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6'-*epi*-FORTIMICINS

OBSERVATIONS RELEVANT TO THE MECHANISM OF THE REDUCTIVE AMINATIONS OF KETONES WITH SODIUM CYANOBOROHYDRIDE AND AMMONIUM ACETATE†

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Abstract—Fortimicin B (2) has been converted to 6'-*epi*-fortimicin B (5) and 6'-*epi*-fortimicin A (4) both of which have the same diaminosugar moiety as that present in gentamicin C_2 (3). The syntheses of the 6'-*epi*-fortimicins and their antibacterial activities are reported.

Although reductive amination of 1,2'-di-N-benzyloxycarbonyl-6'-oxo-fortimicin B-4,5-carbamate (17) with sodium cyanoborohydride and ammonium acetate in methanol gave a mixture of 1,2'-di-N-benzyloxycarbonyl-6'-*epi*-fortimicin B-4,5-carbamate (18) and 1,2'-di-N-benzyloxycarbonylfortimicin B-4,5-carbamate (14), attempted reduction of 1,2'-di-N-benzyloxycarbonyl-6'-iminofortimicin B-4,5-carbamate (16), under identical conditions gave only recovered imine. The significance of these results relative to the mechanism of the reductive amination of carbonyl compounds with sodium cyanoborohydride and amine salts is discussed.

DISCUSSION

The determination of the structures of fortimicin A (1) and fortimicin B (2)[1] established that the diaminosugar moiety, present in both, differed from that present in gentamicin C_2[2] (3) only in the configuration of the secondary carbon ($C_{6'}$) of the aminoethyl side chain. In the context of a program of chemical modification of the fortimicin antibiotics, carried out with the object of preparing fortimicin derivatives with improved therapeutic properties, we have carried out the syntheses, from fortimicin B, of the 6'-*epi*-fortimicins A and B (4 and 5).

Our general plan for the preparation of the 6'-*epi*-fortimicins involved preparation of a 6'-oxofortimicin 9

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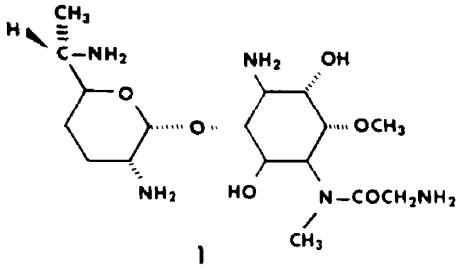

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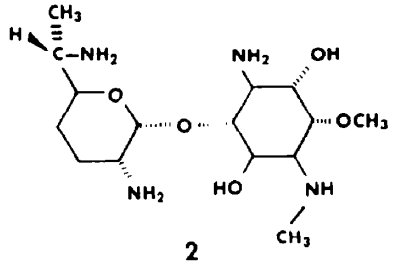

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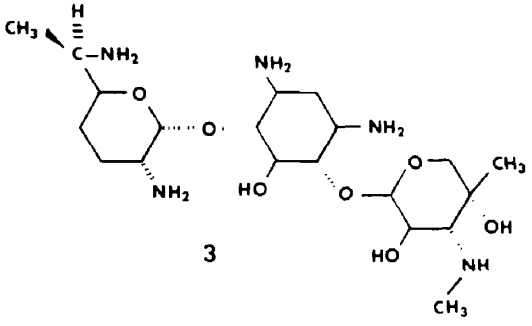

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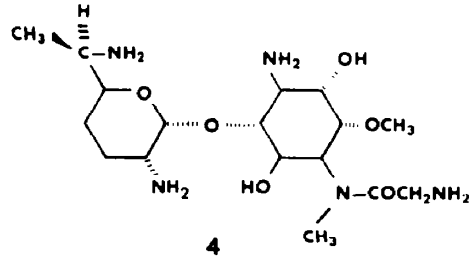

