STUDIES ON A NEW AMINOGLYCOSIDE ANTIBIOTIC, DACTIMICIN

II. ISOLATION, STRUCTURE AND CHEMICAL DEGRADATION

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A new aminoglycoside antibiotic, dactimicin produced by a *Dactylosporangium matsuzakiense* SF-2052 has been isolated by column chromatography on a cation-exchange resin and CM-Sephadex. The structure of dactimicin was determined to be 4-amino-1,4-dideoxy-3-*O*-(2,6-diamino-2,3,4,6,7-pentadeoxy-β-L-*lyxo*-heptopyranosyl)-1-[(*N*-formimidoylglycyl)-methylamino]-6-*O*-methyl-L-*chiro*-inositol. Alkaline hydrolysis of dactimicin afforded 1-*N*-(*N*-formylglycyl)fortimicin B, fortimicin B, fortimicin A and an acyl migration product, 2′-*N*-(*N*-formylglycyl)fortimicin B.

Dactimicin is the first aminoglycoside antibiotic of *Dactylosporangium* origin, which was previously designated as substance SF-2052[1]. The producing strain and the fermentation are described in the preceding report[2]. This paper is concerned with isolation, structure determination and chemical degradation under alkaline conditions.

Isolation of Dactimicin

Dactylosporangium matsuzakiense SF-2052 was fermented by the procedure described in the previous paper[2]. The broth of 96-hour culture was filtered successively at pH 2 and at pH 5, and the antibiotic in the filtrate (130 liters, 110 mcg/ml) was adsorbed on a column of Amberlite IRC-50 (a mixture of Na^+ form (4 liters) and H^+ form (1 liter)). The column was washed with water (25 liters) and eluted with 0.4 N HCl (20 liters). The eluate that was active against *B. subtilis* was adjusted to

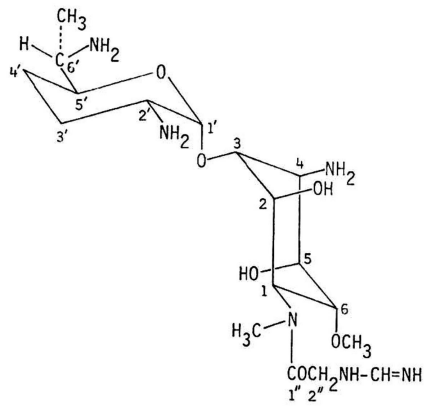


Table 1. Physico-chemical properties of dactimicin sulfate (**1**).

Appearance	White powder
Melting point	176~178°C (dec.)
$[\alpha]_D^{25}$	+81° (*c* 1, H_2O)
UV spectrum	End absorption
Molecular formula	$C_{18}H_{36}N_6O_6 \cdot 2H_2SO_4 \cdot H_2O$
Elementary analysis	Found: C 33.42, H 6.64, N 12.98, S 9.78
	Calcd.: C 33.43, H 6.50, N 13.00, S 9.90
IR (KBr)	3400, 1710, 1643, 1520, 1110 and 1060 cm^{-1}

pH 6.0 and passed through a column of activated carbon (1.2 liters). After washing with water (3 liters), the column was eluted with 50% aqueous acetone. The eluate was concentrated, and applied to a mixed bed of Amberlite IRC-50 (Na^+ form, 0.8 liters; H^+ form, 0.2 liters). Elution with 0.4 N HCl, followed by neutralization and desalting over activated carbon gave the crude solution of dactimicin. This was subjected to a column chromatography using CM-Sephadex C-25 (Na^+, 600 ml), previously equilibrated with 0.1 M NaCl. After washing with 0.3 M NaCl (3 liters), the antibiotic was eluted with 0.5 M NaCl. The active fractions were combined and desalted by the use of activated carbon. The concentrated dactimicin hydrochloride thus obtained was converted into the sulfate (4.2 g) by passing through a column of Amberlite IRA-400 (SO_4^{--}, 400 ml), and lyophilization. Dactimicin differed from other aminoglycosides in that the free base form was unstable. A stable salt was therefore characterized. The physico-chemical properties of dactimicin sulfate are summarized in Table 1. It is soluble in water, less soluble in methanol, and almost insoluble in other solvents. It showed positive ninhydrin, GREIG-LEABACK and LEMIEUX reactions, but negative SAKAGUCHI, FEHLING and biuret reactions. The molecular formula of $C_{18}H_{36}N_6O_6 \cdot 2H_2SO_4 \cdot H_2O$ was established from the elemental analysis and the number of carbon atoms in CMR.

Structure of Dactimicin

The structure of dactimicin was first examined by analyzing the IR spectrum. The IR of dactimicin sulfate (**1**) revealed a band at 1710 cm^{-1} which has not previously been seen in any known aminoglycoside antibiotics. The intensity and location of the 1710 cm^{-1} band suggested the existence

Fig. 1. Amino acid analysis of acid hydrolyzate of dactimicin (**1**).

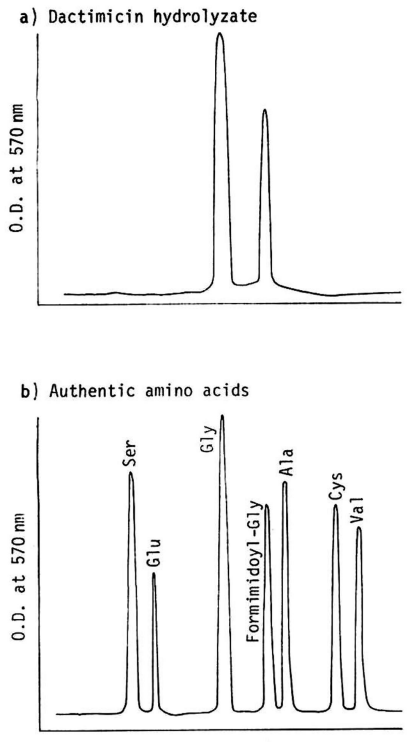


Table 2. 25 MHz CMR parameters of **1** and **3** in D_2O solution.

