NOCARDICIN A, A NEW MONOCYCLIC β-LACTAM ANTIBIOTIC

II. STRUCTURE DETERMINATION OF NOCARDICINS A AND B

MASASHI HASHIMOTO, TADAAKI KOMORI and TAKASHI KAMIYA

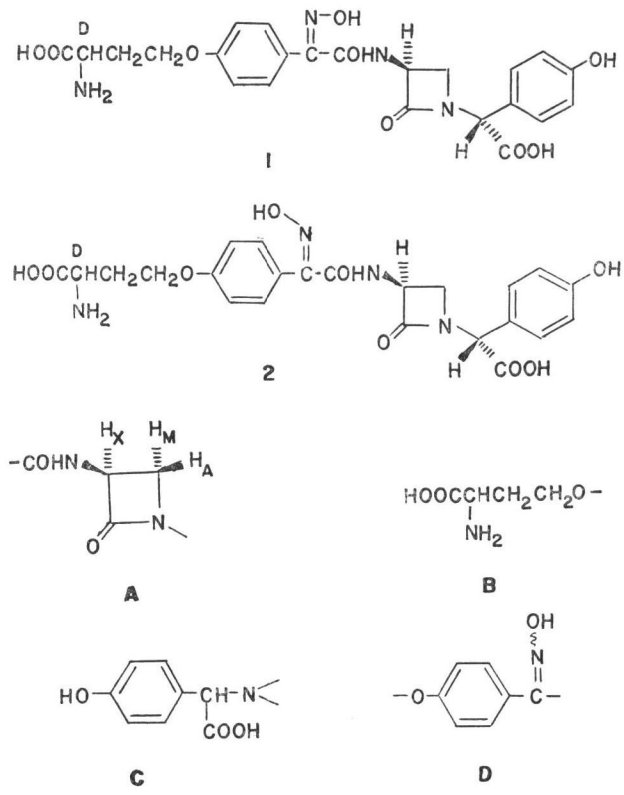
Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.
1-6, 2-chome, Kashima, Yodogawa-ku, Osaka 532, Japan

(Received for publication April 30, 1976)

The structures of nocardicins A and B, novel monocyclic β-lactam antibiotics produced by a strain of *Nocardia*, have been established as **1** and **2**, respectively, on the basis of spectroscopic and chemical evidence. They are the first examples of monocyclic β-lactam antibiotics possessing relatively high antimicrobial activity, and are stereochemically and biologically related to penicillins and cephalosporins.

The excellent antimicrobial activity displayed by the penicillins and cephalosporins has generated active interest in the search for other β-lactam-containing antibiotics in fermentation cultures.[1] As part of a broad antibiotic program in our laboratories, a search was undertaken specifically for inhibitors of bacterial cell wall biosynthesis which led to the discovery of a novel monocyclic β-lactam antibiotic, nocardicin A (**1**). The fermentation, isolation and characterization of this antibiotic have been reported in the preceding paper of this series.[2] More recently, a second antibiotic, nocardicin B (**2**), was isolated as a minor component from the same culture.[3] A preliminary bioassay showed that **1** is active against a variety of gram-negative bacteria, with especially high activity against *Proteus* and *Pseudomonas* species, while **2** shows reduced activity.[3] In this paper, we wish to report the structure determination of these two antibiotics.*

Fig. 1



Nocardicin A (**1**), $C_{23}H_{24}N_4O_9$ (elemental analysis), mp 214~216°C (dec), $[\alpha]_D$ −135° (H_2O),** positive ninhydrin test, was isolated as a

* For a preliminary account of this work, see ref. 4.

** The $[\alpha]_D$ measurements were performed on the sodium salts (see experimental section).

Fig. 2

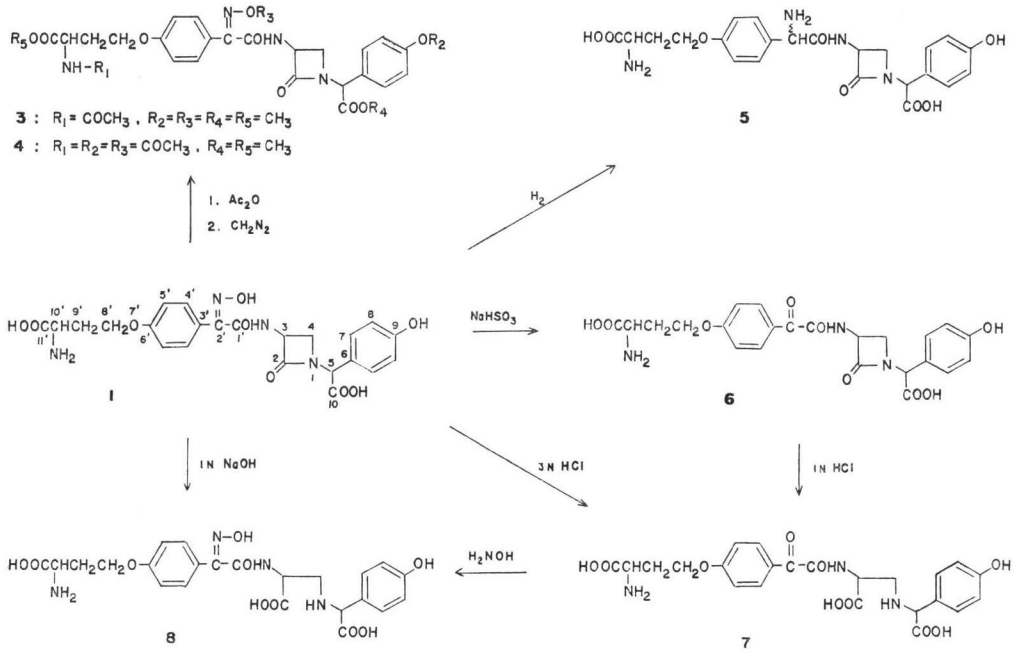


Table 1. ^{1}H NMR Data* of nocardicins and their derivatives

Compound	Solvent	3-H	4β-H	4α-H	5-H	8-H 7-H	5′-H 4′-H	8′-H	9′-H	10′-H	NHCO
1 (Na salt)	D_2O	5.01dd (5,2)	3.14dd (6,2)	3.97dd (6,5)	5.33s	6.91d 7.23d (9)	6.99d 7.42d (9)	4.22t (6)	2.39m	3.81t (6)	
1	DMSO-d_6	4.96m	3.08dd (5,2)	3.83t (5)	5.26s	6.76d 7.14d (9)	6.95d 7.42d (9)	4.14t (6)	2.19m	3.57t (6)	9.12d (8)
2 (Na salt)	D_2O	4.90dd (5,2)	2.99dd (6,2)	3.95dd (6,5)	5.27s	7.02d 7.19d (9)	6.89d 7.36d (9)	4.23t (6)	2.38m	3.71t (6)	
2	DMSO-d_6	4.91m	3.15dd (5,2)	3.74t (5)	5.24s	6.75d 7.13d (9)	6.94d 7.44d (9)	4.15t (6)	2.18m	3.55t (6)	8.81d (8)
6	D_2O ($NaHCO_3$)	5.00dd (5,2)	2.94dd (6,2)	4.00dd (6,5)	5.35s	6.93d 7.23d (9)	6.99d 7.89d (9)	4.27t (6)	2.43m	3.78t (6)	
7	D_2O-DCl	5.12dd (8,5)	3.54q (13,8) 3.86q (13,5)		5.34s	7.01d 7.47d (9)	7.07d 8.01d (9)	4.39t (5)	2.25m	4.49t (6)	
8 (Na salt)	D_2O	4.06dd (7,6)	3.17q (12,7) 3.48q (12,6)		4.70s	6.90d 7.29d (9)	6.95d 7.44d (9)	4.20t (5)	2.37m	3.94dd (6,5)	