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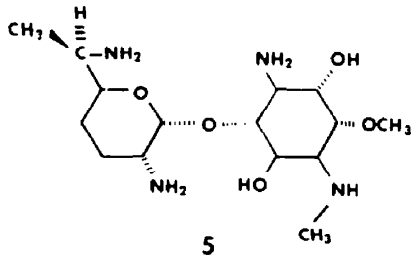

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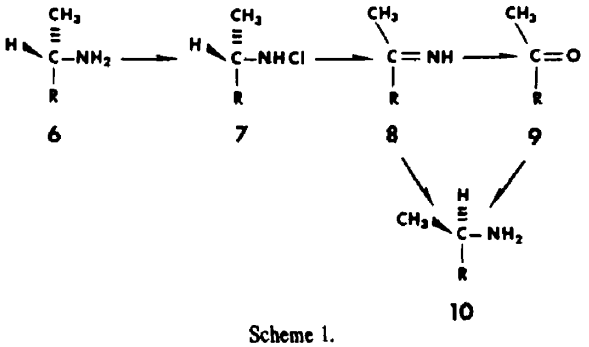


Scheme 1.

by a Ruschig degradation[3] (Scheme 1). I t was hoped that suitable conditions could be found to effect reductive amination of **9** with the desired stereochemistry to form the 6'-*epi* derivative **10**. Our first objective involved conversion of fortimicin B (**2**) to a derivative with a free 6'-amino group, and with the amino groups at C_1, C_4 and $C_{2'}$ protected by acyl groups. This was accomplished by first converting fortimicin B to 1,2' - di - N - benzyloxycarbonylfortimicin B (**11**).[4] Treatment of the latter with excess N - (2,2,2 - trichloroethoxycarbonyloxy)phthalimide gave the 4,6' - di - N -(2,2,2 - trichloroethoxycarbonyl) derivative **12** which was detected by thin layer chromatography. The latter was cyclized without purification to 1,2' - di - benzyloxycarbonyl - 6' - N - (2,2,2 - trichloroethoxycarbonyl) fortimicin B-4,5-carbamate (**13**) on treatment with sodium bicarbonate in refluxing, aqueous methanol. Cleavage of the 2,2,2 - trichloroethoxycarbonyl group of **13** with zinc in glacial acetic acid gave 1,2' - di - N - benzyloxycarbonylfortimicin B-4,5- carbamate (**14**). The latter **14** was converted to the 6'-N-chloroamine **15** with N-chlorosuccinimide in methylene chloride. Dehydrohalogenation of **15** with triethylenediamine in dry ethanol gave the 6'-imine **16**. Mild acid-catalyzed hydrolysis of the latter **16** gave the 6'-oxo derivative **17**. Although the intermediates, with the exception of the 4,6' - di - N - (2,2,2 - trichloroethoxycarbonyl) derivative and the labile 6'-N-chloro derivative, were purified by chromatography for analysis, the imine **16** and the ketone **17** of adequate purity for further reactions, were prepared from 1,2' - di - N - benzyloxycarbonylfortimicin B (**11**) without purification at any step.

The imine **16** and the 6'-oxo derivative **17** were characterized by signals in their CMR spectra at 180.7 and 207.9 ppm, respectively, due to their C_6-carbon atoms, and by singlets in their PMR spectra at δ2.05 and δ2.06, respectively, due to their C_6-Me protons.

Reductive amination of the 6'-oxo derivative **17** by the method of Borch *et al.*[5] gave the 6'-epi and 6'-normal amines **18** and **14** in isolated yields of 18% and 12% respectively, based on 1,2' - di - N - benzyloxycarbonylfortimicin B (**11**). In contrast, attempted reduction of the 6-imine **16** under the same conditions gave recovered imine **16**.