	Dactimicin sulfate (**1**)	Deformimidoylglycyldactimicin (**3**) sulfate	Fortimicin A sulfate	Fortimicin B sulfate
C–1′	95.4	96.4	95.4	96.0
C–2′	51.6	51.6	51.7	51.9
C–3′	21.6	21.6	21.6	21.5
C–4′	26.3	26.3	26.3	26.3
C–5′	70.8	70.9	70.9	71.0
C–6′	49.4	49.4	49.4	49.4
6′–CH_3	15.0	15.4	15.0	15.1
C–1	51.7	57.8	51.8	58.0
C–2	71.5	65.8	71.6	66.8
C–3	74.5	73.9	74.5	74.3
C–4	54.1	53.3	54.1	53.8
C–5	66.2	65.3	66.3	66.3
C–6	72.5	74.7	72.4	74.5
OCH_3	56.8	57.1	56.8	57.9
NCH_3	31.9	31.9	32.0	32.4
$glyCH_2$	44.3		41.3	
glyCO	169.0		168.3	
–CH =NH	155.7			

of an imino function. The 100 MHz PMR spectrum of **1** in D_2O resembled in many aspects that of fortimicin A[3] showing an anomeric proton at 5.34 ppm and three methyl signals assigned to C-CH_3 (1.33 ppm), N-CH_3 (3.16 ppm) and O-CH_3 (3.49 ppm) groups. However, there appeared an additional signal at 7.98 ppm, which was absent in the spectrum of fortimicin A. The observation of the proton signal at 7.98 ppm and the IR band at 1710 cm^{-1} suggested the presence of the formimidoyl group in **1**. Drastic acidic hydrolysis of **1** by refluxing with 6 N HCl for 20 hours gave glycine, but mild acidic hydrolysis in 3 N HCl at 100°C for 3 hours afforded an amino acid (**2**), in addition to glycine.

Compound **2** was identical with synthetic formimidoylglycine[4] by amino acid analysis (Fig. 1) and PPC. In order to determine the carbon skeleton, **1** was treated with 1 N NaOH at 90°C for 1 hour to give the deacylated dactimicin (**3**). Its molecular formula $C_{15}H_{32}N_4O_5$, $[\alpha]_D^{25}$ +25.1° (*c* 1, H_2O), CMR (Table 2) and mass spectrum were quite consistent with those of fortimicin B[3], which had been derived from fortimicin A. Further identification was carried out by the following chemical reactions. Acetylation of **3** with acetic anhydride in methanol gave tetra-*N*-acetyldeacyldactimicin (**4**). Methanolysis of **4** with 6 N hydrogen chloride in anhydrous methanol followed by re-*N*-acetylation and chromatographic separation gave the methyl glycosides (**5**) and (**6**). Those were identified as methyl 2,6-bis-(acetamido)-2,3,4,6,7-pentadeoxy-β-L-*lyxo*-heptopyranoside[3,5] and its α-anomer by comparing with authentic samples. Thus, the identity of deacyldactimicin (**3**) and fortimicin B was established. It was concluded from this that **1** was the acyl derivative of fortimicin B as in the case of fortimicin A[3]. The point of attachment of the formimidoylglycine was determined from the CMR spectrum of **1** shown in Table 2. The 51.7 ppm resonance, which was assigned to the C-1 carbon, was markedly deshielded as compared to that of **3** (fortimicin B), suggesting that the amino group at C-1 was acylated. A resonance at 155.7 ppm in **1**, which was not observed in fortimicin A, was attributed to the carbon of formimidoyl group. The methylene carbon of the formimidoylglycyl group at 44.3 ppm was shifted downfield by 3 ppm when compared to that of fortimicin A bearing the glycyl group at C-1. This shift was explained as a downfield β-shift arising from the formimidoyl substitution of the amino group of the glycyl moiety. Other signals for **1** were in agreement with those of fortimicin A within experimental error. This showed that **1** was 1-*N*-formimidoylglycylfortimicin B. This conclusion was further confirmed by the examination of 400 MHz PMR spectrum of **1** (Fig. 2). Spin decouping made it possible to assign the respective protons of **1** as shown in Table 3.

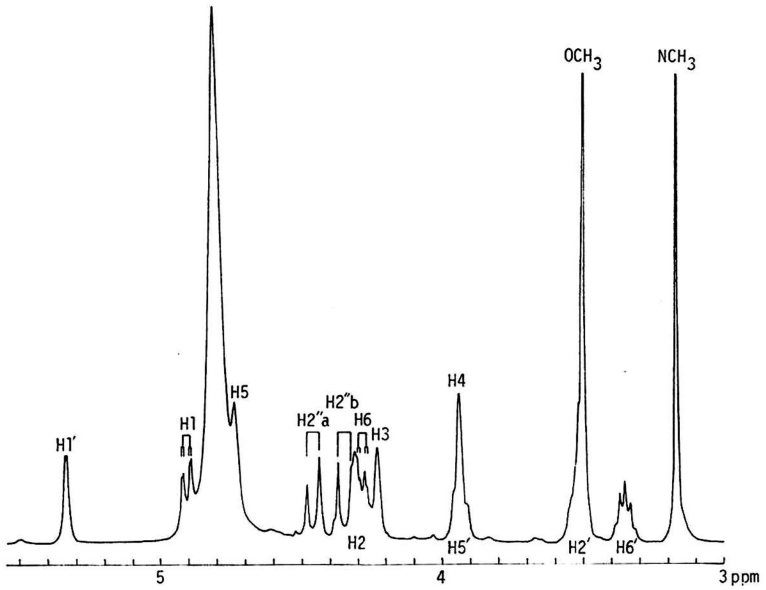
The methine proton of the formimidoyl group was observed at 7.98 ppm as a singlet and the

Table 3. 400 MHz PMR parameters for dactimicin sulfate (**1**) in D_2O solution.