* Values are given in parts per million δ : s=singlet; d=doublet; dd=double doublet; m=multiplet; q=quartet; t=triplet; br=broadened signal. Figures in parentheses are coupling constants in hertz.

major component from the fermentation broth of *Nocardia uniformis* subsp. *tsuyamanensis* ATCC 21806. From the same culture broth, nocardicin B (**2**), $C_{23}H_{24}N_4O_9$ (elemental analysis), mp 262~264° C (dec), $[\alpha]_D$ −162° (H_2O), positive ninhydrin test, was isolated as a minor product.

Potentiometric titration of **1** revealed the presence of five ionizable groups, which were further characterized by the following reactions: acetylation of **1** with Ac_2O in MeOH at 0°C and subsequent methylation with diazomethane gave the monoacetyl-tetramethyl derivative **3**, while acetylation of **1** with Ac_2O in H_2O (pH 9~10, room temperature), followed by methylation with diazomethane gave the triacetyl-dimethyl derivative **4**. These results indicate that **1** contains one amino, two carboxyl and two weakly acidic hydroxyl groups.

The ^1H nmr analysis (see Table 1), together with uv and ir studies of **1** revealed all partial units of the structure. The ^1H nmr spectrum shows an AMX system at 3.14, 3.97 and 5.01 ppm ($J_{AM}=6$ Hz, $J_{AX}=2$ Hz, $J_{MX}=5$ Hz) which is attributed to the geminal protons (H_A and H_M) and the adjacent one (H_X) in partial structure *A*. Proton X is also coupled to the amide proton when measured in DMSO-d_6 (NH, 9.12 ppm, doublet, J=8 Hz). The relatively small coupling constants observed for protons A, M and X are consistent with a β-lactam ring system,* whose presence was also supported by an ir carbonyl band at 1730 cm^{-1} (sodium salt of **1**). A 2H multiplet at 2.39 ppm coupled to a 2H triplet (J=6 Hz) at 4.22 ppm and a 1H triplet (J=6 Hz) at 3.81 ppm suggested the presence of the homoserine unit (partial structure *B*), these signals being assigned to 9′–H, 8′–H and 10′–H, respectively. Two sets of AB systems centered at 7.07 ppm (4H, J=9 Hz, 8–H and 7–H) and 7.22 ppm (4H, J=9 Hz, 5′–H and 4′–H) indicated the presence of two *para* substituted aromatic groups. The 2H signal of 4′–H at 7.42 ppm was shifted down-field to 7.89 ppm in the spectrum of the keto derivative **6** (see Table 1). These aromatic groups were further characterized as a *para* alkylated phenol (*e.g.*, partial structure *C*) and a conjugated alkoxyphenyl derivative (*e.g.*, partial structure *D*) on the basis of uv spectra: λmax 220 nm (ε, 21000) and 272 nm (ε, 16000) in EtOH-H_2O and, λ max 245 nm (ε, 23500) and 285 nm (ε, 11300) in EtOH - 0.1N NaOH. A 1H singlet at 5.33 ppm in the ^1H nmr spectrum of **1** was assigned to the benzylic methine proton 5–H of partial structure *C*.

These partial structures were further corroborated by the ^{13}C nmr analysis of **1** (see Table 2). The proton-decoupled spectrum shows 23 carbon signals, in which six signals in the up-field region were easily identified by an off-resonance experiment. The three triplets at 30.63, 47.02 and 66.01 ppm were directly assigned to the three methylene carbons 9′–C, 4–C and 8′–C, respectively, while the three doublets at 54.17, 54.90 and 61.58 ppm were assigned to the three methine carbons 10′–C, 5–C and 3–C, respectively, by comparison with the corresponding signals in degradation products (see below). Of the twelve aromatic carbon signals due to partial structures *C* and *D*, the six signals at 123.95, 128.68 (doublet, two C), 115.88 (doublet, two C) and 160.53 ppm were assigned to the 3′–C, 4′–C, 5′–C and 6′–C in partial structure *D*, and the other six signals at 127.46, 131.04 (doublet, two C), 116.54 (doublet, two C) and 156.41 ppm to the 6–C, 7–C, 8–C and 9–C in partial structure *C*, respectively, by comparison with the spectra of the degradation compounds (see Table 2).

The ^{13}C nmr spectrum shows five additional signals in the down-field region. Four of them, at 166.84, 168.54, 174.73 and 176.61 ppm, were attributed to the amide (1′–C), β-lactam (2–C) and two carboxyl (11′–C and 10–C) groups, respectively, while the remaining signal at 153.74 ppm, because of its relatively high chemical shift, was assigned to the oxime group (2′–C), which constitutes one of the two weakly acidic hydroxyl functions (the other being the phenolic hydroxyl). This assignment was supported by the nmr spectrum of keto derivative **6**, in which the 2′–C signal was shifted down-field to 188.38 ppm.

* For data of geminal and vicinal coupling constants of substituted β-lactams, see ref. 5.

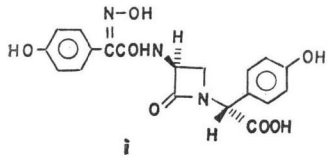
Table 2. ^{13}C NMR Data[a] of nocardicin A and its derivatives

	1[b] (Na salt)	**6**[b] (Na salt)	**8**[b] (Na salt)	**7**[c]	**10**[c]	**11**[c]
9′-C	30.63	30.63	30.63	33.55	34.76	
4-C	47.02	46.77	47.62	48.47		49.44
8′-C	66.01	66.19	66.07	66.43	66.79	
10′-C	54.17	53.93	54.17	54.05	54.42	
5-C	54.90	54.78	52.17	55.75		55.02
3-C	61.58	61.45	66.07	67.16		67.16
6-C	127.46	127.34	124.37[d]	129.16		129.65
7-C	131.04	131.10	131.23	129.53		129.65
8-C	116.54	116.54	117.09	117.51		117.03
9-C	156.41	156.46	157.80	160.47		158.53
3′-C	123.95	126.01	123.95[d]	125.77	125.77	
4′-C	128.68	136.02	128.86	133.90	133.29	
5′-C	115.88	115.57	115.88	115.57	115.94	
6′-C	160.53	164.47	160.59	164.84	164.72	
2′-C	153.74	188.38	154.10	190.21	196.63	
1′-C	166.84	166.17	166.78	166.90	183.52	
2-C	168.54	168.36				177.70
10-C	176.61	176.61	173.39[e] 174.00 174.79	180.50[f] 181.34		179.76
11′-C	174.73	174.79		176.85	174.18	

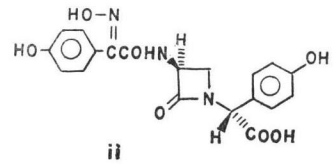
[a]: Values are given in parts per million δ. [b]: In D_2O solution. [c]: In D_2O-NaOD solution.
[d]: Assignments may be interchanged. [e,f]: Not assigned.