To ensure that the failure to effect reduction of the imine **16** was not due to a difference in the pH of the medium from that of the successful reductive amination of the ketone **17**[6] which was carried out under the same conditions, the following experiments were carried out. The pure ketone **17**, the pure imine **16**, and a 1:1 mixture

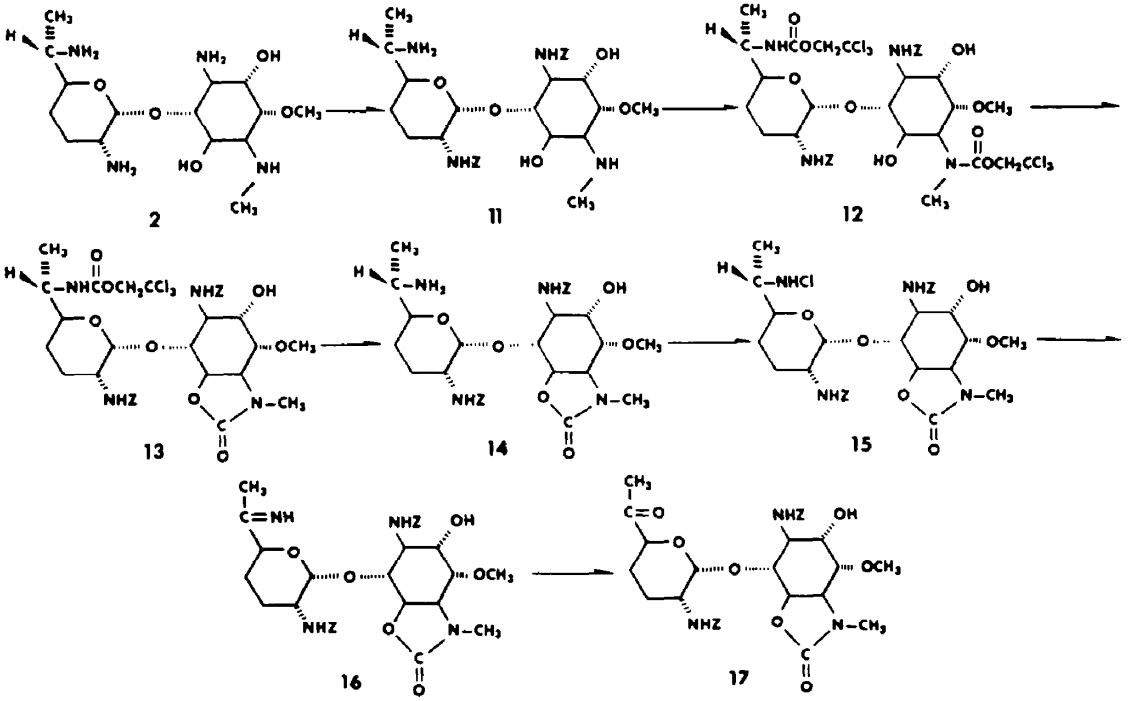


Table 1. Treatment of 1,2' - di - N - benzyloxycarbonyl - 6' - oxo - fortimicin B - 4,5 - carbamate (17) and 1,2' - di - N - benzyloxycarbonyl - 6' - iminofortimicin B - 4,5 - carbamate (16) and a mixture of 16 and 17 with sodium cyanoborohydride and ammonium acetate in methanol

	Ketone (g) 17	Imine (g) 16	$NaBH_3CN$ (g)	NH_4OAc (g)	CH_3OH (ml)	Initial[2] pH	Final[2] pH	% Recovery of Imine 16	Total % Yield Amines 14 & 18[5]
Run 1[1]	0.400	-----	0.060	0.605	7.7	8.0	7.9 (17 hr)	--------	46[6]
Run 2	-----	0.400	0.060	0.605	7.7	7.7	7.9 (17 hr)	93[3]	-------
Run 3[1]	0.400	0.400	0.120	0.121	15.5	7.9	8.0 (19 hr)	95[4]	70[7]

1. TLC assay showed that reductive amination of the ketone 17 was complete within one hour.

2. Apparent pH's determined with a Miler-Markson, Model 80, Digital Mini pH Meter.

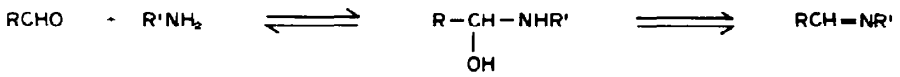
3. Total product isolated by extraction, PMR, IR and TLC identical with those of starting material.

