FORMADICINS, NEW MONOCYCLIC β-LACTAM ANTIBIOTICS OF BACTERIAL ORIGIN

II. ISOLATION, CHARACTERIZATION AND STRUCTURES

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New monocyclic β-lactam antibiotics, formadicins A, B, C and D, were isolated from the culture filtrate of *Flexibacter alginoliquefaciens* sp. nov. YK-49 by various types of column chromatography and preparative reverse-phase HPLC. Their structures were determined by spectroscopic analyses and degradation studies. They have a nocardicin-type skeleton and a formylamino group at the 3- or 12-position. Formadicins A and B each have a D-glucuronide moiety and give formadicins C and D, respectively, upon hydrolysis using β-D-glucuronidase.

In a screening program for new inhibitory antibiotics of cell wall synthesis from bacterial strains, sulfazecins[1], and bulgecins[2,3], from *Pseudomonas acidophila* and *P. mesoacidophila*, and three types of new cephem antibiotics, cephabacins $F_{1\sim6}$, $H_{1\sim6}$[4,5] and $M_{1\sim6}$[6] from *Lysobacter lactamgenus* YK-90, *Xanthomonas lactamgena* YK-280, YK-278[7] and YK-431[8] have been discovered and reported.

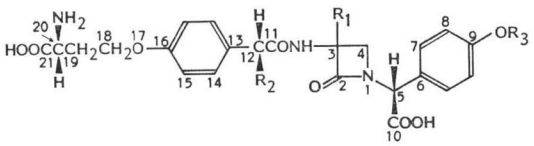
We isolated the new monocyclic β-lactam antibiotics formadicins A, B, C and D from the culture filtrate of a new bacterial species, *Flexibacter alginoliquefaciens* sp. nov. YK-49[9]. They have a nocardicin-type skeleton and a formylamino group at the 3- or 12-position. Formadicins A and B also have a β-D-glucuronide moiety binding to the phenoxy group. This is the first example of β-lactam antibiotics having a sugar moiety from natural sources. These components showed specific antibacterial activities *in vitro* and *in vivo* and remarkable stabilities to various types of β-lactamases[9]. This paper deals with the isolation, characterization and structural elucidation of formadicins A, B, C and D as shown in Table 1[10].

Isolation and Characterization

Fig. 1 shows the isolation procedure of formadicins A, B, C and D (**1**~**4**, respectively). These amphoteric (acidic as a whole molecule), water-soluble antibiotics were isolated by column chromatographies using anion-exchange resins, activated carbon and anion-exchange Sephadex and by preparative HPLC. After treatment of the filtrate with anion-exchange resins, two fractions were separated by QAE-Sephadex chromatography. The divided fractions were successively separated into two components each by rechromatography on QAE-Sephadex or preparative reverse-phase HPLC. The active fractions were detected by antimicrobial activity using a strain hypersensitive to β-lactam antibiotics, *P. aeruginosa* C 141, and by TLC and HPLC. Their mobilities are shown in Table 2.

Antibiotics **1** and **2** were obtained as freeze-dried disodium salts of white powders and **3** and **4** as monosodium salts of colorless crystals. They show positive color reactions to ninhydrin and

Table 1. Structures of formadicins A, B, C and D.



Formadicin	R_1	R_2	R_3
A	NHCHO	OH	D-Glucuronic acid
B	H	NHCHO	D-Glucuronic acid
C	NHCHO	OH	H
D	H	NHCHO	H

Ehrlich reagents and negative color reactions to Greig-Leaback and Dragendorff reagents. Barton reactions are positive for **3** and **4** and negative for **1** and **2**. Formadicins are easily soluble in water, soluble in dimethyl sulfoxide and sparingly soluble in methanol, acetone or ethyl acetate. The stabilities of **1**~**4** at 60°C in phosphate buffers (P.B.) of various pH are shown in Table 3. Components **2** and **4** were stable in acidic to basic pH regions. But **1** and **3** were relatively unstable in acidic or basic pH regions, compared with **2** and **4**.

The physico-chemical and spectral data of **1**~**4** are summarized in Table 4. The molecular formulae were determined from elemental analyses, molecular ion peaks in secondary ion mass spectrometry (SI-MS) and carbon numbers in ^{13}C NMR spectrometry to be $C_{30}H_{32}N_4O_{16}Na_2$ in **1**, $C_{30}H_{32}N_4O_{15}Na_2$ in **2**, $C_{24}H_{25}N_4O_{10}Na$ in **3** and $C_{24}H_{25}N_4O_9Na$ in **4**. The UV spectra showed two maxima, at 224~226 and 268~271 nm, and one shoulder at 277 nm. The CD spectra had strong negative Cotton effect ([θ] −113,000~−89,800) at 230~232 nm in **1** and **3** and ([θ] −194,000~−181,000) at 229 nm in **2** and **4**. These characteristic UV and CD spectral data apparently suggested that formadicin components have a chromophore similar to the monocyclic β-lactams, nocardicins[11,12]. The IR spectra of **1**~**4** indicated that the absorptions originated from β-lactam carbonyl functions at

Fig. 1. Isolation procedure of formadicins.