Hydrogenation of **1** over 10% Pd-C gave an isomeric mixture of amines **5*** in which the newly introduced methine protons appear at 5.02 (singlet) and 5.06 (singlet) ppm, supporting the presence of the oxime group in **1**. Additionally, treatment of **1** with $NaHSO_3$ afforded the keto derivative **6*** which shows a uv absorption maximum at 300 nm (ε, 15400) characteristic of a *para*-alkoxyphenyl keto acid derivative. Hydrolysis of **6** with 1 N HCl gave compound **7** identified by its ^1H nmr spectrum which shows an ABX system at 3.56, 3.86 and 5.12 ppm ($J_{AB}=13$ Hz, $J_{AX}=8$ Hz and $J_{BX}=5$ Hz) due to 4–H (two protons) and 3–H, instead of the AMX system of **1** and **6**. Compound **7** was also obtained by direct hydrolysis of **1** with 3 N HCl. Reaction of **7** with NH_2OH yielded compound **8**, which was also obtained from **1** by treatment with 1 N NaOH. This sequence of reactions conclusively establishes the presence of the oxime group in **1**. The ease of hydrolysis of **1** and **6** in the above reaction

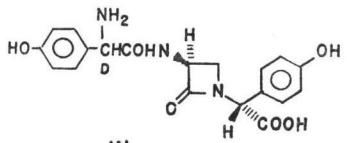
* Very recent experiments in our laboratories have revealed the presence of five additional minor antibiotic components, nocardicins C,D,E,F and G in the same fermentation filtrate.[6] Nocardicins C and D are identical with one of isomeric amines **5** and keto compound **6**, respectively, while E, F and G have been shown to be **i**, **ii** and **iii**, respectively, by analysis of their spectral data and syntheses from 3-ANA (**20**) (see below). Details will be reported elsewhere.



i

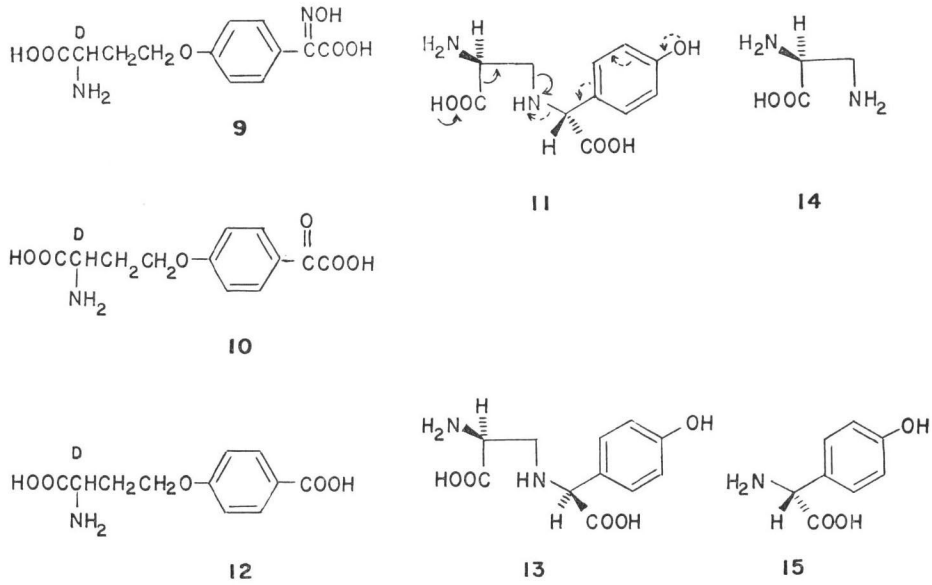


ii



iii

Fig. 3



sequence also indicates the presence of the β-lactam moiety in **1**.

Final confirmation of the full structure of nocardicin A (**1**) was obtained from acid degradation experiments. Hydrolysis of **1** in refluxing 6 N HCl gave compounds **9**, **10** and **11**, which were fully characterized by further degradation reactions as follows: Oxime acid **9** was again hydrolyzed with refluxing 6 N HCl to give keto acid **10**, which reverted to **9** upon treatment with NH_2OH and was further degraded to acid **12** by oxidation with H_2O_2. Prolonged heating of **1** with 6 N HCl gave, in addition to **10** and **11**, acid **12**, **13** (the epimer of **11**), L-$\alpha\beta$-diaminopropionic acid (**14**) and DL-*p*-hydroxyphenylglycine. The formation of **12** most likely results from the decarbonylation of keto acid **10**. The geneses of $\alpha\beta$-diaminopropionic acid and *p*-hydroxyphenylglycine can be rationalized as shown by the arrows in structure **11**. In support of this latter explanation, **11** was treated with refluxing 6 N HCl to generate L-$\alpha\beta$-diaminopropionic acid (**14**) and partially racemized D-*p*-hydroxyphenylglycine (**15**) (optical purity 54%). Thus, the prolonged heating described above led to complete racemization of *p*-hydroxyphenylglycine and partial epimerization of compound **11**. These chemical data are in full agreement with the structure **1** of nocardicin A.

The absolute configurations at the 3-C and 5-C were established to be L and D, respectively, from the optical rotation data obtained on degradation products L-$\alpha\beta$-diaminopropionic acid (**14**) and D-*p*-hydroxyphenylglycine (**15**). The absolute configuration of the homoserine moiety was determined to be D by the formation of D-α-aminobutyrolactone (**16**) and cyclohexanecarboxylic acid (**17**) from the hydrogenation of **12** over ADAMS catalyst in 3 N HCl.

