THE STRUCTURES OF FORTIMICINS C, D, AND KE

TAKAO IIDA, MORIYUKI SATO, ISAO MATSUBARA, YASUKI MORI and KUNIKATSU SHIRAHATA*

Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.
3-6-6 Asahimachi, Machida, Tokyo 194, Japan

(Received for publication October 13, 1978)

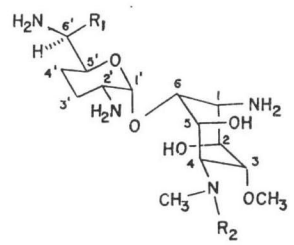
Fortimicins C, D, and KE are new aminocyclitol antibiotics produced by a mutant of fortimicin-producing organisms. Their structures have been determined by ^{1}H and ^{13}C NMR and mass spectra and chemical degradations. Fortimicin C is 4-N-hydantoylfortimicin B, fortimicin KE is 6′-demethylfortimicin B, and fortimicin D is 4-N-glycylfortimicin KE. The last two antibiotics have purpurosamine C instead of 6-epi-purpurosamine B.

Fortimicins are a novel family of aminocyclitol antibiotics produced by *Micromonospora* sp.[1,2] Two compounds, fortimicins A(FM-A) (**1**) and B(FM-B) (**2**), of this family have been described earlier and their structures have been determined by physical and chemical methods by the Abbott group[3]. Single crystal X-ray analysis has also been performed on FM-B by SASADA's group in conjunction with us[4]. They are pseudodisaccharides and incorporate a novel diaminocyclitol.

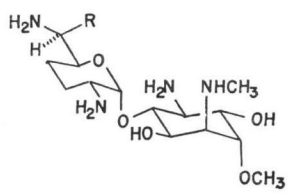
The organisms and their mutants have been known to elaborate other minor components in addition to a major component FM-A. Three new fortimicins were isolated from the fortimicin mixture produced by a mutant, *M. olivoasterospora* CS-26. Isolation and their antibacterial activities are reported in the preceding paper[5]. The present paper describes the structural elucidation of the new fortimicins.

Fortimicin C(FM-C) (**3**) is unstable in alkaline solution. Treatment of this antibiotic with barium hydroxide gave FM-B, $C_{15}H_{32}N_4O_5$, and a fragment as a white powder. The former was identified by tlc, MS, ^{1}H NMR, and $[\alpha]_D$. The latter was negative to ninhydrin and its molecular formula was assigned as $C_3H_6N_2O_3$ by a high resolution MS measurement. It was identified finally as hydantoic acid by comparison of its IR spectrum with that of an authentic sample.

The mass spectrum of **3** showed a weak ion at *m/e* 406, a high resolution measurement of which indicated the composition $C_{17}H_{36}N_5O_6$. This is consistent with the protonated molecular ion of FM-A. The molecular ion, therefore, could not be obtained by the usual electron impact ionization method. Finally it was determined by aid of field desorption mass spectrometry,



	R_1	R_2
FM-A(1)	CH_3	$COCH_2NH_2$
FM-C(3)	CH_3	$COCH_2NHCONH_2$
FM-D(4)	H	$COCH_2NH_2$



	R
FM-B(2)	CH_3
FM-KE(5)	H

* To whom inquiries should be addressed.

which showed a protonated molecular ion at *m/e* 449 as the base peak. On the basis of the field desorption mass spectrum and elemental analyses, $C_{18}H_{36}N_6O_7$ was deduced as the molecular formula.

The ^{13}C NMR spectrum of FM-C free base showed all the eighteen carbons' signals, fifteen of which, attributed to the FM-B skeleton, had the same chemical shifts within 0.2 ppm of those of the corresponding carbons of FM-A (Table 1). The remaining three signals can be easily assigned to the carbons in the hydantoyl group.