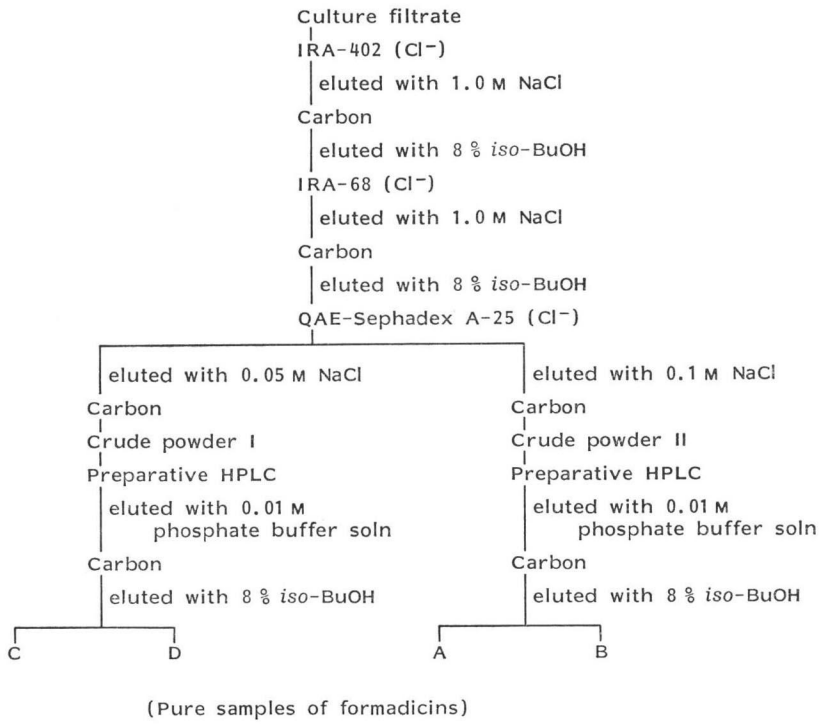


Table 2. Mobilities of formadicins on TLC and HPLC.

TLC	Rf value			
Solvent system	A	B	C	D
BuOH - AcOH - H_2O (1 : 1 : 1)	0.54	0.60	0.63	0.68
CH_3CN - 3% $(NH_4)_2SO_4$ (1 : 1)	0.56	0.66	0.74	0.79
PrOH - H_2O (2 : 1)	0.19	0.21	0.40	0.44

Adsorbent: Cellulose f (Tokyo Kasei).
Detection: Bioautography using *Pseudomonas aeruginosa* C 141 and UV absorbance at 254 nm.

HPLC	Retention time (minutes)			
Mobile phase	A	B	C	D
10% MeOH - 0.01 M P.B. (pH 3), 2 ml/minute	2.5	3.1	4.9	5.9

Column: ODS-Sil, YMC A-312 (Yamamura Chem. Lab.).
Detection: UV absorbance at 220 nm.
Equipment: Liquid chromatograph 638-50 (Hitachi).

Table 3. Stabilities of formadicins in aqueous solutions.

Compound	Time (hours)	Residual rate (%)			
		pH 3.1	pH 5.0	pH 7.0	pH 9.2
A	1	88	99	100	78
	4	61	98	98	39
B	1	103	98	99	87
	4	96	104	99	86
C	1	95	101	100	83
	4	73	101	101	44
D	1	96	91	94	99
	4	93	91	97	101

Concentration: 100 μg/ml in 0.05 M P.B.
Temperature: 60°C.
Detection: HPLC.

Table 4. Physico-chemical properties of formadicins.

Property	A		B		C		D	
Appearance	White powder		White powder		Colorless crystals		Colorless crystals	
MP					>190°C (dec)		>200°C (dec)	
$[\alpha]_D$ (*c*)	−79.1° (0.58)		−158° (0.51)		−74.1° (0.41)		−188° (0.49)	
SI-MS $(M+H)^+$	751		735		553		537	
Anal	Found	Calcd	Found	Calcd	Found	Calcd	Found	Calcd
C	45.99	45.81	46.72	46.76	51.22	51.34	52.78	52.85
H	4.89	4.61	4.78	4.71	4.95	4.67	4.80	4.80
N	6.97	7.12	7.49	7.27	9.93	9.98	10.18	10.27
Na	5.6	5.84	5.9	5.97	3.7	4.09	3.7	4.21
Molecular formula	$C_{30}H_{32}N_4O_{16}Na_2 \cdot 2H_2O$		$C_{30}H_{32}N_4O_{15}Na_2 \cdot 2H_2O$		$C_{24}H_{25}N_4O_{10}Na \cdot 1/2H_2O$		$C_{24}H_{25}N_4O_9Na \cdot 1/2H_2O$	
UV λ_{max} nm (ε)	224 (24,500), 269 (2,000), 277 (sh 1,600)		224 (23,300), 268 (2,100), 277 (sh 1,600)		226 (24,100), 271 (2,500), 277 (sh 2,100)		226 (23,700), 271 (2,300), 277 (sh 2,000)	
CD $[\theta]$ (nm)	−113,000 (230)		−194,000 (229)		−89,800 (232)		−181,000 (229)	
IR ν_{max} (cm^{-1})	1760		1740		1760		1740	

Table 5. ^{1}H NMR spectra of formadicins.

Position	A	B	C	D
3-H		4.8*		4.8*
4-H	3.98 (d, 6.3)	3.74 (t, 5.7)	3.99 (d, 6.5)	3.73 (t, 5.7)
	3.58 (d, 6.3)	3.08 (dd, 5.7, 2.4)	3.52 (d, 6.5)	3.05 (dd, 5.7, 2.5)
5-H	5.32 (s)	5.34 (s)	5.29 (s)	5.27 (s)
7-H	7.27 (d, 8.8)	7.31 (d, 8.5)	7.16 (d, 8.6)	7.22 (d, 8.7)
8-H	7.13 (d, 8.8)	7.14 (d, 8.5)	6.86 (d, 8.6)	6.91 (d, 8.7)
14-H	7.31 (d, 8.8)	7.32 (d, 8.8)	7.29 (d, 8.6)	7.33 (d, 8.7)
15-H	7.01 (d, 8.8)	7.01 (d, 8.8)	6.99 (d, 8.6)	7.02 (d, 8.7)
12-H	5.11 (s)	5.38 (br s)	5.11 (s)	5.39 (br s)
18-H	4.24 (m)	4.22 (m)	4.24 (m)	4.23 (m)
19-H	2.43 (m)	2.43 (m)	2.45 (m)	2.44 (m)
	2.29 (m)	2.31 (m)	2.34 (m)	2.32 (m)
20-H	3.91 (dd, 8.0, 4.5)	3.97 (dd, 7.5, 4.5)	3.98 (dd, 7.6, 4.4)	3.97 (dd, 7.6, 4.6)
CHO	8.14 (s)	8.11 (d, 1.0)	8.15 (s)	8.12 (d, 1.0)
1′-H	5.12 (d, 7.0)	5.14 (d, 6.5)		
2′-4′-H	3.62 (m×3)	3.62 (m×3)		
5′-H	3.90 (d, 9.3)	3.96 (d, 9.3)		