Proton	Chemical shifts (ppm)	Coupling constants (Hz)	Proton	Chemical shifts (ppm)	Coupling constants (Hz)
H1′	5.33	$J_{1',2'}=3.0$	H1	4.90	$J_{1,2}=2.2$
H2′	3.52		H2	4.30	$J_{2,3}=3.0$
H3′,H4′ (3H)	1.95, 2.05		H3	4.22	$J_{3,4}=3.0$
H4′	1.54		H4	3.93	$J_{4,5}=3.0$
H5′	3.92	$J_{5',6'}=8.2$	H5	4.73	$J_{5,6}=3.5$
H6′	3.35	$J_{6',CH_3}=6.7$	H6	4.28	$J_{1,6}=11.5$
CH_3–6′	1.32		OCH_3	3.50	
H2″	4.33, 4.45	$J_{2'',2''}=17.0$	NCH_3	3.17	
–CH=NH	7.98				

Chemical shifts were measured in D_2O using DSS as the internal reference.

Fig. 2. 400 MHz PMR spectrum of dactimicin sulfate (1) in D_2O.



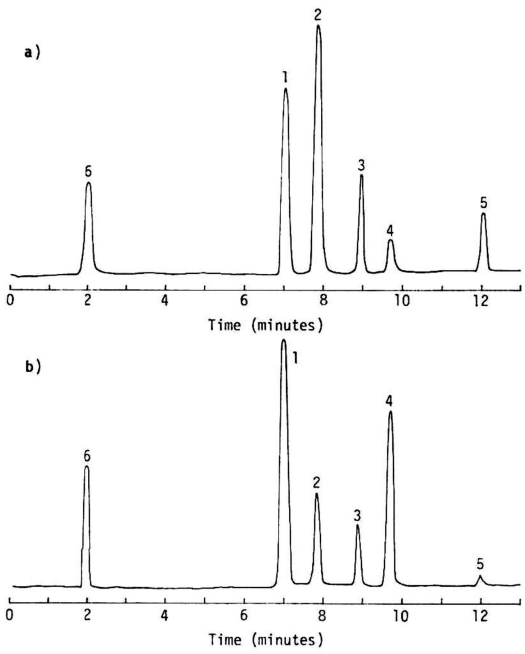
methylene protons of the formimidoyl moiety were observed at 4.33 and 4.45 ppm as an AB quartet (J=17 Hz). It was noted that among the six cyclitol ring protons, a large coupling constant due to diaxial proton orientation was observed only between H-1 and H-6 (J=11.5 Hz). This showed that substituents at C-2, C-3, C-4 and C-5 in the cyclitol moiety had axial orientations, while the other substituents at C-1 and C-6 had equatorial orientations. Taking into consideration the above analysis, the absolute structure of **1** was shown to be 4-amino-1,4-dideoxy-3-*O*-(2,6-diamino-2,3,4,6,7-pentadeoxy-β-L-*lyxo*-heptopyranosyl)-1-[(*N*-formimidoylglycyl)methylamino]-6-*O*-methyl-L-*chiro*-inositol.

Alkaline Degradation of Dactimicin

Like other aminoglycosides, dactimicin (**1**) is stable in acidic and neutral aqueous solution. However, owing to the presence of formimidoyl group, the antibiotic shows alkaline instability, which is not common among aminoglycosides. It was therefore of interest to investigate the pathway of alkaline degradation of **1**. As described already, drastic alkaline hydrolysis gave quantitatively fortimicin B, but mild alkaline treatment gave a complex mixture, which could not be clearly separated by conventional TLC. Quantitative analysis of the mixture was accomplished by using reverse-phase, ion-pair HPLC coupled with the fluorescent detection.[6] The HPLC analysis of the mild alkaline hydrolyzate of **1** is shown in Fig. 3. Six peaks 1~6 were observed in Fig. 3a. The first peak 6 with the shortest retention time was assigned to glycine, which was formed by the degradation of formimidoylglycyl moiety. As judged from the retention time, peaks 1, 2, 3, and 5 were assumed to be fortimicin B, 1-*N*-(*N*-formylglycyl)fortimicin B, fortimicin A and intact dactimicin, respectively.

Peak 4 was a new peak corresponding to an unknown constituent. Verification of the identification of the respective peaks was made by direct isolation of each substance from the alkaline hydrolyzate. An aqueous alkaline solution (pH 10) of **1** was allowed to stand at 37°C for 10 hours. The resulting mixture was neutralized and chromatographed over CM-Sephadex C-25. The component

Fig. 3. HPLC analysis of alkaline hydrolyzate of dactimicin (1).



Column: TSK-Gel LS-410 (2.6 mm×250 mm)
Solvent: 0.005 M PIC B-7+0.2 M Na_2SO_4
Flow rate: 0.8 ml/minute
Alkaline hydrolysis condition:

a) Alkaline aqueous solution of **1** at pH 10 was stood at 37°C for 10 hours.

b) stood at 37°C for 45 hours.

O-Phthalaldehyde reagent (OPA) was consisted with 10 ml of fluesin, 2 ml of 2-mercaptoethanol and 100 ml of Titrizol in 1 liter of distilled water. OPA flow rate was 1.6 ml/minute.

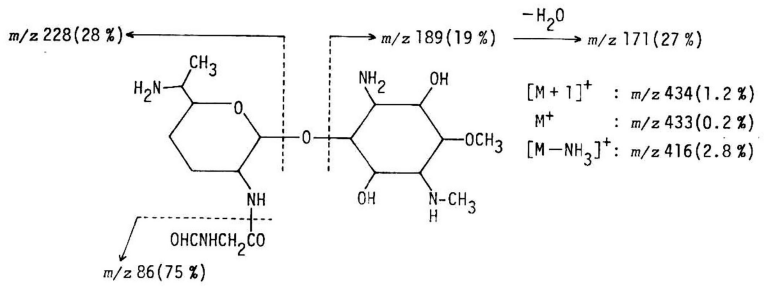
1) Fortimicin B, 2) 1-*N*-(*N*-Formylglycyl)fortimicin B, 3) Fortimicin A, 4) 2′-*N*-(*N*-Formylglycyl)fortimicin B, 5) Dactimicin

Table 4. 25 MHz CMR parameters of **7** and **10** in D_2O solution.