As the ^{13}C NMR chemical shifts of FM-A and B are the one of the most useful data to determine the structures of fortimicins, we have reinvestigated the assignments reported by the Abbott group[3]. Irradiation at 4.19 ppm caused the 72.9 ppm signal to be a sharp singlet and irradiation at 4.13 ppm caused both of the 73.6 and 72.9 ppm signals to be singlets (single frequency decoupling ^{13}C NMR). Irradiation at 3.97 ppm kept the 73.6 ppm signal singlet but caused the 72.9 ppm signal to be a doublet. As the signals at 4.17 and 4.14 ppm are attributed to H-5 and H-3, respectively, the 72.9 and 73.6 ppm signals must be assigned to C-5 and C-3, respectively. The pmr chemical shifts of FM-A and B, and the results of single frequency decoupling experiments are shown in Table 2.

Table 1. The ^{13}C NMR chemical shifts of fortimicins*.

Fortimicins	A	B	C	D	KE
pD	11.5	11.3	11.7	11.1	11.0
Carbons					
CO	176.4	—	173.2 162.1	176.5	—
1′	100.0	102.5	100.0	100.1	102.3
6	78.4	84.0	78.2	78.5	83.7
5′	74.9	75.1	75.0	71.3	71.3
3	73.7	79.9	73.5	73.6	79.9
5	72.9	71.3	72.8	73.0	71.3
2	71.1	71.1	71.1	71.1	71.3
OCH_3	56.4	59.3	56.3	56.4	59.3
1	55.4	53.8	55.4	55.5	53.8
4	52.5	60.9	52.7	52.5	60.9
6′	50.6	50.6	50.5	45.7	45.8
2′	50.2	50.4	50.1	50.2	50.4
$GlyCH_2$	43.4	—	43.0	43.4	—
4-NCH_3	32.2	35.4	32.3	32.3	35.4
4′	27.4	27.4	27.4	28.3	28.3
3′	26.9	27.0	26.9	26.8	27.0
6′-CH_3	18.5	18.5	18.5	—	—

* Assignments were confirmed by means of off-resonances, pD-titrations, and selective proton decouplings.

The assignments of C-2′ and 6′ were determined by comparison of the chemical shifts of FM-A and D, and FM-B and KE. The 50.2 and 45.7 ppm signals of FM-D are assigned to C-2′ and C-6′, respectively (see below). The 50.2 ppm signal of FM-A, therefore, should be assigned to C-2′ instead of C-6′, the chemical shift of the latter must be 50.6 ppm. Table 1 shows the ^{13}C NMR assignments which are also confirmed by pD-titration experiments (β-shifts).

The location of the substituent of FM-C was disclosed to be on the nitrogen atom at position 4 by consideration of the chemical shifts of C-4(52.7 ppm) and the 4-N-methyl carbon(32.3 ppm) in comparison with those of FM-A. Derivation of FM-C from FM-B has been done and will be reported elsewhere.

The mass spectrum of fortimicin D(FM-D) (**4**) exhibited a weak but distinct protonated molecular ion at *m/e* 392, shown by a high resolution measurement to have the composition $C_{16}H_{34}N_5O_6$, proving that the molecular formula is $C_{16}H_{33}N_5O_6$. Hydrolysis of **4** with barium hydroxide afforded a disaccharide($C_{14}H_{30}N_4O_5$) (**5**), a small amount of a rearrangement product (**6**), and glycine, which was identified by comparison of its tlc, amino acid analysis, and IR spectrum with those of an authentic sample.

Table 2. PMR parameters of FM-A and B free base and single frequency decoupling ^{13}C NMR signals.

Proton	FM-A			FM-B		
	Chemical shift (J in Hz)	Position irradiated	Singlet carbon	Chemical shift (J in Hz)	Position irradiated	Singlet carbon
1′	4.86 (d, 2.9)	4.90	100.0	5.07 (d, 3.7)		
2′	3.1~2.6			3.0~2.7		
3′ & 4′	1.9~1.1			1.9~1.2		
5′	3.7~3.2	3.59	74.9	3.8~3.4		
6′	3.1~2.6			3.0~2.7		
1	3.7~3.2			2.98 (t, 5.6)	2.98	53.8
2	4.39 (t, 3)	4.39	71.1	3.8~3.4	3.65	71.1
3	4.14 (dd, 11.6, 3)	4.13	73.7	3.8~3.4	3.65	79.9
4	4.91 (dd, 11.6, 3.2)	4.90	52.5	3.08 (dd, 4.4, 2.9)	3.08	60.9
5	4.17 (t, 3.2)	4.19	72.9	3.99 (dd, 9.3, 4.4)	3.99	71.3
6	3.89 (dd, 3.2, 2.2)	3.89	78.4	3.6~3.3	3.46	84.0
N-Me	3.06(s)			2.38(s)		
O-Me	3.44(s)			3.46(s)	3.46	59.3
Gly-CH_2	3.52(s)	3.59	43.4			