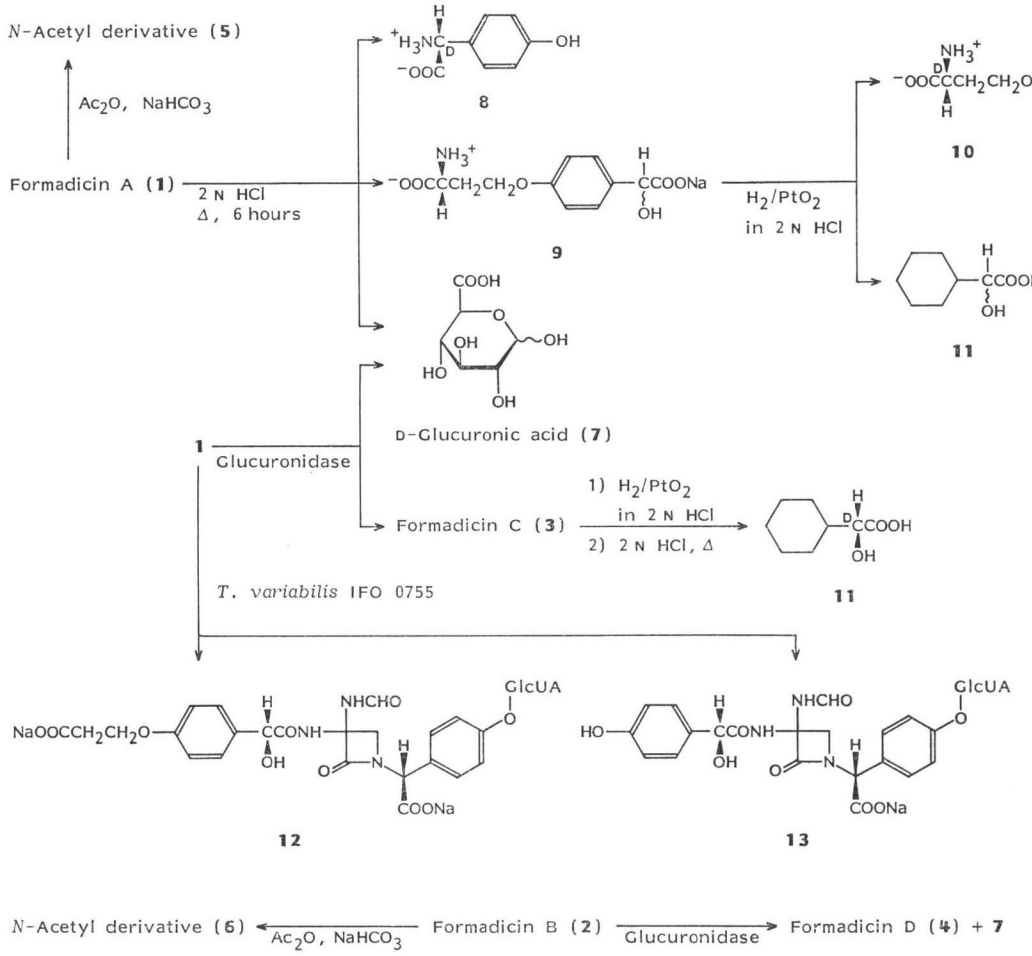
* Signals are overlapped with DHO signals.

Table 6. ^{13}C NMR spectra of formadicins.

Assignment	A	B	C	D
10-C	178.09 s	178.45 s	178.01 s	178.62 s
Glu-CO	177.60 s	178.30 s		
11-C	177.59 s	174.94 s	177.56 s	174.88 s
21-C	176.88 s	177.00 s	176.84 s	176.82 s
Lac-CO	166.96 s	171.17 s	166.94 s	170.92 s
HCONH	166.75 d	166.60 d	166.78 d	166.49 d
16-C	161.01 s	161.13 s	161.03 s	161.10 s
9-C	159.47 s	159.51 s	158.38 s	158.37 s
13-C	133.95 s	132.15 s	133.92 s	131.08 s
6-C	132.43 s	131.13 s	129.46 s	129.62 s
7-C	132.54 d*	132.97 d*	132.62 d*	132.98 d*
14-C	131.33 d*	131.75 d*	131.29 d*	131.69 d*
8-C	119.89 d*	119.86 d*	118.51 d*	118.52 d*
15-C	117.85 d*	118.15 d*	117.81 d*	118.10 d*
O-CH-O	103.11 d	103.05 d		
Glu, -CHOH	78.99 d	79.08 d		
〃	78.17 d	78.20 d		
〃	75.59 d	75.65 d		
〃	74.53 d	74.61 d		
12-C	75.95 d	58.74 d	75.90 d	58.66 d
3-C	74.53 s	57.21 d	74.47 s	57.10 d
18-C	68.07 t	68.10 t	68.03 t	68.06 t
5-C	63.57 d	63.56 d	63.50 d	63.53 d
20-C	56.22 d	56.29 d	56.27 d	56.22 d
4-C	56.05 t	49.36 t	55.98 t	49.17 t
19-C	32.64 t	32.67 t	32.64 t	32.63 t

* Duplicate.

Fig. 2.



1760 cm^{-1} in **1** and **3** and at 1740 in **2** and **4**. The latter was almost the same absorption as that of nocardicin A. But the –C–O– absorptions at the region of 1000 to 1100 cm^{-1} in **1** and **2** were clearly absent from that of nocardicin A. Formadicins were also found to have a skeleton similar to nocardicins from their ^{1}H and ^{13}C NMR spectra as shown in Tables 5 and 6, although the signals of a formylamino group were observed at δ 8.11~8.15 ppm (each 1H, s or d) in the ^{1}H NMR spectra and at 166.49~166.78 ppm (each d) in the ^{13}C NMR spectra. Furthermore, these NMR spectra indicated the presence of a sugar moiety in **1** and **2**. These findings clearly showed that formadicins are new monocyclic β-lactams.