	1-*N*-(*N*-formylglycyl)-fortimicin B (**7**) pD 3.0	2′-*N*-(*N*-formylglycyl)-fortimicin B (**10**) pD 3.0	Dactimicin (**1**) pD 3.0	Deformimidoylglycyldactimicin (**3**) pD3.0
C–1′	95.2	97.6	95.4	96.4
C–2′	51.7	51.9	51.6	51.6
C–3′	21.6	22.5	21.6	21.6
C–4′	26.4	27.3	26.3	26.3
C–5′	70.8	70.6	70.8	70.9
C–6′	49.4	49.1	49.4	49.4
6′–CH_3	15.2	15.4	15.0	15.4
C–1	51.7	57.6	51.7	57.8
C–2	71.5	66.0	71.5	65.8
C–3	74.4	73.9	74.5	73.9
C–4	54.1	53.6	54.1	53.3
C–5	66.3	65.8	66.2	65.3
C–6	72.5	74.7	72.5	74.7
OCH_3	56.9	57.4	56.8	57.1
NCH_3	32.2	31.6	31.9	31.9
glyCH_2	41.3	41.9	44.3	
glyCO	171.5	171.1	169.0	
–CHO	165.5	165.6		
–CH =NH			155.7	

corresponding to peak 2 (**7**) was isolated by eluting with 0.3 M NaCl, and the structure, 1-*N*-(*N*-formylglycyl)fortimicin B, was determined from the PMR and mass spectral analysis. To further confirm this structure, an authentic sample was synthesized by the following procedure. Coupling of 4,2′,6′-tri-*N*-*t*-butoxycarbonylfortimicin B (**8**) with the 1-hydroxybenzotriazole ester of *N*-formylglycine, in tetrahydrofuran, gave 1-*N*-(*N*-formylglycyl)-4,2′,6′-tri-*N*-*t*-butoxycarbonylfortimicin B (**9**). Acid treatment of this product (**9**) afforded 1-*N*-(*N*-formylglycyl)fortimicin B. The synthetic compound and **7** gave identical mass, PMR and CMR spectra. Interestingly enough, 1-*N*-(*N*-formylglycyl)-fortimicin B is a natural product isolated from the culture broth of *Micromonospora olivoasterospora* SF-1854[7]. Further elution of the CM-Sephadex C-25 column with 0.5 M NaCl gave two products corresponding to peaks 3 and 1 in the HPLC. These products were confirmed to be fortimicin B and A by direct comparison with authentic samples. For the isolation of the unknown component 4, an aqueous alkaline solution of **1** was allowed to stand for 3 days at 37°C. Degradation mixtures were chromatographed over Amberlite CG-50 (NH_4^+), developing with 0.1 N NH_4OH. The compound (**10**), which gave peak 4 in the HPLC was obtained as its free base. It exhibited an amide carbonyl band at 1640 cm^{-1}, suggesting the presence of an *N*-acyl group. The PMR spectrum in D_2O showed

Scheme 1. Mass spectral fragmentation* of **10**.

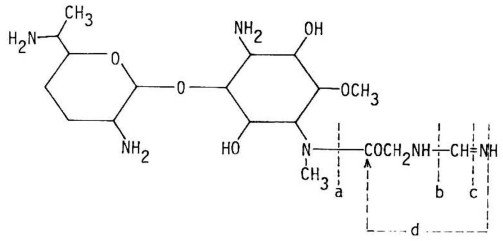


* Figures in parentheses indicate abundance relative to the base peak at *m/z* 44.

an anomeric proton at 5.26 ppm (d, J=3.8 Hz), three methyl groups at 1.22 ppm (C-CH_3), 2.38 ppm (*N*-methyl) and 3.46 ppm (*O*-CH_3), and a formyl proton at 8.15 ppm. The chemical shift of *N*-methyl group in **10** was close to that of fortimicin B, but shifted to higher field than that of fortimicin A, indicating that the C-1 amino group was not substituted with an acyl group.