The physicochemical properties of the disaccharide were superimposable upon those of fortimicin KE(FM-KE) (**5**), which is also a fermentation product isolated along with FM-D.

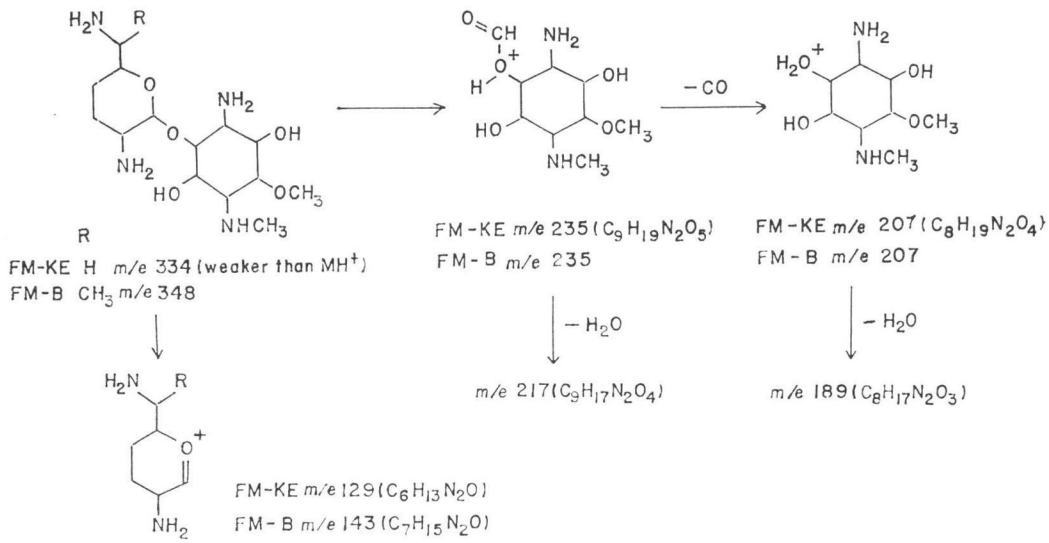
The ^1H NMR spectrum of FM-KE free base at pD 11.0 resembled closely that of FM-B measured under the identical conditions except that the former lacked a secondary methyl signal which was observed at 1.09 ppm in the latter. In addition, comparison of ^{13}C NMR spectra of both compounds (Table 1) provided the evidence that FM-KE is 6′-demethyl FM-B as follows: first, all the chemical shifts attributed to the fortamine carbons as well as C-1′, C-2′, and C-3′ of the purpurosamine moiety of FM-KE are consistent with those of FM-B within 0.3 ppm. Secondly, the ^{13}C NMR spectrum of FM-KE did not show a C-methyl signal, whereas that of FM-B did at 18.5 ppm. Finally, FM-KE has a carbon atom having a 45.8 ppm chemical shift assignable to CH_2NH_2. The corresponding carbon atom of FM-B was observed at 50.6 ppm.

Further confirmative evidence of the structure of FM-KE was obtained by its mass spectrum, showing prominent peaks at *m/e* 335, 317, 235, 217, 207, 189, and 129. Fragmentations are shown in Chart 1. Only the purpurosamine fragment was shifted by 14 amu(CH_2) from the corresponding fragment of FM-B. The dideoxysugar moiety of FM-KE is presumed to be purpurosamine C, a component of gentamicin C_{1a}[6].