The stereochemistry of the oxime group in nocardicin A was established to be *syn* to the acylamino group (Z isomer) by comparison of the physical data for nocardicin A (**1**) with those for nocardicin B (**2**). The ^1H nmr spectra of **1** and **2** are superimposable, suggesting that they are stereoisomers. Further, treatment of **2** with $NaHSO_3$ also gave the keto derivative **6**, indicating that **2** is a stereoisomer of **1** at the oxime function. The ^1H nmr spectrum of **1** in DMSO-d_6 shows the amide proton at 9.12 ppm

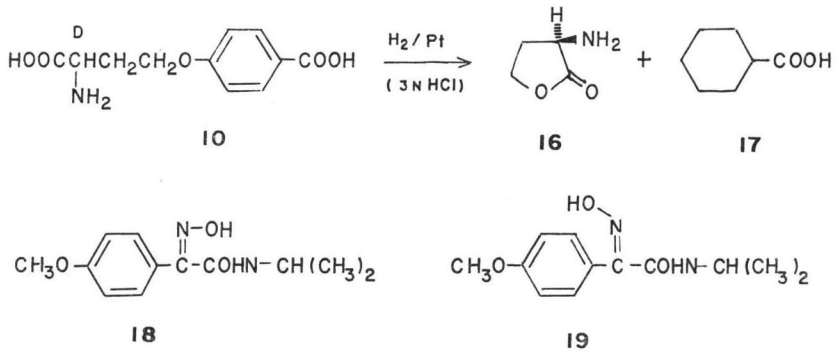
(as described above), while in **2** it is at 8.81 ppm (doublet, J=8 Hz). This 0.31 ppm downfield shift in the amide proton of **1** suggested that an internal hydrogen bonding exists between the oxime oxygen and the amide proton in **1**. This is possible only if the oxime OH is *syn* to the amide group. This behavior was observed also in model compounds **18** and **19**. Thus, compound **18** which has the oxime OH *syn* (Z) to the amide group shows the amide proton at 8.30 ppm, while the *anti* (E) isomer **19** shows it at 8.00 ppm. This chemical shift difference (0.30 ppm) is in good agreement with that observed in the case of nocardicins A and B.

The above conclusion was also supported by the following uv studies: the uv spectra due to partial structure *D* of nocardicins A and B were calculated by subtraction of the absorbance of *p*-hydroxyphenylglycine from those of nocardicins: **1**, $\lambda_{max}^{EtOH-H_2O}$ 270 nm (ε, 14900) and $\lambda_{max}^{EtOH-0.1N\ NaOH}$ 283 nm (ε, 9500); **2**, $\lambda_{max}^{EtOH-H_2O}$ 267 nm (ε, 8900) and $\lambda_{max}^{EtOH-0.1N\ NaOH}$ 275 nm (ε, 9400). Comparing these differential spectra then, the absorption maximum of **1** is at a higher wavelength and has a larger extinction coefficient in both neutral and basic media than the maximum observed for **2**. This agrees with the behavior of model compounds **18** and **19**: **18** (*syn* isomer), $\lambda_{max}^{EtOH-H_2O}$ 270 nm (ε, 15800) and $\lambda_{max}^{EtOH-0.1N\ NaOH}$ 283 nm (ε, 11400); **19** (*anti* isomer), $\lambda_{max}^{EtOH-H_2O}$ 267 nm (ε, 9800) and $\lambda_{max}^{EtOH-0.1N\ NaOH}$ 275 nm (ε, 9500). These data also confirmed that the oxime function is *syn* (Z) to the amide group in **1** and *anti* (E) in **2**.

The structures of nocardicins A and B were thus established as being **1** and **2**, respectively. Nocardicin A shows relatively high *in vivo* activity against a variety of gram-negative bacteria including *Pseudomonas* and indole-positive *Proteus* species,*1 while nocardicin B is somewhat weaker.[3]

These antibiotics possess several unique features: (1) they are the first examples of a monocyclic β-lactam antibiotic possessing relatively high antimicrobial activity;*2 (2) they contain an oxime function whose *syn* (Z) relation to the acylamino group is favored for antimicrobial activity in nocardicins;*3 (3) they contains a *p*-hydroxyphenylglycine unit which is rare in nature;*4 (4) their structures are stereochemically related to the penicillin molecule: *i.e.*, the presence of the L-configuration of the

Fig. 4



*1 Detailed antimicrobial activity of nocardicin A will be reported in the following paper of this series.[7]

*2 Numerous compounds containing monocyclic β-lactam rings have been synthesized hitherto from a viewpoint of modification of the natural penicillins and cephalosporins. However, removal of the thiazolidine ring in penicillins and the thiazine ring in cephalosporins eliminates or greatly diminishes their biological activity.

*3 Aside from semisynthetic compounds,[8] few natural products bearing an oxime group have been isolated from microorganisms,[9] although hydroxamic acid-containing microbial products are common.[10] This unusual oxime group may be derived biogenetically from an amino group *via* a hydroxylamine by enzymatic oxidations.

*4 To our knowledge, vancomycin is the only example of a microbial product containing a *para* oxygenated phenylglycine unit.[11]

3-acylamino side chain and the D-configuration of the 5-carboxyl group; and (5) like the penicillins and cephalosporins, they are inhibitors of bacterial cell wall biosynthesis.[2]

These features of nocardicins are unique additions to the structure-activity relationships in the β-lactam antibiotic field. Chemical modification of nocardicins and preparation of new 3-acyl derivatives of 3-aminonocardicinic acid (3-ANA) (**20**) are in progress.*

Experimental

Melting points were measured on a Thomas-Hoover apparatus and are uncorrected. ^{1}H nmr spectra were obtained at 100 MHz on a Jeol PS-100 spectrometer. ^{13}C nmr spectra were taken at 25.1 MHz on the same instrument using pulsed FT. Chemical shifts (δ) are reported as parts per million from tetramethylsilane. Infrared and ultraviolet spactra were recorded using a Hitachi 215 spectrophotometer and a Hitachi 323 spectrophotometer, respectively. Optical rotations were measured on a JASCO DIP-SL automatic polarimeter. Thin-layer chromatography (tlc) was carried out on Eastman chromagram sheets (solvent, *n*-BuOH - AcOH - H_2O 4: 1: 2); the spots were detected by spraying with a 0.5% ninhydrin solution in *n*-BuOH.

Nocardicin A (**1**): For isolation of nocardicin A, see experimental section in ref. 2: mp 214~216°C (dec); pKa (in 50% DMSO) 3.2, 4.5, 10.0, 11.6, 12.7; ir (nujol) 2100~3650, 1730, 1655, 1610 cm^{-1}; uv $\lambda_{max}^{EtOH-H_2O}$ 220 nm (ε, 21000), 272 (16000), $\lambda_{max}^{EtOH-0.1N\ NaOH}$ 245 nm (ε, 23500), 285 (11300).

Anal. Calcd. for $C_{23}H_{24}N_4O_9\ H_2O$: C 53.28; H 5.05; N 10.81.
Found: C 53.50; H 4.89; N 10.74.

The sodium salt was prepared as follows: A stirred suspension of nocardicin A in H_2O was adjusted to pH 7 by addition of 1 N NaOH. The resulting solution was concentrated and the residue was recrystallized from 70% EtOH: mp 234~235°C (dec); $[\alpha]_D$ −135° (*c* 1.0, H_2O); ir (nujol) 2200~3700, 1730, 1645, 1605 cm^{-1}; nmr (see Tables 1 and 2).