4. Isolated by column chromatography as described in the experimental section. Yield based on starting imine 16.

5. The discrepency in the yields is believed to be due to loss on the chromatography column. The higher yield was obtained by elution with a more polar solvent system. See footnotes 6 and 7.

6. Eluted with a solvent system composed of methylene chloride-ethanol-methanol-concentrated ammonium hydroxide (18:1:1:0.1). Yield based on starting ketone 17.

7. Eluted with methylene chloride-methanol-concentrated ammonium hydroxide (9:4:0.4). Yield based on starting ketone 17.



Scheme 2.

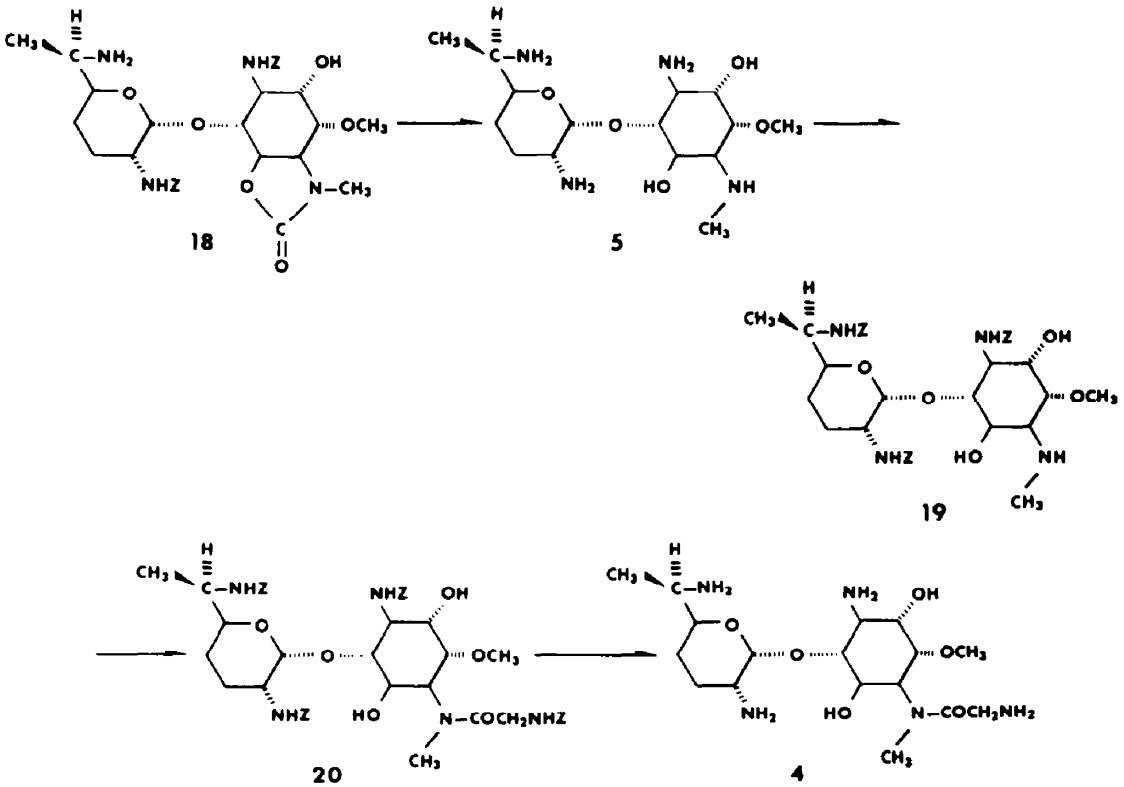
of ketone and imine were treated in parallel with sodium cyanoborohydride and ammonium acetate in methanol with identical ratios and concentrations of total fortimicin derivatives and reagents (Table 1). The initial apparent pH's of the solutions were between 7.7 and 8.0. The final apparent pHs after 17–19 hr were between 7.9 and 8.0. Tlc determinations established that reductive amination of the ketone **17** was complete within 1 hr for both the reaction of the pure ketone and the reaction carried out with the 1:1 mixture of ketone and imine. Products were isolated after 17–19 hr. As reported above the imine was recovered in 93% yield from the reaction with the pure imine. In addition, the imine **16** was recovered in 95% yield based on starting imine by chromatography of the product mixture obtained from the reaction of the 1:1 mixture of ketone and imine.