Location of the glycyl group in FM-D was also established by means of its ^{13}C NMR spectrum. The chemical shifts of the purpurosamine carbons of FM-D were consistent with those of FM-KE within 0.2 ppm except for the anomeric carbon (the anomeric carbon of FM-D resonated at 2.2 ppm higher field than that of FM-KE. The same phenomenon was observed between FM-A and B.) On the other hand the chemical shifts of all the remaining carbons including the glycyl group were consistent with those of FM-A within 0.1 ppm. The glycyl group, therefore, must be attached to the nitrogen atom at position 4 in the fortamine moiety.

The structure and formation of the rearrangement product (**6**), $C_{16}H_{33}N_5O_6$, will be reported very soon[7].

Chart 1. Major MS fragmentations of FM-KE and FM-B (Compositions in parentheses were confirmed by high resolution measurements).



The stereochemistry was determined by the following experiments. Mercaptolysis of tetra-N-acetylfortimicin KE (**7**) with hydrochlolic acid and ethanethiol, followed by re-N-acetylation, gave N,N′-diacetylpurpurosamine C diethyl dithioacetal (**8**) and 1-N-acetylfortamine (**9**). The $[\alpha]_D^{25}$ +30.6° (*c* 0.3, MeOH) of **8** was identical essentially with the reported value, +27.4°(*c* 0.3, MeOH), for the same compound derived from gentamicin C_{1a}[8]. Similarly the $[\alpha]_D^{25}$ −66.1° (*c* 0.5, MeOH) of **9** was also in agreement with that of the corresponding compound derived from FM-B. These results define all the stereochemistry including absolute configurations of FM-D and KE.

Experimental

Low and high resolution mass spectra were obtained on a JEOL JMS-01SG-2 spectrometer operating at 30 eV and a field desorption mass spectrum was taken on the same model with a FD ion source. ^1H and ^{13}C NMR spectra were determined on a JEOL PS 100 or a JEOL PFT 100A spectrometer in the FT mode and chemical shifts for ^1H NMR are reported in ppm downfield from internal DSS unless otherwise stated. The chemical shifts of ^{13}C NMR spectra were measured in D_2O from internal dioxane (67.4 ppm) and are reported in ppm downfield from TMS. IR spectra were taken on a Shimadzu IR-27G spectrometer. Elemental analyses were obtained on a Yanagimoto CHN Corder MT-1. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. Reported pD values are uncorrected pH meter readings of deuterium oxide solutions with an Okakura model AH 21 pH meter.

Fortimicin C (**3**)

$[\alpha]_D^{25}$ +84° (*c* 0.1, H_2O). ^1H NMR(deuteriochloride, D_2O) 5.33(d, J=4, 1′-H), 4.07(br s, $COCH_2N$), 3.50(s, OCH_3), 3.16(s, NCH_3), and 1.34 (d, J=6.5, 6′-CH_3); (free base, D_2O, Varian T-60 spectrometer) 4.90 (d, J=4, 1′-H), 4.07 (br s, $COCH_2N$), 3.49 (s, OCH_3), 3.13 (s, NCH_3), and 1.12 (d, J=6.5, 6′-CH_3). ^{13}C NMR(pD=0.7) 162.3(CO×2), 95.2(C1′), 74.3(C6), 72.6(C3), 71.6(C5), 70.8(C5′), 66.3(C2), 56.9 (OCH_3), 54.2(C1), 51.7(C4 & C6′), 49.4(C2′), 43.0(glyCH_2), 32.1(NCH_3), 26.3(C4′), 21.6(C3′), and 15.2(6′-CH_3). IR(KBr): 3400, 2900, 1625, 1570, 1450, 1350, 1100, and 1030 cm^{-1}.

Anal. (%), Found: C 44.84, H 8.19, N 17.36.
Calcd for $C_{18}H_{36}N_6O_7 \cdot 2H_2O$: C 44.61, H 8.32, N 17.35.