Structural Determination

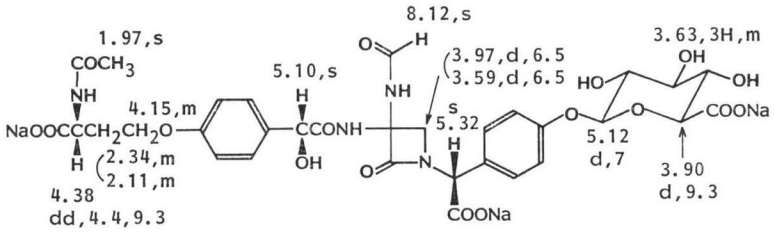
Fig. 2 shows the reaction and degradation pathways of formadicins. Compounds **1** and **2** gave *N*-acetyl derivatives as trisodium salts, **5**; $[\alpha]_D$ −79.6° (*c* 0.52) and **6**; $[\alpha]_D$ −157° (*c* 0.44), respectively, by acetic anhydride in 2% $NaHCO_3$. The differences of the ^{13}C NMR signals between **1** and **3** or **2** and **4** at δ 103 ppm (anomeric), 74~79 ppm (4 carbons, –CHO–) and 178 ppm (carboxyl) (Table 6) suggested the presence of a uronic sugar moiety in **1** and **2** and therefore, the trial for the hydrolysis

Table 7. ^{13}C NMR spectral data of the decomposition products.

Carbon No.	8	9	7*	
5	60.82 d			
6	128.89 s			
7	132.51 d			
8	118.91 d			
9	159.29 d			
10	176.25 s			
11		182.42 s		
12		77.26 d		
13		136.43 s		
14		131.43 d		
15		117.69 d		
16		160.55 s		
18		68.09 t		
19		32.69 t		
20		56.39 d		
21		176.95 s		
			α-	β-
1′			93.09 d	96.88 d
2′			71.87 d	74.50 d
3′			73.18 d	76.10 d
4′			72.28 d	72.05 d
5′			71.27 d	75.30 d
6′			173.78 s	172.83 s

* 25.2 MHz. Internal standard: dioxane (67.40 ppm).

Fig. 3.



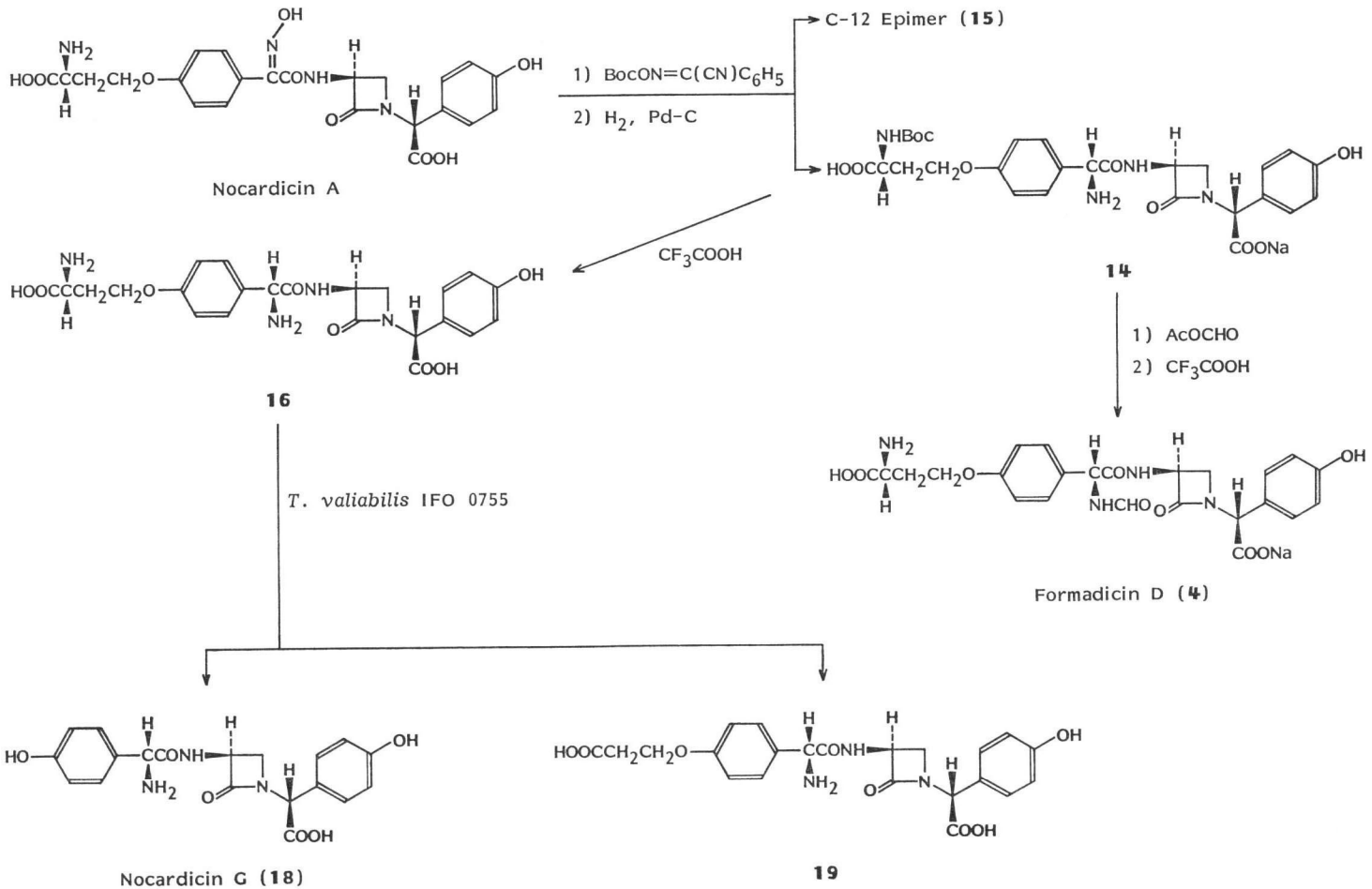
Aromatic 7.30, 7.28 7.13, 7.00, 2H×4, d, 8.8

by β-D-glucuronidase was carried out. Compound **1** or **2** afforded D-glucuronic acid (**7**), mp 161~163°C, $[\alpha]_D$ +36.5° (*c* 1.0), and **3** or **7** and **4** in good yields under usual conditions.