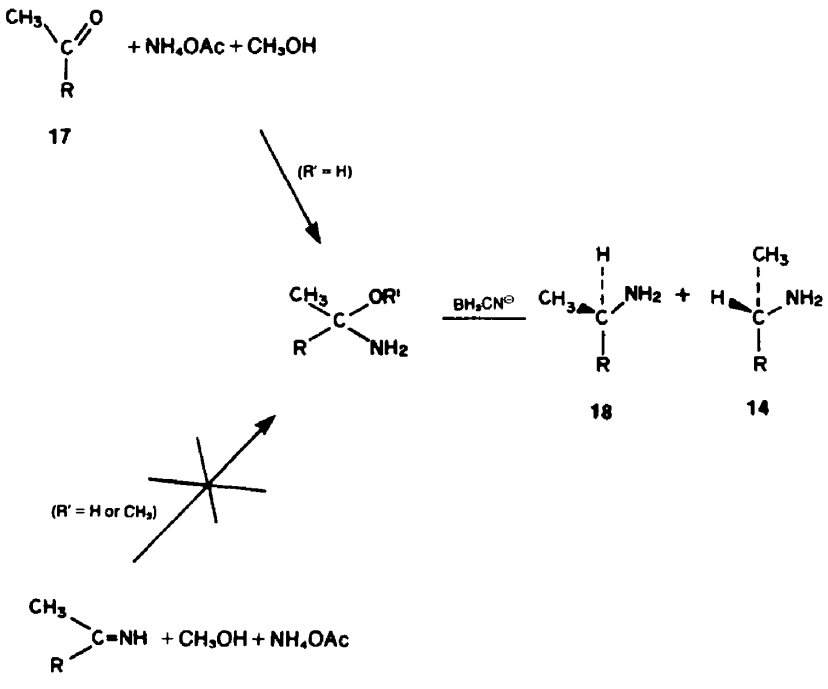
The recovery of the imine from conditions which effect reductive amination of the ketone proves that, contrary to the proposed mechanism[5] of reductive amination of ketones with sodium cyanoborohydride and ammonium acetate in methanol, the imine **16** is not an intermediate in the reductive amination of the ketone **17**. The proposal[5] that imines were the intermediates reduced in reductive aminations of aldehydes and ketones with sodium cyanoborohydride and amines was based on the fact that the reactions of such carbonyl compounds with amines give rise to imines. It is known, however, that the imines are formed via geminal hydroxyamines[7] (Scheme 2). Since in the present case the results exclude the imine **16** as the intermediate in the reductive amination of the ketone **17**, we propose that the intermediate involved is the geminal hydroxyamine (Scheme 3). Failure of the imine **16** to be reduced under the reductive amination conditions may then be interpreted as a consequence of its failure to undergo conversion to the geminal hydroxyamine or the corresponding geminal methoxyamine (Scheme 3). We thus propose that reductive aminations of aldehydes and ketones with amines and sodium cyanoborohydride may occur, in general, via geminal hydroxyamines rather than imines.

Consistent with this mechanism is the report by Kapnang *et al.*[8] that bis (methoxymethyl) amines which were prepared and isolated, were quantitatively reduced by sodium cyanoborohydride in methanol to the corresponding dimethylamines. For precedence these authors pointed out the ease of cleavage by nucleophiles of the C–O bonds of carbinolamine ethers.