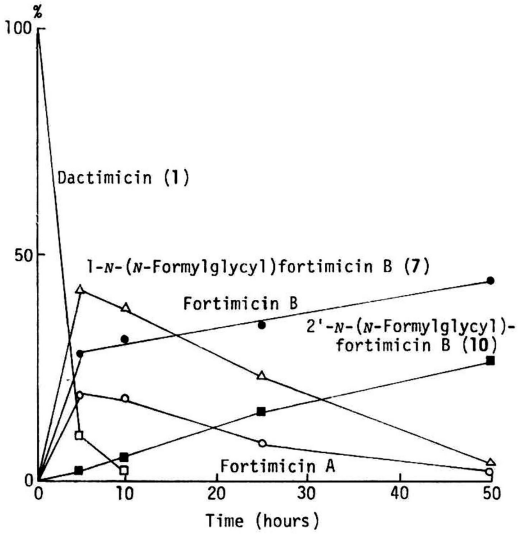
These spectral data suggested that compound **10** was to be a positional isomer of the *N*-formylglycyl moiety of **7**. The mass spectrum of the free base **10** gave ions at *m/z* 433 (molecular ion), *m/z* 228 (aminosugar ion), *m/z* 189 (aminocyclitol ion) and *m/z* 86 (*N*-formylglycyl cation) as shown in Scheme 1. The sugar fragment ion at *m/z* 228 instead of *m/z* 143 in **7** and **3** indicated that the *N*-formylglycyl group was attached to the aminosugar moiety. The carbon resonance of **10** due to the *N*-formylglycyl group were observed at 41.9 ppm (methylene carbon), 165.6 ppm (formyl carbon) and 171.1 ppm (carbonyl carbon). More significantly, C-1′ and C-3′ signals were shifted downfield relative to those of fortimicin B. Thus, the *N*-formylglycyl group was placed on the C-2′ amino group. The structure of **10** was finally confirmed by the synthesis described below. Treatment of 1,4,6′-tri-*N*-benzyloxycarbonylfortimicin B (**11**), which was prepared from 2′-*N*-*t*-butoxycarbonylfortimicin B by reacting with the 1-hydroxybenzotriazole ester of *N*-formylglycine in tetrahydrofuran, gave 2′-*N*-(*N*-formylglycyl)-1,4,6′-tri-*N*-benzyloxycarbonylfortimicin B (**13**). Catalytic hydrogenolysis of this compound afforded 2′-*N*-(*N*-formylglycyl)fortimicin B. The synthetic compound and **10** gave identical mass, PMR and CMR spectra. The formation of **10** could be rationalized by the intramolecular acyl migration of the *N*-formylglycyl group from the C-1 to the C-2′ amino group during alkaline treatment. A similar acyl migration involving a glycyl group was recently reported by TADANIER *et al.* for which they proposed "the isofortimicin rearrangement"[8,9]. Three degradation products, **3** (fortimicin B), fortimicin A and **7** were formed by the cleavage at the position a, b and c, respectively (Scheme 2). Fig. 4 summarizes the time-course of formation of these products estimated by HPLC. At the initial stage of degradation, the formation of **7** predominated, followed by fortimicin B, fortimicin A and **10** in that order.

Scheme 2.

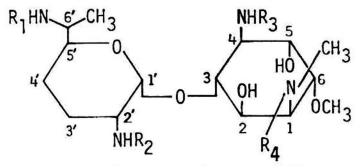


Thus, the relative ease of cleavage at this stage was c>a>b. After 5 hours of treatment, **7** and fortimicin A began to decrease, while **10** and fortimicin B increased, probably by the consumption of **7** and fortimicin A. The increase of **10** was remarkable after 10 hours when **1** disappeared completely.

Fig. 4. Time-course of alkaline degradation products of **1** by HPLC analysis.



Scheme 3.



	R_1	R_2	R_3	R_4
1	H	H	H	$COCH_2NHCH{=}NH$
3	H	H	H	H
4	Ac	Ac	Ac	Ac
7	H	H	H	$COCH_2NHCHO$
8	Boc	Boc	Boc	H
9	Boc	Boc	Boc	$COCH_2NHCHO$
10	H	$COCH_2NHCHO$	H	H
11	Cbz	Boc	Cbz	Cbz
12	Cbz	H	Cbz	Cbz
13	Cbz	$COCH_2NHCHO$	Cbz	Cbz

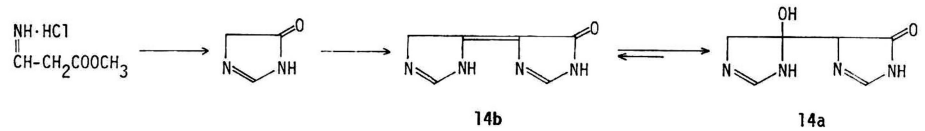
In addition to the above-mentioned degradation pathways, another cleavage d, was taken into consideration (Ref. to Scheme 2). This cleavage involves the nucleophilic attack of the imino group of the formimidoyl function on the amide-carbonyl, resulting in the formation of a cyclization product, 2-imidazoline-4-one[12] or its dimer (**14**)*. A comprehensive search for this compound (**14**) in the degradation products of **1** gave no evidence for its formation by the HPLC analysis. Thus, it was concluded that this type of cyclization reaction, which occurred readily in the case of the formimidoylglycine ester, did not take place in the formimidoylglycine amide moiety present in **1**. In contrast to the alkaline instability, dactimicin was relatively stable in acidic or neutral aqueous solution (pH 4~7) as shown in Table 5. When the aqueous solution (pH 6) of **1** was kept for 10 hours at room temperature, only 4% of **7**, 3% of fortimicin B and less than 1% of other degradation products were detected by HPLC.

Table 5. pH Stability of dactimicin (**1**).

	Residual activity (%)
pH 5	98
pH 6	96
pH 7	92
pH 8	51
pH 10	7

A solution of dactimicin (0.1 mg/ml) was kept in various pH solutions of 0.05 M phosphate buffer at 25°C for 24 hours. Residual activity was assayed by the paper disc method on *B. subtilis*.

Scheme 4.



* 2-Imidazoline-4-one was synthesized and was unstable, and easily converted to dimer[10] (**14**) showing, λ_{max} 326 nm in the UV. The compound (**14**) has been briefly mentioned in the reference[10], without isolation. It was found that compound (**14**) existed mainly in the hydrated form (a), rather than the dehydrated form (b).

Experimental

Methods of Analysis

Melting points were obtained on a Yamato capillary apparatus and are uncorrected. Spectral data were obtained as follows; optical rotation with a Perkin-Elmer 141 polarimeter, IR spectra in KBr tablets with a Hitachi model 215 IR spectrometer, mass spectra with a M-80 mass spectrometer, amino acid analysis with a Hitachi 835 amino acid analyzer, HPLC analysis with a Hitachi 638 HPLC analyzer, 25.16 MHz CMR spectra using a Varian XL-100 spectrometer and dioxane as the internal standard in D_2O, 100 MHz PMR spectra using a Varian XL-100 spectrometer and TMS as the external reference in D_2O, and 400 MHz PMR spectra in D_2O using a JEOL FX-400 spectrometer and DSS as the internal reference.