Fortimicin D (**4**)

$[\alpha]_D^{25}$ +121° (*c* 0.5, H_2O). ^1H NMR(D_2O, pD=10.1) 4.92 (d, J=4, 1′-H), 3.53 (br s, $COCH_2N$), 3.45 (s, OCH_3), 3.07 (s, NCH_3); (pD=1.0) 5.33 (d, J=4, 1′-H), 4,09 (br s, $COCH_2N$), 3.51 (s, OCH_3), and 3.16 (s, NCH_3). ^{13}C NMR(pD=1.0) 168.7(CO), 95.4(Cl′), 74.4(C6), 72.5(C3), 71.5(C5), 66.6(C2), 66.2(C5′), 56.9(OCH_3), 54.2(Cl), 51.8(C4), 49.4(C2′), 43.5(C6′), 41.4(glyCH_2), 32.1(NCH_3), 26.7(C4′), and 21.7(C3′). MS(*m/e*): 329 (MH^+, high resolution 392.2495, $C_{16}H_{34}N_5O_6$ requires 392.2509), 374, 292, 274, 264, 246, and 129. IR(KBr): 3400, 2900, 1635, 1570, 1470, 1350, 1315, 1100, and 1020 cm^{-1}.

Fortimicin KE (**5**)

$[\alpha]_D^{25}$ +28.5° (*c* 0.5, H_2O). ^1H NMR (D_2O, pD=11.0) 5.04 (d, J=4, 1′-H), 3.48 (s, OCH_3), and 2.40(s, NCH_3); (pD=0.8) 5.40 (d, J=4, 1′-H), 3.52 (s, OCH_3), and 2.87 (s, NCH_3). ^{13}C NMR (pD=1.0) 96.5 (C1′), 74.6 (C6), 73.9 (C3), 66.8 (C5′), 65.9 & 65.4 (C5 & C2), 57.9 (OCH_3), 57.2 (C1), 53.4 (C4), 49.2 (C2′), 43.5 (C6′), 31.8 (NCH_3), 26.5 (C4′), and 21.8 (C3′). MS (*m/e*): 335 (MH^+, high resolution 335.2311, $C_{14}H_{31}N_4O_5$ requires 335.2294), other peaks, see text. IR (KBr): 3350, 2920, 1580, 1470, 1370, 1330, 1090, and 1035 cm^{-1}.

Hydrolysis of FM-C

A solution of 11 mg of FM-C in 2 ml of 0.1 N aqueous barium hydroxide was heated at 100°C for 1 hour. To the hydrolysate were added several pieces of dry ice and the resulting precipitate was removed by filtration. The filtrate was applied to a column containing 5 ml of Amberlite CG-50 (NH_4^+) resin and eluted with water. The water eluate was charged on a column of 5 ml of Diaion SK 1-B (H^+) and eluted with water. Freeze-drying of the eluate gave 2.3 mg of hydantoic acid (87%). MS (*m/e*): 118.0377 (M^+), $C_3H_6N_2O_3$ requires 118.0378.

The CG-50 column was then eluted with 0.3 N ammonium hydroxide, and the eluate was freeze-dried. The residue was dissolved in a minimum portion of water, acidified (pH 2) with 1 N hydrochloric acid, and freeze-dried to afford 12 mg of FM-B hydrochloride(98%), identical in all respects with the product obtained from the fermentation.

Hydrolysis of FM-D

A solution of 332 mg of FM-D in a 0.1 N barium hydroxide solution was heated at 100°C for 3 hours. After cooling and neutralization with several pieces of dry ice, the resulting precipitate was removed by filtration. The filtrate was concentrated to a small volume under reduced pressure, and then applied to a column of Amberlite CG-50 (NH_4^+) resin (20 ml). After it had been washed with 100 ml of water, the column was eluted successively with 10 ml each of 0.1 N, 0.2 N, 0.3 N, 0.4 N and 0.5 N ammonium hydroxide. The first ninhydrin-positive fractions were combined and concentrated. Freeze-drying of the residue gave 28 mg of a rearrangement product (**6**).

The second ninhydrin-positive fractions were combined, evaporated *in vacuo*, and then dissolved in small volume of water. Freeze-drying of the solution gave 257 mg of amorphous FM-KE identical in all respects with the product obtained from the fermentation.

The washing(100 ml) was concentrated, then applied to a column of Dowex 1×4(OH^-) resin(10 ml). After washing the column with water, elution with 0.5 N hydrochloric acid gave ninhydrin-positive fractions, which were combined and concentrated to dryness to leave 66 mg of glycine hydrochloride as an amorphous powder. Recrystallization from ethanol afforded an analytical sample, mp 184~186°C. IR (KBr): 3420, 3000, 1740, 1610, 1490, 1420, 1250, 1035, 905, and 860 cm^{-1}.