Upon acidic hydrolysis by refluxing in 2 N HCl for 6 hours, **1** afforded **7**, D-*p*-hydroxyphenylglycine (**8**), mp 220°C (dec), $[\alpha]_D$ −84.0° (*c* 0.50), and a mandelic acid derivative, **9**, mp 210~220°C (dec), $[\alpha]_D$ +9.1° (*c* 0.58). Compound **9** was successively hydrogenolyzed over platinum oxide, giving D-homoserine (**10**), mp 199°C (dec), $[\alpha]_D$ +8.6° (*c* 0.50), and racemic hexahydromandelic acid (**11**), $[\alpha]_D$ 0° (*c* 0.5). Since racemization of the C-12 carbon was assumed to occur in the step of acid hydrolysis, **3** was hydrolyzed in 2 N HCl after hydrogenolysis over platinum oxide, yielding D-hexahydromandelic acid (**11**), mp 126~128°C, $[\alpha]_D$ −23.0° (*c* 0.40, AcOH). The ^{13}C NMR spectral data of these degradation products are shown in Table 7. The degradation data unambiguously identified the absolute configurations to be 5*R*, 12*R*, 20*R* and 5′*S*.

Microbial transformation of **1** using the yeast, *Trigonopsis variabilis* IFO 0755, afforded a deamino-decarboxy compound, **12**, $[\alpha]_D$ −85.4° (*c* 0.41), and a hydroxyphenyl compound, **13**, $[\alpha]_D$ −63.5° (*c*

Fig. 4.


Nocardicin A
1) BocON=C(CN)C6H5
2) H2, Pd-C
C-12 Epimer (15)
14
CF3COOH
16
1) AcOCHO
2) CF3COOH
Formadicin D (4)
T. valiabilis IFO 0755
Nocardicin G (18)
19

0.46). The former reaction has been known in the case of cephalosporins[13], but the latter cleavage reaction of an ether bond is, to our knowledge, a rare case in microbial transformation studies.

All of these findings led to the determination of the structures of **1** and **3**. The ^{1}H NMR spectrum of **5** partially confirmed with proton spin-decoupling studies was assigned as shown in Fig. 3. The binding position of the formylamino group in **1** and **3** was elucidated on the basis of the splitting patterns of the 4-methylene signals at δ 3.97 ppm (d, J=6.5 Hz) and 3.59 (d, J=6.5 Hz) in **5**. The β-configuration of the uronic acid was elucidated by the coupling constant at 5.12 ppm (d, J=7 Hz). The structures of **1** and **3** were thus determined as depicted in Table 1. The absolute configuration at the 3-position may be α- in biosynthetic consideration of these antibiotics.

The structures of **2** and **4** were clarified by derivatization from nocardicin A* as shown in Fig. 4. On hydrogenation over palladium-charcoal, a *N-tert*-butoxycarbonyl (Boc) derivative of nocardicin A afforded two epimers at the 12-position, **14**, $[\alpha]_D$ $-108°$ (*c* 0.50), and **15**, $[\alpha]_D$ $-33.8°$ (*c* 0.50). The protecting group was separately eliminated upon treatment with trifluoroacetic acid, giving the corresponding 12-amino-epimers, **16**, mp 200~220°C (dec), $[\alpha]_D$ $-162°$ (*c* 0.51), and **17**, mp 180~210°C (dec), $[\alpha]_D$ $-53.7°$ (*c* 0.49). From the value of the specific rotation in nocardicin C ($-95°$)[12], these compounds are thought to be the pure epimers of nocardicin C. Compound **16** was transformed using *T. variabilis* into a dehomoseryl compound, **18**, $[\alpha]_D$ $-200°$ (*c* 0.40, 1% $NaHCO_3$) and a deamino-decarboxy compound, **19**, mp 180~185°C (dec), $[\alpha]_D$ $-184°$ (*c* 0.31). The specific rotation of **18** agreed with that of nocardicin G ($-205°$)[12]. By the same transformation, **17** gave a 12-epimer of nocardicin G, **20**, mp 200~225°C (dec), $[\alpha]_D$ $-96.3°$ (*c* 0.32, 1% $NaHCO_3$) and **21**, mp 205~217°C (dec), $[\alpha]_D$ $-77.1°$ (*c* 0.32, 1% $NaHCO_3$). Nocardicin G has been strereospecifically synthesized[12], and therefore, the relations among **14**~**21** including the absolute configurations at the 12-position could be clarified. Compound **14** was treated with acetic-formic anhydride and then deprotected, giving a formylamino derivative. This compound and naturally occurring **4** showed the same retention time on HPLC and IR, SI-MS, CD and NMR spectra. The structures of **2** and **4** were thus determined to be as shown in Table 1.

Formadicins were active against Gram-negative bacteria *in vitro*. Of them, **1** showed comparably stronger protective effects than nocardicin A on mice experimentally infected with *Escherichia coli* O-111 and *Proteus vulgaris* GN 4712[9]. The preliminary acute toxicities of these antibiotics were more than 1,000 mg/kg in mice by subcutaneous administration.

Nocardicins are the first examples of monocyclic β-lactam antibiotics and sulfazecins are the second ones. Both are mainly active against Gram-negative bacteria, but differ in the ease with which their biological activities can be enhanced by chemical modification[14,15]. This difference may arise from the substitution effects at the 3- or 4-position. Another new antibiotic belonging to the sulfazecin-type, SQ 28,332[16], is mainly active against Gram-positive bacteria. It has no substitution function at the 3-position. Formadicins A and C, which have the 3-formylamino group, are highly resistant to the β-lactamases, as clarified by biological characterization of cephabacin series. The introduction of the formylamino group into the α-position of β-lactam carbonyl led to appear remarkable improvement of the activities for Gram-negative bacteria and the stabilities for β-lactamases. This modification will be much more applied to synthesize clinically valuable β-lactam antibiotics, although Beecham researchers carry on the development of the penicillin derivative[17] before discovery

* Nocardicin A being used herein was prepared in our laboratories.

of this novel finding from natural sources.