More favorable stereoselectivity in formation of the desired 6'-*epi* derivative **18** was achieved by direct reduction of the 6'-imine **16** with sodium borohydride which gave the 6'-*epi* amine **18** in 41% yield and the 6'-normal amine **14** in 3% yield based on 1,2' - di - N - benzyloxycarbonylfortimicin B (**11**).

Hydrolysis of 1,2' - di - N - benzyloxycarbonyl - 6' - *epi* - fortimicin B-4,5-carbamate (**18**) with potassium hydroxide gave 6'-*epi*-fortimicin B (**5**). The latter **5** was converted to 6'-*epi*-fortimicin A (**4**) by the procedure previously employed for conversion of fortimicin B (**2**) to fortimicin A (**1**).[9] 6'-*epi*-Fortimicin B (**5**) was converted to 1,2',6' - tri - N - benzyloxycarbonyl - 6' - *epi* -





Scheme 3.

fortimicin B (**19**) with N - (benzyloxycarbonyloxy)succinimide. Acylation of **19** with N - (N - benzyloxycarbonylglycyloxy)succinimide gave 1,2',6',2'' - tetra - N - benzyloxycarbonyl - 6' - *epi* - fortimicin A (**20**). Catalytic hydrogenolysis of the benzyloxycarbonyl groups of **20** with Pd–C in 0.2 N hydrochloric acid gave 6'-*epi*-fortimicin A (**4**) as the tetrahydrochloride salt. The latter was converted to the disulfate salt with AG1-X2 (SO_4^{2-}-form) anion exchange resin.

The CMR parameters of 6'-*epi*-fortimicin B (**5**), fortimicin B (**2**)[1] and gentamicin C_2 (**3**)[10] are listed in Table 2. CMR spectra were recorded at various pH values in order to determine the pertinent chemical shift changes due to protonation of the amino groups. The data were plotted[11] to correlate chemical shifts at highest and lowest pH values. Comparison of the spectra of **5**, **2** and **3** was made at the lowest pH to minimize variations in chemical shifts which might be caused by incomplete protonation at higher pH. Comparison of the spectrum of 6' - *epi*-fortimicin B with fortimicin B showed good agreement of the chemical shifts of the aminocyclitol carbons. The chemical shifts of the $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$ and $C_{7'}$ carbons of the diaminosugar of 6'-*epi*-fortimicin B were all in excellent agreement with those of gentamicin C_2. In contrast the chemical shifts of the $C_{2'}$, $C_{4'}$ and $C_{7'}$ carbons of 6'-*epi*-fortimicin B differed significantly from those of fortimicin B. That the chemical shift of the anomeric carbon of 6'-*epi*-fortimicin B was in slightly better agreement with that of fortimicin B than with that of the anomeric carbon of the diaminosugar of gentamicin C_2 may reflect the fact that the fortimicins have a common aminocyclitol moiety. This is

Table 2. CMR parameters[1]

	Fortimicin B (2)			6'-epi-Fortimicin B (5)			Gentamicin C (3)			
C	pD 1.8	pD 10.3	δ-Shift	pD 1.2	pD 10.2	δ-Shift	pD 2.9	pD 10.1	δ-Shift[2]	Free base[3]
1	53.5	53.8		53.5	53.9					
2	65.5	71.2	5.7	65.4	71.2	5.8				
3	74.1	79.9	5.8	74.7	79.9	5.2				
4	58.1	60.8		57.9	60.8					
5	66.6	71.2	4.6	66.5	71.2	4.7				
6	74.2	84.1	9.9	74.1	84.1	9.0				
1'	96.0	102.5	6.5	96.7	102.7	6.0	95.5	101.1	5.6	102.6
2'	51.9	50.6		50.4	50.5		50.4	50.7		51.0
3'	21.5	27.0	5.5	21.8	27.0	5.2	21.9	27.0	5.1	27.1
4'	26.3	27.3		23.9	25.9		23.9	26.0		26.2
5'	71.0	75.1	4.1	69.7	74.4	4.7	69.6	74.4	4.8	74.5
6'	49.4	50.4		49.4	50.0		49.6	50.2		50.3
7'	15.1	18.5	3.4	13.2	18.6	5.4	13.1	19.0	5.9	19.2
NCH_3	32.3	35.4		32.2	35.4					
OCH_3	56.1	59.2		57.5	59.2					

[1] 25.2 MHz in D_2O. pD values are uncorrected pH measurements on D_2O solutions.
[2] Determined from values obtained in these laboratories.
[3] Reference 8.

