloroacetoxyimino)acetic acid (0.58 g) in a manner similar to that used for **6c** (0.24 g, 31.0%): mp 228—231 °C (dec.). $[\alpha]_D^{24}$ −192° (c=1.0, H_2O). IR (Nujol): 1745 (β-lactam C=O), 1690 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 3.25 (1H, dd, J=3, 5 Hz, 4β-H), 3.90 (1H, t, J=5 Hz, 4α-H), 5.50 (1H, s, ArCHCOO), 6.80 (2H, d, J=8 Hz, ArH), 6.85 (2H, d, J=8 Hz, ArH), 7.28 (2H, d, J=8 Hz, ArH), 7.50 (2H, d, J=8 Hz, ArH). *Anal.* Calcd for $C_{19}H_{17}N_3O_7$: C, 57.14; H, 4.29; N, 10.52. Found: C, 56.95; H, 4.20; N, 10.48.

3-[2-(2-Amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]nocardicinic Acid (6e)——3-ANA (0.24 g) was acylated with 2-(2-trifluoroacetamido-1,3-thiazol-4-yl)-2-methoxyiminoacetic acid (0.27 g) in a manner similar to that used for **6c** to give an oil (0.28 g), which was used in the following reaction without further purification. This oil (0.24 g) and AcONa (0.34 g) were dissolved in H_2O (4 ml) and the solution was stirred for 8 h at room temperature. The reaction mixture was adjusted to pH 3.2 with 5% HCl and after the precipitate had been removed by filtration, the filtrate was evaporated and the residue was dissolved in H_2O (3 ml). The solution was subjected to column chromatography on XAD2 eluted with H_2O. The fractions containing the target compound were collected and evaporated. The residue was triturated with EtOH to give **6e** as a pale yellow powder (0.17 g, 40.5%): mp 144—148 °C. IR (Nujol): 1740 (β-lactam C=O), 1660 cm^{-1}. ^{1}H-NMR (D_2O + $NaHCO_3$) δ: 3.16 (1H, dd, J=3, 5 Hz, 4β-H), 3.88 (1H, t, J=5 Hz, 4α-H), 5.06 (1H, dd, J=3, 5 Hz, 3α-H), 5.32 (1H, s, ArCHCOO), 6.82 (1H, s, thiazole ring H), 6.92 (2H, d, J=8 Hz, ArH), 7.24 (2H, d, J=8 Hz, ArH). *Anal.* Calcd for $C_{17}H_{17}N_5O_6S \cdot 2H_2O$: C, 44.83; H, 4.64; N, 15.38. Found: C, 45.27; H, 4.21; N, 15.41.

3-[(4-Hydroxyphenyl)glyoxyloylamino]nocardicinic Acid (6f) Sodium Salt——3-ANA (0.47 g) was acylated with (4-hydroxyphenyl)glyoxylic acid (0.37 g) in a manner similar to that described for **6c** to give a crude powder (0.46 g). This powder was dissolved in acetone and then treated with a solution of sodium 2-ethylhexanonate in acetone. The mixture was evaporated to give an oil, which was triturated with Et_2O to give **6f** as a pale yellow powder (0.17 g, 20.8%): mp 220—225 °C. IR (Nujol): 1740 (β-lactam C=O), 1660, 1600 cm^{-1}. ^{1}H-NMR (D_2O) δ: 3.08 (1H, dd, J=3, 5 Hz, 4β-H), 3.87 (1H, t, J=5 Hz, 4α-H), 5.08 (1H, dd, J=3, 5 Hz, 3α-H), 5.43 (1H, s, ArCHCOO), 6.77 (2H, d, J=8 Hz, ArH), 6.95 (2H, d, J=8 Hz, ArH), 7.32 (2H, d, J=8 Hz, ArH), 7.38 (2H, d, J=8 Hz, ArH). *Anal.* Calcd for $C_{19}H_{15}N_2NaO_7 \cdot H_2O$: C, 53.77; H, 4.04; N, 6.60. Found: C, 53.75; H, 3.81; N, 6.55.

3-[D-(4-Hydroxyphenyl)glycylamino]nocardicinic Acid (6g)——*N*-(*tert*-Butoxycarbonyl)-D-(4-hydroxyphenyl)-glycine succinimidyl ester (0.95 g) was added to a solution of 3-ANA (0.472 g) and $NaHCO_3$ (0.17 g) in 50% aqueous acetone (20 ml). The mixture was stirred for 8 h at room temperature and then the acetone was evaporated off. The remaining aqueous layer was washed with AcOEt, adjusted to pH 2 with 10% HCl and extracted with AcOEt. The extract was washed with H_2O, dried over $MgSO_4$ and evaporated to give a powder (0.62 g, 65.0%). This powder (0.50 g) was dissolved in trifluoroacetic acid (3 ml) under ice-bath cooling and the mixture was stirred for 1 h at the same temperature. After evaporation of the trifluoroacetic acid, the residue was dissolved in H_2O. The solution was subjected to column chromatography on XAD2 eluted with H_2O. The fractions containing the target compound were collected and evaporated. The residue was triturated with Et_2O and AcOEt to give **6f** as a powder (0.31 g, 70.0%): mp 205—209 °C. $[\alpha]_D^{24}$ −205° (c=1.0, 1% $NaHCO_3$). IR (Nujol): 1745 (β-lactam C=O), 1670, 1600 cm^{-1}. ^{1}H-NMR (D_2O) δ: 3.07 (1H, dd, J=3, 5 Hz, 4β-H), 3.82 (1H, t, J=5 Hz, 4α-H), 4.97 (1H, dd, J=3, 5 Hz, 3α-H), 5.28 (1H, s, ArCHCOO), 6.80 (2H, d, J=8 Hz, ArH), 6.90 (2H, d, J=8 Hz, ArH), 7.10 (2H, d, J=8 Hz, ArH), 7.28 (2H, d, J=8 Hz, ArH). *Anal.* Calcd for $C_{19}H_{19}N_3O_6 \cdot 1/2H_2O$; C, 57.87; H, 5.11; N, 10.65. Found: C, 57.62; H, 5.14; N, 10.39.

3-(D-Phenylglycylamino)nocardicinic Acid (6h)——This compound was prepared from 3-ANA (0.47 g) and *N*-(*tert*-butoxycarbonyl)-D-phenylglycine succinimidyl ester (0.91 g) in a manner similar to that used for **6g**, (0.25 g, 35.0%): mp 193—196 °C. IR (Nujol): 1740 (β-lactam C=O), 1695, 1610 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 2.89 (1H, dd, J=3, 5 Hz, 4β-H), 3.81 (1H, t, J=5 Hz, 4α-H), 5.27 (1H, s, ArCHCOO), 6.74 (2H, d, J=8 Hz, ArH), 7.16 (2H, d, J=8 Hz, ArH), 7.40 (5H, s, ArH). *Anal.* Calcd for $C_{19}H_{19}N_3O_5$: C, 61.78; H, 5.19; N, 11.38. Found: C, 61.99; H, 5.19; N, 11.51.

References and Notes

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