Experimental

The specific rotations, UV and CD spectra were measured at approx 22~25°C in H_2O unless otherwise stated. The CD spectra were measured by Jasco J-20A with DP-501N. The IR spectra were measured in KBr pellet. The values in the ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra using Jeol GX-400 were recorded in ppm downfield from DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate). All spectra were measured in D_2O unless otherwise stated. The SI-mass spectra were measured on Hitachi M-80 A mass spectrometer with xenon ion beam source. The samples were supplied by glycerol matrix.

Isolation of Formadicins (1~4)

The culture broth of *F. alginoliquefaciens* YK-49 (1,150 liters, pH 5.4) was filtered with Hyflo-Super Cel. The filtrate (1,200 liters) was loaded onto Amberlite IRA-402 (Cl^- type, 40 liters) and active substances were eluted with 1 M NaCl (400 liters). The eluate was applied to activated carbon chromatography (20 liters) eluting with 8% *iso*-BuOH (160 liters). The eluate was chromatographed on Amberlite IRA-68 (Cl^- type, 10 liters) and active fractions were eluted with 1 M NaCl (80 liters). The eluate was desalted with activated carbon (10 liters) and concentrated. The concentrate (1.5 liters) was applied to QAE-Sephadex A-25 (Cl^- type, 3 liters) and eluted with 0.05 M NaCl (21 liters) and 0.1 M NaCl (20 liters). Two eluates were separately desalted with activated carbon and followed by concentration and lyophilization to give the crude powder containing **3** and **4** (13 g) from the former eluate and the crude powder containing **1** and **2** (30.3 g) from the latter eluate.

The crude powder containing **1** and **2** (56.9 g) in H_2O (500 ml) was loaded on QAE-Sephadex (3 liters) and eluted with 0.08 M NaCl. Each fraction detected by HPLC was individually desalted with activated carbon to give freeze-dried **1** (22 g) and the mixture of **1** and **2** (15.3 g). The mixture was again applied to QAE-Sephadex (1.2 liters) and eluted with 0.08 M NaCl to divide two fractions. Each fraction was separately desalted with activated carbon and followed by concentration and lyophilization to afford disodium salts of **1** (3.7 g) and **2** (2.0 g) as white powders.

The crude powder containing **3** and **4** (10 g) in water (500 ml) was applied to Amberlite IRA-68 column and eluted with 0.1M NaCl. The desalted eluate was concentrated to give the crude powder containing **3** and **4** (2.7 g). This powder was loaded onto QAE-Sephadex and eluted with 0.05 M NaCl. The desalted eluate was concentrated and freeze-dried to give the powder (482 mg). The powder was purified with preparative HPLC using YMC-Pack S-30 ODS (Yamamura Chem. Lab.) with the mobile phase of 3% MeOH - 0.05 M P.B. (pH 3). The two pure fractions separately desalted were concentrated and crystallized from H_2O to give free types of **3** (14 mg) and **4** (10 mg) as colorless crystals.

N-Acetylation of 1 and 2 (5, 6)

Ac_2O was added (70 μl) to a soln of **1** (107 mg) in 2% $NaHCO_3$ (10 ml) and the mixture was stirred for 2 hours at 0°C. After adjustment to pH 6.9, the reaction mixture was passed through a column of Diaion HP-20 (20 ml). The effluent was applied to activated carbon (10 ml) and eluted with 8% *iso*-BuOH. The eluate was concentrated and lyophilized to give **5** (92 mg) as a white powder: SI-MS *m/z* 815 (M+H); UV λ_{max} 224 nm (ε 26,800), 269 (2,200), 275 (sh, 1,800); IR 1760, 1620 cm^{-1}.

Anal Calcd for $C_{32}H_{33}N_4O_{17}Na_3 \cdot 2H_2O$: C 45.18, H 4.38, N 6.59, O 35.74, Na 8.11.
Found: C 45.10, H 4.28, N 6.91.

By the same procedure, **2** (100 mg) was converted to **6** (103 mg): SI-MS *m/z* 799 (M+H); UV λ_{max} 226 nm (ε 28,300), 269 (1,900), 275 (sh, 1,700); IR 1745, 1610 cm^{-1}.

Anal Calcd for $C_{32}H_{33}N_4O_{16}Na_3 \cdot 4H_2O$: C 44.14, H 4.75, N 6.44, O 36.75, Na 7.92.
Found: C 43.93, H 4.86, N 6.50.

Hydrolysis of 1 and 2 by β-D-Glucuronidase (3, 4)

To a soln of **1** (3.0 g) in 0.02 M P.B. (pH 4.5, 3.0 liters) was added a commercial sulfatase (Type H-1, Sigma, containing β-glucuronidase activity, 15 g) and the mixture was shaked for 24 hours at

37°C. After treatment of the reaction mixture with acetone (12 liters), the concentrate of the filtrate was applied to activated carbon chromatography (150 ml) and eluted as two fractions of the effluent and the eluate of 8% *iso*-BuOH. The concentrate of the eluate was loaded on QAE-Sephadex A-25 (Cl^- type, 150 ml) and eluted with 0.03 M NaCl. The eluate (pH 7.2) was desalted with activated carbon and crystallized from EtOH - H_2O to give **3** (1.03 g). The concentrate of the effluent (pH 2.2) was again applied to activated carbon (150 ml) and eluted with H_2O and *iso*-BuOH. The concentrated eluate (pH 11) was loaded onto Amberlite IRA-402 (AcO^- type, 50 ml) and eluted with 0.5 M AcOH. The eluate was concentrated and crystallized from EtOH - H_2O to give **7** (315 mg).