Anal. Calcd. for $C_{23}H_{23}N_4NaO_9$: C 52.88; H 4.44; N 10.72; Na 4.40.
Found: C 52.56; H 4.49; N 10.77; Na 4.05.

Nocardicin B (**2**): Isolation of **2** will be reported shortly[3]: mp 262~264°C (dec); ir (nujol) 3200 (br), 1740, 1710, 1660, 1630, 1615 cm^{-1}; uv $\lambda_{max}^{EtOH-H_2O}$ 224 nm (ε, 24600), 270 (9700), $\lambda_{max}^{EtOH-0.1N\ NaOH}$ 245 nm (ε, 26000), 280 (11100).

Anal. Calcd. for $C_{23}H_{24}N_4O_9$: C 55.20; H 4.83; N 11.20.
Found: C 55.34; N 4.80; N 10.86.

The sodium salt was prepared in the same way as for nocardicin A; mp 257~260°C (dec); $[\alpha]_D$ −162° (*c* 1.0, H_2O); ir (nujol) 3300 (br), 1703, 1662, 1630, 1605, 1590 cm^{-1}; nmr (see Table 1).

Anal. Calcd. for $C_{23}H_{23}N_4NaO_9\ 2H_2O$: C 49.46; H 4.87; N 10.03; Na 4.12.
Found: C 49.51; H 4.52; N 9.98; Na 4.51.

Monoacetyltetramethylnocardicin A (**3**): To a cooled suspension of **1** sodium salt (500 mg) in 30 ml of MeOH was added 1 ml of Ac_2O and the mixture was stirred for 4 hours at 0°C. The resulting solution was diluted with H_2O and extracted with EtOAc. The extract was dried and evaporated to give a crude product (550 mg), which was dissolved in 15 ml of MeOH and treated with ethereal diazomethane overnight in a refrigerator. The usual work-up and purification by column chromatography on silicic acid ($CHCl_3$) to give 350 mg of **3** as an oil: ir ($CHCl_3$) 3450, 1760, 1745, 1677, 1610 cm^{-1}; nmr ($CDCl_3$) δ 1.96 (3H, s, $COCH_3$), 2.27 (2H, m, 9-H), 3.17 (1H, dd, J=5 and 2 Hz, 4β-H), 3.73 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.02 (2H, t, J=6 Hz, 8-H), 4.72 (1H, br.q, J=6-7 Hz, 10'-H), 4.95 (1H, m, 3-H), 5.63 (1H, s, 5-H), 6.78 and 7.19 (4H, ABq, J=9 Hz, 8-H and 7H), 6.89 and 7.47 (4H, ABq, J=9 Hz, 5'-H and 4'-H).

Triacetyldimethylnocardicin A (**4**): To a cooled solution of **1** sodium salt (500 mg) in 5 ml of H_2O was added dropwise 1 ml of Ac_2O over 1 hour with stirring and keeping the reaction mixture in the pH

* Preparation of **20** from nocardicin A will be reported elsewhere.

range 9~10 with 1 N NaOH. The reaction mixture was acidified with 1 N HCl and extracted with EtOAc. The extract was dried and evaporated to give a crude oil, which was dissolved in 10 ml of MeOH and treated with ethereal diazomethane overnight in a refrigerator. The usual work-up and purification by column chromatography on silicic acid ($CHCl_3$) to give 300 mg of **4** as an oil: ir (film) 3280 (br), 1740 (br), 1655 (br), 1605 cm^{-1}; nmr ($CDCl_3$) δ 1.95 (3H, s, $COCH_3$), 2.04 (3H, s, $COCH_3$), 2.27 (3H, s, $COCH_3$), 2.24 (2H, m, 9'-H), 3.28 (1H, dd, J=5 and 2 Hz, 4β-H), 3.73 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 4.00 (2H, t, J=6 Hz, 8'-H), 4.70 (1H, br.q, J=6—7 Hz, 10'-H), 5.10 (1H, m, 3-H), 5.61 (1H, s, 5-H).

Hydrogenation of nocardicin A (**1**): A solution of **1** sodium salt (500 mg) in 6 ml of H_2O was hydrogenated over 10% Pd-C (150 mg) in the usual manner. After the catalyst was filtered off, the filtrate was acidified to pH 3 with dilute HCl and 6 ml of acetone was added to give 380 mg of **5** as crystals: mp 220~225°C (dec); ir (nujol) 2200~3700, 1725, 1690, 1610 (br), 1585 (br) cm^{-1}; uv $\lambda_{max}^{H_2O}$ 229 nm (ε, 22800), 273 (2200); nmr (D_2O-$NaHCO_3$) δ 2.33 (2H, m, 8-H), 2.99 (1H, m, 4β-H), 3.68 (2H, t, J=6 Hz, 9'-H), 3.92 (1H, dd, J=6 and 5 Hz, 4α-H), 4.12 (2H, t, J=6 Hz, 10'-H), 4.85 (1H, m, 3-H), 5.02 and 5.06 (1H, two s, 2'-H), 5.24 (1H, s, 5-H).

Anal. Calcd. for $C_{23}H_{26}N_4O_9$ $2H_2O$: C 52.87; H 5.97; N 10.72.
Found: C 53.02; H 5.62; N 10.67.

Conversion of nocardicin A (**1**) to **6**: To a solution of **1** sodium salt (500 mg) in 10 ml of H_2O was added 500 mg of $NaHSO_3$ and the mixture was stirred for 3 hours at 80°C. After cooling, the solution was acidified to pH 3 with dilute HCl and concentrated to about 5 ml to separate crystals, which were collected and washed with H_2O to give 330 mg of **6**: mp 230~235°C (dec); $[\alpha]_D$ −186° (*c* 1.0, 1% $NaHCO_3$); ir (nujol) 3460, 3200 (br), 1735, 1675, 1645, 1610, 1600 cm^{-1}; uv $\lambda_{max}^{EtOH-H_2O}$ 227 nm (ε, 19500), 300 (15400); nmr (see Tables 1 and 2).

Anal. Calcd. for $C_{23}H_{23}N_3O_9$ $1/2H_2O$: C 55.87; H 4.90; N 8.50.
Found: C 55.52; H 4.82; N 8.74.

Acid hydrolysis of keto derivative **6**: A suspension of **6** (100 mg) in 10 ml of 1 N HCl was stirred for 20 hours at room temperature. After the resulting solution had been evaporated, the residue was dissolved in a small amount of H_2O and neutralized to pH 3 with dilute NaOH and the crystalline precipitate was collected, washed with H_2O and then acetone to yield 57 mg of **7**: mp 203~205°C (dec); ir (nujol) 2200~3200, 1730 (br), 1650, 1600 cm^{-1}; uv $\lambda_{max}^{EtOH-H_2O}$ 229 nm (ε, 17500), 300 (14800); nmr (see Tables 1 and 2).

Anal. Calcd. for $C_{23}H_{25}N_3O_{10}$ H_2O: C 52.97; H 5.21; N 8.06.
Found: C 53.14; H 5.37; N 8.13.