Deformimidoylglycyldactimicin (3)

A solution of 980 mg of dactimicin (**1**) in 100 ml of 0.5 N NaOH was refluxed at 95°C for 1.5 hours. The reaction mixture was neutralized with 5 N HCl and then charged onto a column of Amberlite CG-50 (NH_4^+). After washing with water, the column was developed with 0.2 N NH_4OH. Fractions containing **3** were combined and concentrated to afford a white powder of **3** (409 mg): mp 105~107°C; $[\alpha]_D^{25}$ +25.1° (*c* 1, H_2O); MS: *m/z* 349 (M^++1), 348 (M^+), 189, 143; PMR (D_2O): δ 1.18 (3H, d, J=6.8 Hz, 6′-CH_3), 1.4~2.6 (4H, m, H3′, H4′), 2.50 (3H, s, NCH_3), 3.10 (1H, dd, $J_{3,4}$=9.5 Hz, $J_{4,5}$=9.5 Hz, H4), 3.18 (1H, dd, $J_{1,2}$=4.5 Hz, $J_{6,1}$=3.0 Hz, H1), 3.58 (3H, s, OCH_3), 3.60 (1H, t, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.5 Hz, H3), 3.78 (1H, t, $J_{1,6}$=3.0 Hz, $J_{5,6}$=3.0 Hz, H6), 3.82 (1H, dd, $J_{4,5}$=9.5 Hz, $J_{5,6}$=3.0 Hz, H5), 4.10 (1H, dd, $J_{1,2}$=4.5 Hz, $J_{2,3}$=9.5 Hz, H2), 5.18 (1H, d, $J_{1',2'}$=3.8 Hz, H1′).

Anal. Calcd. for $C_{15}H_{32}N_4O_5$: C 51.71, H 9.26, N 16.08.
Found: C 51.63, H 9.35, N 15.97.

Tetra-*N*-acetyldeformimidoylglycyldactimicin (4)

To a solution of 400 mg of **3** in 40 ml of absolute methanol, 8 ml of acetic anhydride was added at 0°C, and the mixture was stirred at 0~5°C for 0.5 hours and then at room temperature for 5 hours. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column (120 ml). The column was eluted with chloroform - methanol (10: 1) and fractions containing **4** were collected and evaporated to give 610 mg (**4**) as a white powder: $[\alpha]_D^{25}$ +93.2° (*c* 1, MeOH); IR: 1640, 1530 cm^{-1}: MS: *m/z* 516 (M^+).

Anal. Calcd. for $C_{23}H_{40}N_4O_9$: C 53.49, H 7.75, N 10.85.
Found: C 53.32, H 7.68, N 10.70.

Methanolysis of Tetra-*N*-acetyldeformimidoylglycyldactimicin (4)

A solution of 600 mg of **4** in 150 ml of 6 N HCl in dry methanol was heated under refluxed for 15 hours. The reaction mixture was evaporated to dryness to leave a syrup. The syrup was dissolved in water and passed through a column of Amberlite IRA-400 (OH^-). The effluent was concentrated to dryness and the residue (406 mg) was dissolved in 20 ml of anhydrous methanol, and was acetylated by treating with 3 ml of acetic anhydride at room temperature. The mixture was concentrated, and chromatographed on a column of silica gel (120 ml), developing with chloroform - methanol (1: 1). Effluents were collected in 15 ml of fraction. Fractions No. 46~80 were combined and evaporated to afford 940 mg of methyl 2,6-bis(acetamido)-2,3,4,6,7-pentadeoxy-β-L-*lyxo*-heptopyranoside (methyl 2,6-di-*N*-acetyl-6-*epi*-α-purpurosaminide B) (**5**), which crystallized from chloroform, mp 218~219°C; $[\alpha]_D^{25}$ +63.3° (*c* 1, MeOH); MS: *m/z* 259 (M^++1); PMR ($CDCl_3$): δ 1.19 (3H, d, J=7 Hz, 6-CH_3), 2.00 (6H, s, *N*-$COCH_3$), 3.37 (3H, s, OCH_3), 4.58 (1H, d, J=3.5 Hz, H1).

Anal. Calcd. for $C_{12}H_{22}N_2O_4$: C 55.80, H 8.58, N 10.84.
Found: C 55.63, H 8.61, N 10.65.

From fractions No. 140~172, 21 mg of 2,6-bis(acetamido)-2,3,4,6,7-pentadeoxy-α-L-*lyxo*-heptopyranoside (methyl 2,6-di-*N*-acetyl-6-*epi*-β-purpurosaminide B) (**6**) were crystallized from chloroform; mp 211~213°C; $[\alpha]_D^{25}$ −109° (*c* 1, MeOH); MS: *m/z* 259 (M^++1); PMR ($CDCl_3$): δ 1.22 (3H, d, J=6.8 Hz, 6-CH_3), 2.00 (6H, s, *N*-$COCH_3$), 3.48 (3H, s, OCH_3), 4.17 (1H, d, J=8 Hz, H1).

Anal. Calcd. for $C_{12}H_{22}N_2O_4$: C 55.80, H 8.58, N 10.84.
Found: C 55.69, H 8.27, N 10.71.

1-*N*-(*N*-Formylglycyl)fortimicin B (**7**) from the Alkaline Hydrolyzate of **1**

A solution of 700 mg of **1** in 140 ml of water adjusted to pH 10 with 1 N NaOH, and kept at 37°C. The reaction process was monitored by HPLC. After 10 hours, the reaction mixture was adjusted to pH 5.0 with 5 N HCl and chromatographed over CM-Sephadex C-25 (Na^+, 150 ml). The column was developed with 0.3 M NaCl and the eluate were collected in 17 ml fractions. Fractions No. 21~32 which gave peak 2 in the HPLC were desalted over carbon, and were passed through a column of Amberlite IRA-400 (OH^-, 20 ml) at 5°C. The effluent was concentrated and freeze-dried to give 120 mg of the free base of **7** as white powder. $[\alpha]_D^{25}$ +89.2° (*c* 1, H_2O); IR: 3400, 1650, 1620, 1485 cm^{-1}; MS: *m/z* 434 (M^++1), 433 (M^+), 416, 274, 256, 143, 126, 86; PMR (D_2O): δ 1.10 (3H, d, *J*=7 Hz, 6-CH_3), 3.12 (3H, s, NCH_3), 3.43 (3H, s, OCH_3), 5.27 (1H, d, *J*=4 Hz, H1'), 8.16 (1H, s, CHO).