Anal Calcd for $C_6H_{10}O_7$: C 37.12, H 5.19, O 57.69.
Found: C 37.01, H 5.12.

By the similar method, **2** (200 mg) was converted to **4** (59 mg) and **7** (22 mg).

Acidic Hydrolysis of **1** (**7**, **8**, **9**)

A soln of **1** (1.0 g) in 2 N HCl (100 ml) was refluxed for 6 hours. The concentrated residue was suspended in H_2O (100 ml), filtered, neutralized and passed through a column of Amberlite XAD-2. The concentrate of the effluent was loaded on activated carbon and eluted with H_2O and 8% *iso*-BuOH. The *iso*-BuOH eluate was concentrated to give the powder of **9** (54 mg). The H_2O eluate was divided into two fractions. The fraction eluting fast was concentrated and adjusted to pH 11. The soln was applied to Amberlite IRA-402 (AcO^- type, 20 ml) and eluted with 0.5 M AcOH. The eluate was concentrated and crystallized from EtOH - H_2O to yield **7** (17 mg). The fraction eluting late was passed through a column of QAE-Sephadex A-25 (Cl^- type, 40 ml). The column was washed with H_2O and eluted with 0.05 M NaCl. The passed fraction was concentrated and crystallized from EtOH - H_2O to yield **8** (136 mg).

Anal Calcd for $C_8H_9NO_3$: C 57.48, H 5.43, N 8.38, O 28.71.
Found: C 57.32, H 5.39, N 8.45.

The eluate was desalted with activated carbon to afford additional **9** (151 mg) as colorless crystals: SI-MS *m/z* 292 (M+H); UV λ_{max} 227 nm (ε 96,400), 271 (1,050), 277 (sh, 900); IR 1610 cm^{-1}.

Anal Calcd for $C_{12}H_{14}NO_6Na \cdot 1/2H_2O$: C 48.01, H 5.04, N 4.67, O 34.64, Na 7.66.
Found: C 48.19, H 4.90, N 4.71.

Hydrogenation of **9** (**10**, **11**)

A soln of **9** (300 mg) in 2 N HCl (15 ml) was hydrogenated over PtO_2 (200 mg) with hydrogen for 7 hours at room temp. The filtrate was extracted with ether and the extract was washed, dried and concentrated. The residue was crystallized from hexane - ether to yield racemic **11** (76 mg) as colorless crystals. The aqueous layer was concentrated and loaded on Dowex 50WX2 (H^+ type, 20 ml). The elution of 0.2 N NH_4OH was concentrated and crystallized from EtOH - H_2O to give **10** (44 mg) as colorless crystals.

Anal Calcd for $C_4H_9NO_3$: C 40.33, H 7.62, N 11.76, O 40.30.
Found: C 40.34, H 7.69, N 11.58.

Hydrogenation and Acidic Hydrolysis of **3** (**11**)

A soln of **3** (250 mg) in 2 N HCl (12.5 ml) was hydrogenated over PtO_2 (125 mg) with hydrogen for 5 hours at room temp. After filtration of the soln, the filtrate was refluxed for 5 hours. The soln was extracted with ether and followed by reextraction with 2% $NaHCO_3$. The aqueous layer was reextracted at pH 2 with ether. The concentrate of the extract was crystallized from hexane - ether to yield **11** (19 mg) as colorless crystals.

Anal Calcd for $C_8H_{14}O_3$: C 60.74, H 8.92, O 30.34.
Found: C 60.64, H 8.97.

Microbial Transformation of **1** (**12**, **13**)

T. variabilis IFO 0755 was cultured according to the method of SERIZAWA *et al.*[13]. Into a soln of **1** (200 mg) dissolved in 0.1 M pyrophosphate buffer (40 ml, pH 8) containing 0.01 M NaN_3, wet cells (15 g) were suspended and shaked for 4.5 hours at 28°C. The reaction mixture was centrifuged and desalted with activated carbon (20 ml). The eluate was applied to QAE-Sephadex A-25 (Cl^- type, 20 ml) eluting with 0.2 M and 0.5 M NaCl. These eluates were individually desalted with activated

carbon to give **13** (46 mg) and **12** (30 mg) as white powders.

12: SI-MS *m/z* 744 (M+H); UV λ_{max} 224 nm (ε 26,200), 269 (2,000), 276 (sh, 1,500); IR 1765, 1620 cm^{-1}.

Anal Calcd for $C_{29}H_{28}N_3O_{16}Na_3 \cdot 6H_2O$: C 40.90, H 4.73, N 4.93, O 41.33, Na 8.10.
Found: C 40.94, H 4.73, N 4.97.

13: SI-MS *m/z* 650 (M+H); UV λ_{max} 223 nm (ε 22,300), 269 (1,900), 276 (sh, 1,700); IR 1760, 1620 cm^{-1}.

Anal Calcd for $C_{26}H_{25}N_3O_{14}Na_2 \cdot 5/2H_2O$: C 44.96, H 4.35, N 6.05, O 38.01, Na 6.62.
Found: C 45.04, H 4.55, N 5.92.

The reaction mixture of **16** (200 mg) treated with the same method was applied to activated carbon (10 ml) and eluted with H_2O and 8% *iso*-BuOH. The *iso*-BuOH eluate containing **18** was concentrated and applied to QAE-Sephadex A-25 (Cl^- type, 10 ml). The residue of the effluent was loaded on preparative HPLC using YMC-Pack SH-343 and eluted with 0.01 M P.B. (pH 5). The pure fractions were desalted with activated carbon to give **18** (18 mg, nocardicin G) as colorless crystals: SI-MS *m/z* 386 (M+H); UV λ_{max} 227 nm (ε 19,800), 270 (2,300), 276 (sh, 2,000); IR 1740, 1695, 1610 cm^{-1}.

Anal Calcd for $C_{19}H_{19}N_3O_6 \cdot 3/2H_2O$: C 55.34, H 5.38, N 10.19, O 29.10.
Found: C 55.50, H 5.21, N 9.57.