Acid hydrolysis of nocardicin A (**1**): A suspension of **1** (500 mg) in 15 ml of 3 N HCl was stirred for 5 hours at room temperature. The resulting solution was adjusted to pH 3 with dilute NaOH. Crystals separated and were collected and washed with MeOH to yield 280 mg of **7**, which was identical to the product obtained from acid hydrolysis of **6**.

Alkaline hydrolysis of nocardicin A (**1**): A solution of **1** sodium salt (500 mg) in 10 ml of 1 N NaOH was stirred for 15 hours at room temperature. The reaction mixture was adjusted to pH 5 by addition of Dowex 50 W ion-exchange resin (H^+ form). After the resin was filtered off, the filtrate was concentrated to dryness and the residue was washed with MeOH to give 450 mg of **8** sodium salt. A portion (300 mg) of this sodium salt was dissolved in a small amount of H_2O and acidified to pH 2 with dilute HCl to separate crystals, which were collected, washed with H_2O and then acetone to yield 210 mg of **8**: mp 203~205°C (dec); ir (nujol) 3380, 3150 (br), 1705(br), 1635 (br), 1620 (sh), 1605 (sh) cm^{-1}; uv $\lambda_{max}^{H_2O}$ 223 nm (ε, 19300), 273 (15100); nmr (see Tables 1 and 2).

Anal. Calcd. for $C_{23}H_{26}N_4O_{10}$ H_2O: C 51.49; H 5.26; N 10.44.
Found: C 51.75; H 5.16; N 10.54.

Reaction of **7** with hydroxylamine: To a suspension of **7** (150 mg) in 20 ml of EtOH - H_2O (1 : 1) was added 350 mg of NH_2OH (free) and the mixture was refluxed for 2 hours. After removal of solvent, the residue was dissolved in a small amount of H_2O and acidified to pH 3 with dilute HCl. Crystals separated and were collected and washed with H_2O to yield 100 mg of **8**. Identical to product obtained above.

Acid degradation of nocardicin A (**1**): (I) A suspension of **1** (1.20 g) in 50 ml of 6 N HCl was refluxed for 1 hour and the resulting brown-colored solution was treated with active carbon and concentrated to dryness. The residue was dissolved in a small amount of H_2O and left at room temperature. Crystals separated and were collected and washed with H_2O to yield 170 mg of **10**: mp 186~189°C (dec); $[\alpha]_D$ −15.1° (*c* 2.7, 1 N NaOH); ir (nujol) 2300~3300, 1740, 1653, 1600 cm^{-1}; uv $\lambda_{max}^{0.1N\ HCl}$ 291 nm (ε, 16200); ^{13}C nmr (see Table 2).

Anal. Calcd. for $C_{12}H_{13}NO_6$: C 53.93; H 4.90; N 5.24.
Found: C 53.91; H 4.89; N 5.21.

The filtrate was again concentrated to dryness, and the residue was dissolved in a small amount of H_2O and left overnight. Crystals separated and were collected and purified by dissolving in dilute NaOH, followed by neutralization with dilute HCl to yield 160 mg of **11**: mp 204~206°C (dec); ir (nujol) 3450, 1655, 1565 (br), 1515 cm^{-1}; uv $\lambda_{max}^{0.1N\ HCl}$ 230 nm (ε, 10500), 273 (1300); nmr (D_2O–DCl) δ 3.52 and 3.76 (2H, octet, AB part of ABX pattern, J_{AB}=13 Hz, J_{AX}=6 Hz, J_{BX}=7 Hz), 4.62 (1H, q, X part of ABX pattern, J=6 and 7 Hz), 5.28 (1H, s), 7.04 and 7.45 (4H, ABq, J=9 Hz); ^{13}C nmr (see Table 2).

Anal. Calcd. for $C_{11}H_{14}N_2O_5\ H_2O$: C 48.52; H 5.92; N 10.29.
Found: C 48.40; H 5.75; N 10.20.

The filtrate was left overnight to precipitate a crystalline solid, which was collected and purified in the same way as for **11** to yield 30 mg of **9**: mp 193~195°C (dec); ir (nujol) 3230, 1715, 1615 (sh), 1605, 1570, 1550 cm^{-1}; uv $\lambda_{max}^{0.1N\ HCl}$ 273 nm (ε, 14200); nmr (D_2O–$NaHCO_3$) δ 2.37 (2H, m), 3.95 (1H, dd, J=7 and 5 Hz), 4.23 (2H, t, J=6 Hz), 7.04 and 7.54 (4H, ABq, J=9 Hz).

Anal. Calcd. for $C_{12}H_{14}N_2O_6$: C 51.06; H 5.00; N 9.93.
Found: C 51.02; H 5.00; N 9.73.

(II) A suspension of **1** (2.00 g) in 80 ml of 6 N HCl was refluxed for 7 hours. The hot, dark-brown colored solution was treated with active carbon and the filtrate was allowed to stand at room temperature. The crystalline precipitate was collected and washed with H_2O to give 290 mg of **12**: mp 290~291°C (dec); ir (nujol) 1850~3300, 1653, 1608, 1585, 1507 cm^{-1}; uv $\lambda_{max}^{0.1N\ HCl}$ 256 nm (ε, 16100).

Anal. Calcd. for $C_{11}H_{13}NO_5$: C 55.23; H 5.48; N 5.86.
Found: C 54.99; H 5.45; N 5.81.

The filtrate was evaporated to dryness, the residue was dissolved in H_2O and allowed to stand for several hours. The crystalline precipitate was collected and washed with H_2O to give 70 mg of **10**. Identical to material obtained above.

After evaporation of the filtrate, EtOH was added to the residue and the crystalline precipitate was collected to yield 250 mg of L-α,β-diaminopropionic acid hydrochloride (**14**): mp 240~242°C (dec); $[\alpha]_D$ +20.3° (*c* 2.5, 1 N HCl) [lit.[12] $[\alpha]_D^{25}$ +25.2° (*c* 2, 1 N HCl)].

Anal. Calcd. for $C_3H_8N_2O_2$ HCl: C 25.63; H 6.45; N 19.93.
Found: C 25.67; H 6.57; N 19.76.

The mp, ir, nmr and tlc were identical with those of an authentic sample.

The filtrate was again evaporated to dryness and the residue was chromatographed over 50 g of cellulose, 20 ml fractions being collected in the following order: 1~85 (*n*-BuOH saturated with H_2O), 86~137 (10% AcOH in *n*-BuOH saturated with H_2O), 138~158 (10% AcOH in H_2O). Fractions 2~8 gave crystals of DL-*p*-hydroxyphenylglycine hydrochloride (20 mg): mp 213~215°C (dec): identical with an authentic sample by mp, ir and tlc.