Anal. Calcd. for $C_{18}H_{35}N_5O_7$: C 49.88, H 8.08, N 16.17.
Found: C 49.53, H 7.86, N 16.02.

CMR spectrum of **7** (Table 5) was very similar that of **1** except for the chemical shift of the acyl group.

A formyl carbon resonance was observed at 165.5 ppm which differed from a formimidoyl carbon which resonates at 155.7 ppm. Continued elution of the column with 0.5 M NaCl gave successively two components corresponding peak 3 and peak 1 in HPLC (Fig. 3). Eightyfive mg of peak 3 compound and 30 mg of peak 1 compound were obtained by a procedure similar to that used for the isolation **7**, and were identified as fortimicin B and fortimicin A, respectively.

Synthesis of 1-*N*-(*N*-Formylglycyl)fortimicin B (**7**)

To an ice-cooled, stirred solution of *N*-formylglycine (66 mg) and 1-hydroxybenzotriazole (90 mg) in 15 ml of tetrahydrofuran was added dicyclohexylcarbodiimide (132 mg), and the mixture was stirred at 5°C for 3 hours. To the reaction mixture was added 4,2',6'-tri-*N*-*t*-butoxycarbonylfortimicin B (347 mg), and the solution was stirred at room temperature for 20 hours. The resulting precipitate was filtered off and the filtrate was evaporated to dryness. The residue was chromatographed on a column of silica gel (50 ml) and developed with chloroform - methanol - conc. NH_4OH (1000: 36: 5). Fractions No. 15~40 (375 ml) were combined and evaporated to give 225 mg of 1-*N*-(*N*-formylglycyl)-4,2',6'-tri-*N*-*t*-butoxycarbonylfortimicin B (**9**): $[\alpha]_D^{25}$ +50.3° (*c* 1, MeOH); PMR ($CDCl_3$): δ 1.19 (3H, d, *J*=7 Hz, 6'-CH_3), 1.44 (9H, s, $OC(CH_3)_3$), 3.13 (3H, s, NCH_3), 3.49 (3H, s, OCH_3), 5.00 (1H, d, *J*=3.5 Hz, H1'), 8.17 (1H, s, CHO).

Anal. Calcd. for $C_{38}H_{67}N_5O_{15}$: C 54.74, H 8.04, N 8.40.
Found: C 54.68, H 7.96, N 8.25.

Two hundred mg of **9** were dissolved in 3 ml of trifluoroacetic acid and allowed to stand for 30 minutes at room temperature. The reaction mixture was concentrated to dryness and the residue was dissolved in 30 ml of cold water and passed through a column of Amberlite IRA-400 (OH^-, 15 ml) at 5°C. The column was washed with 40 ml of cold water and the effluent and washings were combined, concentrated and lyophilized to afford 50 mg of the free base of **7** as a white powder: $[\alpha]_D^{25}$ + 90.3° (*c* 1, H_2O); IR: 3400, 1648, 1620, 1485 cm^{-1}, MS: *m/z* 434 (M^++1), 433 (M^+), 416, 274, 256, 143, 126, 84; PMR ($CDCl_3$) δ 1.11 (3H, *J*=7 Hz, 6'-CH_3), 3.12 (3H, s, NCH_3), 3.44 (3H, s, OCH_3), 5.29 (1H, d, *J*=4 Hz, H1'), 8.16 (1H, s, CHO).

Anal. Calcd. for $C_{18}H_{35}N_5O_7$: C 49.88, H 8.08, N 16.17.
Found: C 49.53, H 7.96, N 16.08.

2'-*N*-(*N*-Formylglycyl)fortimicin B (**10**) from the Alkaline Hydrolyzate of **1**

A solution of 590 mg of **1** in 50 ml of water was adjusted to pH 10.0 with 1 N NaOH and allowed to stand at 37°C for 50 hours. The reaction mixture was neutralized with 5 N HCl and chromatographed over Amberlite CG-50 (NH_4^+, 35 ml). After washing with water, elution was effected with 0.1 N NH_4OH and 15 ml fractions were collected. Fractions No. 11~24 were concentrated and lyophilized to give 50 mg of the free base of **10** as a white powder: $[\alpha]_D^{25}$ +55.2° (*c* 1, H_2O); IR: 3400, 1640, 1500 cm^{-1}; MS; *m/z* 434 (M^++1), 433 (M^+), 416, 235, 228, 207, 189, 171, 86; PMR (D_2O): δ 1.22 (3H, d, *J*=7 Hz, 6'-CH_3), 2.40 (3H, s, NCH_3), 3.46 (3H, s, OCH_3), 5.26 (1H, d, *J*=3.8 Hz, H1), 8.15 (1H, s, CHO).

Anal. Calcd. for $C_{18}H_{35}N_5O_7$: C 49.88, H 8.08, N 16.16.
Found: C 49.63, H 8.01, N 16.03.

Synthesis of 2′-*N*-(*N*-Formylglycyl)fortimicin B (**10**)

To an ice-cooled, stirred solution of 2′-*N*-*t*-butoxycarbonylfortimicin B (600 mg) and sodium carbonate (850 mg) in 40 ml of water, benzyloxycarbonylchloride (1.37 g) in 20 ml of dioxane was added dropwise over a period of 1 hour and stirred at room temperature for 15 hours. The reaction mixture was neutralized with 5 N HCl and extracted with 100 ml of ethyl acetate. The solvent layer was dried over anhydrous sodium sulfate, and concentrated to dryness. The residue was dissolved in 5 ml of diethyl ether and 50 ml of hexane was added. The resulting precipitate was dried to give 1.14 g of white powder of 2′-*N*-*t*-butoxycarbonyl-1,4,6′-tri-*N*-benzyloxycarbonylfortimicin B (**11**); $[\alpha]_D^{25}$ +35.2° (*c* 1, MeOH); PMR (CD_3OD): δ 1.19 (1H, d, J=7 Hz, 6′-CH_3), 1.44 (9H, s, $OC(CH_3)_3$), 3.13 (3H, s, NCH_3), 3.40 (3H, s, OCH_3), 7.35 (15H, m, aromatic).