Anal. (%), Found:	C 21.52, H 5.66, N 12.56.
Calcd for $C_2H_5NO_2 \cdot HCl$:	C 21.33, H 5.33, N 12.44.

Tetra-N-acetylfortimicin KE (**7**)

To a solution of 230 mg of FM-KE in 2.2 ml of anhydrous methanol was added 0.7 ml of acetic anhydride with ice-cooling. After it had been stirred for 5 hours at room temperature, 50 ml of dry ether was added dropwise to the reaction mixture with vigorous stirring. The resulting precipitate was collected by filtration, washed with dry ether and then dried *in vacuo* at 50°C to yield 307 mg of tetra-N-acetyl FM-KE (**7**), mp 140~145°C, $[\alpha]_D^{25}$ +125° (*c* 0.5, H_2O). IR (KBr): 3300, 2950, 1620, 1540, 1370, 1100, and 1030 cm^{-1}. MS (*m/e*) 502 (M^+), 484, 384, 319, 315, 301, 291, 273, 255, and 213.

Anal. (%), Found: C 49.83, H 7.82, N 10.36.
Calcd for $C_{22}H_{38}N_4O_9 \cdot \frac{3}{2}H_2O$: C 49.90, H 7.80, N 10.58.

Mercaptolysis of tetra-N-acetylfortimicin KE

A mixture of 200 mg of tetra-N-acetyl FM-KE, 0.4 ml of ethanethiol, and 0.4 ml of concentrated hydrochloric acid was stirred for 18 hours at room temperature. The excess thiol was removed by evaporation *in vacuo* to give an aqueous residue, which was diluted with 20 ml of water and neutralized by addition of excess lead carbonate. After removal of the resulting precipitate by filtration, the mother liquor was concentrated, then freeze-dried to afford a colorless solid. This material was extracted with 5 ml of hot methanol. The methanol solution was treated with 1 ml of acetic anhydride for 18 hours at room temperature, then concentrated *in vacuo*. Addition of 30 ml of chloroform to the residue(acetic anhydride solution) resulted in formation of a precipitate, which was removed by filtration. The mother liquor was concentrated and purified by silica gel chromatography with a mixture of chloroform - methanol (30: 1) to give 47 mg of N,N'-diacetylpurpurosamine C diethyl dithioacetal (**8**) as colorless solid. A pure sample was obtained by recrystallization from benzene, mp 110~113°C (lit.[8] 109~112°C); ^{1}H NMR (C_5D_5N, TMS) 1.21 and 1.25 (each 3H, t, J=7), 1.78~2.22 (3H, m), 2.10 and 2.18 (each 3H, s), 2.68 and 2.69 (each 2H, q, J=7), 2.60~2.90 (1H, m), 3.65 (2H, m), 4.02 (1H, m), 4.45 (1H, d, J=5), 4.70 (1H, m), 6.38 (1H, br s), 8.56 and 8.65 (1H, d, J=8). MS (*m/e*) 336 (M^+), 307, 277, 216, 201, 142, and 100.

Anal. (%), Found: C 49.83, H 8.65, N 8.31.
Calcd for $C_{14}H_{28}N_2S_2O_3$: C 49.96, H 8.39, N 8.32.

The chloroform-insoluble precipitate was chromatographed on a column of silica gel with the lower layer of a mixture of chloroform - methanol - concentrated ammonium hydroxide (2: 1: 1). After elution of a mixture of by-products, 25 mg of 1-N-acetylfortamine was eluted. Crystallization from a mixture of ether and methanol afforded a pure sample, mp 234~237°C (dec.), $[\alpha]_D^{25}$ −66.1° (*c* 0.5, MeOH). ^{1}H NMR (CD_3OD, TMS) 2.03 and 2.43 (each 3H, s), 3.03 (1H, t, J=4), 3.48 (3H, s) overlapping on 3.48 (1H, dd, J=4.9), and 4.00 (1H, t, J=9). MS (*m/e*) 248 (M^+), 217, 186, 156, and 102. IR (KBr): 3400, 3300, 1650, 1570, 1350, 1080, 1040, 740, and 650 cm^{-1}.