Fractions 31~46 gave crystals of **13** (34 mg): mp 225~228°C (dec); $[\alpha]_D$ −9.4° (*c* 1.2, 0.1 N NaOH); ir (nujol) 2200~3650, 1625, 1605, 1508 cm^{-1}; uv $\lambda_{max}^{0.1N\ HCl}$ 230 nm (ε, 10000), 273 (1100); nmr (D_2O–DCl) δ 3.69 (2H, d, J=6 Hz), 4.63 (1H, t, J=6 Hz), 5.33 (1H, s), 7.03 and 7.45 (4H, ABq, J=9 Hz).

Anal. Calcd. for $C_{11}H_{14}N_2O_5$: C 51.96; H 5.55; N 11.02.
Found: C 51.57; H 5.53; N 10.81.

Fractions 47~81 gave an additional 48 mg sample of L-α,β-diaminopropionic acid hydrochloride (**14**). Fractions 92~158 gave crystals of **11** (50 mg): identical with sample obtained previously.

Acid degradation of **11**: A suspension of **11** (500 mg) in 40 ml of 6 N HCl was refluxed for 3 hours. The resulting solution was treated with active carbon and evaporated to dryness. The residue was

chromatographed over 50 g of cellulose, 50 ml fractions being collected in the following order: 1~30 (*n*-BuOH saturated with H_2O), 31~44 (5% AcOH in *n*-BuOH saturated with H_2O), 45~170 (5% AcOH in H_2O). Fractions 9~10 gave 35 mg of D-*p*-hydroxyphenylglycine hydrochloride (**15**); mp 210~213°C (dec); $[\alpha]_D$ −80.0° (*c* 0.3, 0.1 N HCl) [an optically pure sample (HCl salt) showed $[\alpha]_D$ −149° (*c* 0.5, 0.1 N HCl); lit.[13] $[\alpha]_D^{22}$ −108° (*c* 1, H_2O)]; ir 2300~3200, 1744, 1725, 1695 (sh), 1615, 1517 cm^{-1}.

Anal. Calcd. for $C_8H_9NO_3$ HCl: C 47.19; H 4.95; N 6.88.
Found: C 46.82; H 4.63; N 6.62.

The tlc was identical with that of an authentic sample.

Fractions 35~44 gave crystals of **13** (150 mg): identified by mp, ir and tlc. Fractions 50~86 gave crystals (50 mg) of L-$\alpha\beta$-diaminopropionic acid hydrochloride (**14**): identified by mp, ir and tlc. Fractions 140~167 gave 240 mg of the starting material **11**.

Acid hydrolysis of **9** to **10**: A 5-mg sample of **9** was hydrolyzed with 1 ml of 6 N HCl by refluxing for 1 hour. After concentration of the resulting solution, the residue was crystallized from H_2O to give 3 mg of **10**; identified by mp, ir and tlc.

Conversion of **10** to **9**: A suspension of **10** (130 mg) in 5 ml of H_2O containing 30 mg of $NH_2OH \cdot$ HCl and 40 mg of $NaHCO_3$ was refluxed for 30 minutes. The resulting solution was acidified to pH 3 with dilute HCl to separate crystals, which were collected and washed with H_2O to yield 110 mg of **9**: identified by mp, ir and tlc.

Oxidation of **10** to **12**: To a solution of **10** (25 mg) in 1 ml of 10% NaOH was added dropwise 1 ml of H_2O_2 (30% solution) and the mixture was stirred for 1 hour. The reaction mixture was adjusted to pH 3 with 10% HCl to separate crystals, which were collected and recrystallized from H_2O to yield 17 mg of **12**: identified by mp, ir and tlc.

Hydrogenation of **12**: A solution of **12** (1.00 g) in 150 ml of 3 N HCl was hydrogenated over ADAMS catalyst (1.00 g) in the usual manner. After the catalyst was filtered off, the filtrate was washed with ether and evaporated to dryness. The residue was recrystallized with MeOH-ether to give 340 mg of D-α-aminobutyrolactone hydrochloride: mp 224°C (dec); $[\alpha]_D$ +29.0° (*c* 1.0, 0.1 N HCl) [L-isomer derived from L-homoserine by refluxing in 3 N HCl: $[\alpha]_D$ −29.0° (*c* 1.0, 0.1 N HCl); lit.[14] $[\alpha]_D^{26}$ +26.7° (*c* 5, H_2O) for the D isomer]; ir (nujol) 3450, 1780, 1610, 1505 cm^{-1}.

Anal. Calcd. for $C_4H_7NO_2$ HCl: C 34.91; H 5.86; N 10.18.
Found: C 34.60; H 5.78; N 10.26.

The ether layer was washed with H_2O, dried, and evaporated to give an oil product (400 mg): ir (film) 2300~3600, 1700, 940 cm^{-1}. A 260 mg sample of this oil was dissolved in 8 ml of EtOH-H_2O (5: 3) and adjusted to pH 6 with 1 N NaOH. To this solution was added 550 mg of *p*-bromophenacyl bromide and the mixture was refluxed for 2 hours. After cooling, the resulting crystals were collected and recrystallized from EtOH to yield 310 mg of *p*-bromophenacyl ester of cyclohexanecarboxylic acid (**17**): mp 88~89°C (dec); ir (nujol) 1750, 1695, 1590 cm^{-1}.

Anal. Calcd. for $C_{15}H_{17}BrO_3$: C 55.40; H 5.27; Br 24.57.
Found: C 55.31; H 5.19; Br 25.08.

The mp and ir were identical with those of the sample prepared from authentic cyclohexanecarboxylic acid and *p*-bromophenacyl bromide in the same manner.

Conversion of nocardicin B (**2**) to **6**: To a solution of **2** sodium salt (90 mg) in 2 ml of H_2O containing 100 mg of $NaHCO_3$ was added 100 mg of $NaHSO_3$ and the mixture was stirred for 3 hours at 80°C. After removal of the resulting precipitate (20 mg of the starting material recovered as free acid), the filtrate was acidified (pH 3) with dilute HCl. Crystals separated and were collected and washed with H_2O and then acetone to yield 45 mg of **6**: identified by mp, ir and tlc.

Preparation of model compounds **18** and **19**: To a solution of 4.20 g of ethyl *p*-methoxyphenylglyoxylate (oil: ir (film) 1740 cm^{-1}, prepared from *p*-methoxyphenylglyoxylic acid[15] by treatment with EtOH-HCl) in *i*-PrOH was added 3.60 g of isopropylamine and the mixture was refluxed for 3 hours. After removal of solvent, the residue was dissolved in H_2O and extracted with EtOAc. The extract was washed with dilute HCl, dried, and evaporated to give crystals, which were collected and washed with ether to yield 2.20 g: mp 97~98°C; ir (nujol) 3270, 1670, 1640, 1605 cm^{-1}; uv λ_{max}^{EtOH} 298 nm (ε, 15900).

Anal. Calcd. for $C_{12}H_{15}NO_3$: C 65.14; H 6.83; N 6.33.
Found: C 64.90; H 6.82; N 6.23.