Anal. Calcd. for $C_{44}H_{58}N_4O_{13}$: C 62.12, H 6.82, N 6.59.
Found: C 62.02, H 6.91, N 6.37.

A part of **11** (600 mg) was dissolved in 5 ml of trifluoroacetic acid and stirred at 15°C for 1 hour. The reaction mixture was concentrated to dryness and the residue was dissolved in 100 ml of ethyl acetate and washed with 0.1 N aqueous sodium hydrogen carbonate (50 ml) and then water. The ethyl acetate layer was dried over anhydrous sodium sulfate, and concentrated to give 420 mg of 1,4,6′-tri-*N*-benzyloxycarbonylfortimicin B (**12**); $[\alpha]_D^{25}$ +37.0° (*c* 1, MeOH); IR: 3350, 1690, 1500 cm^{-1}; PMR (CD_3OD): δ 1.19 (1H, d, J=7 Hz, 6′-CH_3), 3.10 (3H, s, NCH_3), 3.40 (3H, s, OCH_3), 7.35 (15H, m, aromatic).

Anal. Calcd. for $C_{39}H_{50}N_4O_{11}$: C 62.40, H 6.67, N 7.47.
Found: C 62.32, H 6.58, N 7.29.

To an ice-cooled, stirred solution of *N*-formylglycine (67 mg) and 1-hydroxybenzotriazole (90 mg) in 20 ml of tetrahydrofuran was added dicyclohexylcarbodiimide (135 mg) and the mixture was stirred at 10°C for 2 hours. To the reaction mixture was added 1,4,6′-tri-*N*-benzyloxycarbonylfortimicin B (**12**) (400 mg) and the solution was stirred at room temperature for 15 hours. The resulting precipitate was filtered off and the filtrate was concentrated, and chromatographed over silica gel (400 ml), which was developed with chloroform - methanol (30: 1). Fractions No. 18~35 were combined (225 ml) and evaporated to give 405 mg of 2′-*N*-(*N*-formylglycyl)-1,4,6′-tri-*N*-benzyloxycarbonylfortimicin B (**13**); $[\alpha]_D^{25}$ +35.7° (*c* 1, MeOH); IR: 3355, 1695, 1638, 1500 cm^{-1}; PMR ($CDCl_3$): δ 1.19 (1H, d, J= 7 Hz, 6′-CH_3), 3.11 (3H, s, NCH_3), 3.40 (3H, s, OCH_3), 7.35 (15H, m, aromatic), 8.17 (1H, s, CHO).

Anal. Calcd. for $C_{42}H_{53}N_5O_{13}$: C 60.36, H 6.35, N 8.38.
Found: C 60.28, H 6.37, N 8.29.

A solution of compound **13** (350 mg) in a mixture of methanol (10 ml), H_2O (4 ml) and acetic acid (0.7 ml) was hydrogenolyzed over 5% palladium - carbon (70 mg) for 3 hours. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was chromatographed over Amberlite CG-50 (NH_4^+, 30 ml), being developed with 0.1 N NH_4OH and collected in 5 ml of fraction. Fractions No. 22~31 were concentrated and lyophilized to give 65 mg of the free base of **10** as a white powder; $[\alpha]_D^{25}$ +54.0° (*c* 1, H_2O); IR: 3400, 1640, 1500 cm^{-1}; PMR (D_2O): δ 1.22 (3H, d, J=7 Hz, 6′-CH_3), 2.39 (3H, s, NCH_3), 3.46 (3H, s, OCH_3), 5.26 (1H, d, J=3.8 Hz, H1′), 8.15 (1H, s, CHO).

Anal. Calcd. for $C_{18}H_{35}N_5O_7$: C 49.88, H 8.08, N 16.17.
Found: C 49.79, H 7.86, N 16.05.

5-(4-Hydroxy-2-imidazolidin-4-yl)-2-imidazoline-4-one (**14**)

A solution of *N*-formimidoylglycine methylester hydrochloride (450 mg) in 40 ml of water was adjusted to pH 9 with 1 N NaOH and stirred at room temperature for 0.5 hour. The reaction mixture was adjusted to pH 4.0 with 1 N HCl to prevent polymerization and concentrated to 3 ml of solution. This was chromatographed over Diaion HP-20 (200 ml), and was developed with water. Five ml fractions were collected. Fractions No. 31~45 were combined and lyophilized to give 56 mg of **14**. The structure of **14** was confirmed to be a hydrated dimer of 2-imidazoline-4-one by PMR and FD-MS as follows: UV (H_2O): 237 sh. ($E_{1cm}^{1\%}$ 160), 326 ($E_{1cm}^{1\%}$ 740) nm; FD-MS: m/z 169 (M^++1), 168

(M^+); δ PMR (D_2O): δ 3.80 (1H, s, methine), 4.22 (2H, methylene), 7.45 (1H, s, N=CH), 7.56 (1H, s, N=CH).

Anal. Calcd. for $C_6H_8N_4O_2$: C 42.86, H 4.76, N 33.33.
Found: C 42.75, H 4.59, N 33.26.

Compound **14** gave a single peak with retention time of 2.5 minutes by HPLC analysis (column: Nucleosil 4.6×150 mm; solvent: 1% AcOH - MeOH - CH_3CN (10: 75: 15); flow rate: 1 ml/minute; detection: UV at 326 nm).

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