Anal. (%), Found: C 48.73, H 8.42, N 10.93.
Calcd for $C_{10}H_{20}N_2O_5$: C 48.38, H 8.12, N 11.28.

Tetra-N-acetylfortimicin B

Tetra-N-acetyl FM-B(340 mg) was derived from 253 mg of FM-B by means of the procedure described above: mp 155~160°C, $[\alpha]_D^{25}$ +90.6° (*c* 0.5, H_2O); MS (*m/e*) 516 (M^+), 498, 398, 315, 301, 291, 273, 255, and 227. IR(KBr) 3300, 2900, 1620, 1530, 1370, 1100, and 1020 cm^{-1}.

Anal. (%), Found: C 51.67, H 7.92, N 10.48.
Calcd for $C_{23}H_{40}N_4O_9 \cdot H_2O$: C 51.77, H 8.14, N 10.38.

1-N-Acetylfortamine

By the procedure described above, 33 mg of 1-N-acetylfortamine was obtained from 200 mg of tetra-N-acetyl FM-B. Recrystallization from a mixture of ether and methanol gave the pure sample, mp 234~237°C (dec), $[\alpha]_D^{25}$ −64.8° (*c* 0.5, MeOH).

Anal. (%), Found: C 48.07, H 8.48, N 11.09.

Acknowledgement

The authors wish to express their gratitude to Dr. Y. ITAGAKI of JEOL for measuring the field desorption mass spectrum. Thanks are also due to Dr. JAMES B. MCALPINE of Abbott Laboratories for discussions.

References

1) NARA, T.; M. YAMAMOTO, I. KAWAMOTO, K. TAKAYAMA, R. OKACHI, S. TAKASAWA, T. SATO & S. SATO: Fortimicins A and B, new aminoglycoside antibiotics. I. Producing organism, fermentation and biological properties of fortimicins. J. Antibiotics 30: 533~540, 1977
2) OKACHI, R.; S. TAKASAWA, T. SATO, S. SATO, M. YAMAMOTO, I. KAWAMOTO & T. NARA: Fortimicins A

and B, new aminoglycoside antibiotics. II. Isolation, physico-chemical and chromatographic properties. J. Antibiotics 30: 541~551, 1977

3) EGAN, R. S.; R. S. STANASZEK, M. CIROVIC, S. L. MUELLER, J. TADANIER, J. R. MARTIN, P. COLLUM, A. W. GOLDSTEIN, R. L. DE VAULT, A. C. SINCLAIR, E. E. FAGER & L. A. MITSCHER: Fortimicins A and B, new aminoglycoside antibiotics. III. Structural identification. J. Antibiotics 30: 552~563, 1977
4) HIRAYAMA, N.; K. SHIRAHATA, Y. OHASHI, Y. SASADA & J. R. MARTIN: Structure of fortimicin B. Acta Cryst. B34: 2648~2650, 1978
5) SUGIMOTO, M.; S. ISHII, R. OKACHI & T. NARA: Fortimicins C, D, and KE, new aminoglycoside antibiotics. J. Antibiotics 32: 868~873, 1979
6) COOPER, D. J.; P. J. L. DANIELS, M. D. YUDIS, H. M. MARIGLIANO, R. D. GUTHRIE & S. T. K. BUKHARI: The gentamicin antibiotics. III. The gross structures of the gentamicin C components. J. Chem. Soc. (C) 1971: 3126~3129, 1971
7) SHIRAHATA, K.; I. IIDA, M. SATO & K. MOCHIDA: On the formation of 2'-N-acylfortimicins by acyl migrations of 4-N-acylfortimicins. Carbohydr. Res. 1980, in press.
8) COOPER, D. J.; M. D. YUDIS, H. M. MARIGLIANO & T. TRAUBEL: The gentamicin antibiotics. II. Separation and degradation of the gentamicin C components. The purpurosamines, a new class of naturally occurring 2,6-diaminomonosaccharides. J. Chem. Soc.(C) 1971: 2876~2879, 1971