A 1.53 g sample of this product was dissolved in 30 ml of *i*-PrOH - H_2O (1: 1) containing 1.67 g of $NH_2OH \cdot HCl$ and 2.10 g of $NaHCO_3$, and the mixture was refluxed for 2 hours. After cooling, the precipitate was collected and recrystallized from EtOAc to yield 690 mg of **18**: mp 189~193°C; ir (nujol) 3280, 1640, 1622, 1600, 1565, 1555, 1510 cm^{-1}; uv $\lambda_{max}^{EtOH-H_2O}$ 270 nm (ε, 15800), $\lambda_{max}^{EtOH-0.1N\ NaOH}$ 283 nm (ε, 11400); nmr (DMSO-d_6) δ 1.13 (6H, d, J=7 Hz), 3.76 (3H, s), 4.05 (1H, m), 6.97 and 7.45 (4H, ABq, J=8 Hz), 8.30 (1H, d, J=8 Hz).

Anal. Calcd. for $C_{12}H_{16}N_2O_3$: C 61.00; H 6.83; N 11.86.
Found: C 60.93; H 6.74; N 11.98.

The filtrate and the mother liquor were combined, washed with H_2O, dried, and evaporated. The residue was chromatographed over 50 g of silica gel ($CHCl_3$) to give, besides an additional 260 mg of **18**, crystals (270 mg) of **19** (recrystallized from EtOAc-hexane): mp 164~165°C (dec); ir (nujol) 3500, 3180, 1655, 1620, 1605 cm^{-1}; uv $\lambda_{max}^{EtOH-H_2O}$ 267 nm (ε, 9800), $\lambda_{max}^{EtOH-0.1N\ NaOH}$ 275 nm (ε, 9500); nmr (DMSO-d_6) δ 1.13 (6H, d, J=7 Hz), 3.76 (3H, s), 3.99 (1H, m), 6.96 and 7.55 (4H, ABq, J=8 Hz), 8.00 (1H, d, J=8 Hz).

Anal. Calcd. for $C_{12}H_{16}N_2O_3$: C 61.00; H 6.83; N 11.86.
Found: C 60.75; H 6.81; N 11.74.

References

1) (a) NAGARAJAN, R.; D. BOECK, M. GORMAN, R. L. HAMILL, C. E. HIGGENS, M. HOEN, W. M. STARK & J. WHITENEY: β-Lactam antibiotics from *Streptomyces*. J. Am. Chem. Soc. 88: 2308~2310, 1971
(b) MILLER, T. W.; R. T. GOEGELMAN, R. G. WESTON, I. PUTTER & F. J. WOLF: Cephamycins, a new family of β-lactam antibiotics. II. Isolation and chemical characterization. Antimicr. Agents & Chemoth. 2: 132~135, 1972
(c) HIGGENS, C. E.; R. L. HAMILL, T. H. SANDS, M. M. HOEHN, N. E. DAVIS, R. NAGARAJAN & L. D. BOECK: The occurrence of deacetoxycephalosporin C in fungi and *Streptomycetes*. J. Antibiotics 27: 298~300, 1974
(d) KITANO, K.; K. KINTAKA, S. SUZUKI, K. KATAMOTO, K. NARA & Y. NAKANO: Production of cephalosporin antibiotics by strains belonging to the genera *Arachnomyces*, *Antixiopsis* and *Spiroidium*. Agr. Biol. Chem. 38: 1761~1762, 1974
(e) TRAXLER, P.; H. J. TREICHLER & J. NÜESCH: Synthesis of N-acetyldeacetoxycephalosporin C by a mutant of *Cephalosporium acremonium*. J. Antibiotics 28: 605~606, 1975
(f) FUKASE, H.; T. HASEGAWA, K. HATANO, H. IWASAKI & M. YONEDA: C-2801X, a new cephamycin-type antibiotic. II. Isolation and characterization. J. Antibiotics 29: 113~120, 1976
2) AOKI, H.; H. SAKAI, M. KOHSAKA, T. KONOMI, J. HOSODA, T. KUBOCHI, E. IGUCHI & H. IMANAKA: Nocardicin A, a new monocyclic β-lactam antibiotic. I. Discovery, isolation and characterization. J. Antibiotics 29: 492~500, 1976
3) KURITA, M. *et al.*: to be published.
4) HASHIMOTO, M.; T. KOMORI & T. KAMIYA: Nocardicin A and B, novel monocyclic β-lactam antibiotics from a *Nocardia* species. J. Am. Chem. Soc. 98: 3023~3025, 1976
5) BARROW, K. D. & T. T. SPOTSWOOD: Stereochemistry and P.M.R. spectra of β-lactams. Tetrahedron Lett. 1965: 3325~3335, 1965
6) HOSODA, J. *et al.*: in preparation.
7) NISHIDA, M. *et al.*: in preparation.
8) O'CALLAGHAN, C. H.; R. B. SYKES, D. M. RYAN, R. D. FOORD & P. W. MUGGLETON: Cefuroxime, a new cephalosporin antibiotic. J. Antibiotics 29: 29~37, 1976.
9) (a) WILEY, P. F.; R. R. HERR, F. A. MACKELLAR & A. D. ARGOUDELIS: Three chemically related metabolites of *Streptomyces*. II. Structural studies. J. Org. Chem. 30: 2330~2334, 1965
(b) SAKAKIBARA, H.; H. NAGANAWA, M. OHNO, K. MAEDA & H. UMEZAWA: The structure of althiomycin. J. Antibiotics 27: 897~899, 1974
(c) BYCROFT, B. W. & R. PINCHIN: Structure of althiomycin, a highly modified peptide antibiotic. J. C. S. Chem. Comm. 1975: 121~122, 1975
10) MAEHR, H.: Antibiotics and other naturally occurring hydroxamic acids and hydroxamates. Pure Appl.

Chem. 28: 603~636, 1971

11) SMITH, G. A.; K. A. SMITH & D. H. WILLIAMS: Structural studies on the antibiotic vancomycin: Evidence for the presence of modified phenylglycine and β-hydroxytyrosine units. J. Chem. Soc. Perkin Trans. I 1975: 2108~2115, 1975.
12) BIRNBAUM, S. M.; R. J. KOEGEL, S. -C. J. FU. & J. P. GREENSTEIN: Optical enantiomorphs of $\alpha\beta$-diaminopropionic acid. J. Biol. Chem. 198: 335~342, 1952
13) LONG, A. A. W.; J. H. C. NAYLOR, H. SMITH, T. TAYLOR & N. WARD: Derivatives of 6-aminopenicillinic acid. XI. α-Amino-*p*-hydroxybenzylpenicillin. J. Chem. Soc. C 1971: 1920~1922, 1971
14) BIRNBAUM, S. M. & J. P. GREENSTEIN: Optical enantiomorphs of homoserine and homocysteine. Arch. Biochem. Biophys. 42: 212~218, 1953
15) ROSS, S. D.; US Patent 2,692,290 (Chem. Abst. 45: 12539d, 1955)