The H_2O eluate containing **19** was applied to QAE-Sephadex (10 ml) and eluted with 0.05~0.1 M NaCl. The eluate (pH 3.4) was desalted with activated carbon to give **19** (21 mg) as colorless crystals: SI-MS *m/z* 458 (M+H); UV λ_{max} 227 nm (ε 20,800), 271 (2,100), 277 (sh, 1,800); IR 1740, 1690, 1615 cm^{-1}.

Anal Calcd for $C_{22}H_{23}N_3O_8 \cdot 3/2H_2O$: C 54.54, H 5.41, N 8.67, O 31.37.
Found: C 54.62, H 5.18, N 8.95.

Compound **17** (200 mg) was also transformed into **20** (9 mg) and **21** (20 mg) by the similar procedure.

20: SI-MS *m/z* 386 (M+H); UV λ_{max} 227 nm (ε 19,400), 270 (2,300), 276 (sh, 2,000); IR 1740, 1690, 1620 cm^{-1}.

21: SI-MS *m/z* 458 (M+H); UV λ_{max} 227 (ε 20,500), 271 (2,100), 277 (sh, 1,800); IR 1730, 1610 cm^{-1}.

Anal Calcd for $C_{22}H_{23}N_3O_8 \cdot 1/2H_2O$: C 56.65, H 5.19, N 9.01, O 29.16.
Found: C 56.83, H 4.98, N 9.12.

Reduction of Nocardicin A (**14~17**)

To a suspension of nocardicin A (free form, 2.0 g) in H_2O (20 ml) and dioxane (20 ml) were added 3 N NaOH (1.21 ml), Boc-ON (Aldrich, 990 mg) and triethylamine (766 μl). The reaction mixture was stirred for 4 hours at room temp and extracted at pH 2 with EtOAc. The extract was concentrated and triturated with ether - hexane to afford Boc-nocardicin A as white powder (2.21 g).

A soln of Boc-nocardicin A (2.0 g) in MeOH (40 ml) was hydrogenated over 10% Pd-C (500 mg) with hydrogen for 6 hours at room temp. The filtered reaction mixture was concentrated and the residue was suspended in H_2O (40 ml) at pH 8.0. The resulting soln was chromatographed 3 times on Diaion HP-20 (100~200 mesh) eluting with H_2O and 10% MeOH. The two pure fractions detected by HPLC using YMC-Pack A 312 with the mobile phase of 8% CH_3CN - 0.01 M P.B. (pH 5.0), were individually combined and lyophilized to give **14** (308 mg, Rt 7.7 minutes) and **15** (311 mg, Rt 7.0 minutes) as white powders. The mixture of **14** and **15** was also obtained (549 mg).

14: UV λ_{max} 228 nm (ε 23,900), 271 (2,400), 279 (sh, 2,000); IR 1740, 1690, 1610 cm^{-1}.

Anal Calcd for $C_{28}H_{33}N_4O_{10}Na \cdot 7/2H_2O$: C 50.07, H 6.00, N 8.34, O 32.16, Na 3.42.
Found: C 50.02, H 5.49, N 8.67.

15: UV λ_{max} 228 nm (ε 25,900), 270 (2,700), 277 (sh, 2,300); IR 1740, 1680, 1610 cm^{-1}.

Anal Calcd for $C_{28}H_{33}N_4O_{10}Na \cdot 7/2H_2O$: C 50.07, H 6.00, N 8.34, O 32.16, Na 3.42.
Found: C 50.22, H 5.85, N 8.65.

A soln of **14** (100 mg) in trifluoroacetic acid (1.0 ml) was stirred for 20 minutes at −10°C. To the reaction mixture was added EtOAc (5.0 ml) and concentrated. The residue in H_2O (5.0 ml) was neutralized to pH 6.4. The solution was desalted with activated carbon and crystallized from acetone -

H_2O to yield **16** (45 mg) as colorless crystals: SI-MS m/z 487 (M+H); UV λ_{max} 228 nm (ε 21,900), 270 (2,100), 276 (sh, 1,900); IR 1745, 1615 cm^{-1}.

Anal Calcd for $C_{23}H_{26}N_4O_8 \cdot 2H_2O$: C 52.87, H 5.79, N 10.72, O 30.62.
Found: C 52.83, H 5.44, N 10.63.

By the similar procedure, **15** (100 mg) gave **17** (45 mg) as colorless crystals: SI-MS m/z 487 (M+H); UV λ_{max} 227 nm (ε 23,000), 270 (2,300), 276 (sh, 2,000); IR 1735, 1700, 1610 cm^{-1}.

Anal Calcd for $C_{23}H_{26}N_4O_8 \cdot 1/2H_2O$: C 55.75, H 5.49, N 11.31, O 27.45.
Found: C 55.81, H 5.76, N 10.95.

Formylation of **14** (**4**)

To a soln of **14** (100 mg) in MeOH (2.0 ml) was added acetic-formic anhydride (200 μl) and the mixture was stirred for 30 minutes at −78°C. After concentration, the residue in trifluoroacetic acid (2.0 ml) was stirred for 20 minutes at −10°C. The mixture was concentrated and loaded on preparative HPLC using YMC-Pack SH-343 with the mobile phase of 8% MeOH - 0.02 M P.B. (pH 3.0). The pure fractions were desalted with activated carbon to give **4** (32 mg) as colorless crystals. This compound was identical with the specimen of natural **4** in the specific rotation, IR, ^1H NMR and SI-mass spectra.

By the same procedure, compound **15** (100 mg) was converted to 12-*epi*-formadicin D (**22**, 38 mg) as colorless crystals: mp 195~210°C (dec), $[\alpha]_D$ −53.9° (c 0.49), SI-MS m/z 537 (M+H); UV λ_{max} 226 nm (ε 24,400), 271 (2,500), 277 (sh, 2,100); IR 1740, 1610 cm^{-1}.

Anal Calcd for $C_{24}H_{25}N_4O_9Na \cdot H_2O$: C 51.99, H 4.91, N 10.10, O 28.85, Na 4.15.
Found: C 51.71, H 4.78, N 10.13.

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