Table 3. *In vitro* antimicrobial activities*

Organism	6'-epi-Fortimicin A (4)	Fortimicin A (1)
Staph. aureus Smith	1.56	0.78
Strep. faecalis 10541	100	50
Enterobacter aerogenes	12.5	3.1
E. coli Juhl	12.5	6.2
E. coli BL-3676	50	25
E. coli 76-2	6.2	3.1
Kleb. pneumoniae 10031	12.5	1.56
Kleb. pneumoniae KY-4262	50	3.1
Providencia 1577	3.1	1.56
Pseudo. aeruginosa BMH #10	3.1	0.78
Pseudo. aeruginosa KY 8512	25	12.5
Pseudo. aeruginosa KY 8516	25	6.2
Pseudo. aeruginosa 209	>100	>100
Pseudo. aeruginosa 27853	50	≤5
Sal. typhimurium Ed. #9	6.2	3.1
Serratia marcescens #003	6.2	1.56
Shigella sonnei 9290	12.5	6.2
Proteus vulgaris JJ	6.2	3.1
Proteus mirabilis Fin #9	6.2	6.2

* The in vitro antibacterial activities were determined by the serial, two-fold dilution method using Mueller-Hinton agar. The antibiotics were assayed as their disulfate salts. Activities are expressed as micrograms of free base per milliliter.

consistent with the observation[11b] that the chemical shifts of the anomeric carbons of glycosides are sensitive to the nature of the alkyl group bonded to the bridging glycosidic oxygen. It has been suggested[11b] that the chemical shifts of anomeric carbons are affected by rotamer populations about the C–O–C glycosidic bonds.

The *in vitro* antimicrobial activities of 6'-*epi*-fortimicin A (4) and fortimicin A (1) against sixteen organisms are listed in Table 3. The overall activity of 6'-*epi*-fortimicin A was less than one half that of fortimicin A.

EXPERIMENTAL

General methods. The general methods are described in a related paper.[4]

N-(2,2,2-*Trichloroethoxycarbonyloxy*)*phthalimide*

A stirring, ice bath-cooled soln prepared from 65.2 g of N-hydroxyphthalimide, 60.8 g Et_3N and 400 ml N,N-dimethylacetamide was treated dropwise with 93.2 g 2,2,2-trichloroethoxycarbonyl chloride. After addition was complete, stirring was continued in the cold for 1 hr and then at room temp for 16 hr. The mixture was poured into 2.5 l ice water and allowed to stand for 1 hr. The crystalline mass which separated was collected on a filter and washed with water. The solid was dissolved in $CHCl_3$ and washed with 5% $NaHCO_3$ aq and dried ($MgSO_4$). Evaporation of the $CHCl_3$ gave an oil which crystallized on trituration with hexane to give 105.4 g N - (2,2,2 - trichloroethoxycarbonyloxy)phthalimide: m.p. 102–103.5°; IR ($CDCl_3$) 1815, 1789 and 1745 cm^{-1}; PMR ($CDCl_3$) δ4.93 (s, CH_2), 7.87 (m, aromatic). (Found: C, 38.92; H, 1.80; N, 4.08; Cl, 31.99. Calc. for $C_{11}H_6NO_5Cl_3$: C, 39.02; H, 1.79; N, 4.14; Cl, 31.42%).

1,2' - *Di* - N - *benzyloxycarbonyl* - 6' - N - (2,2,2 - *trichloroethoxycarbonyl*) - *fortimicin* B-4,5-*carbamate* (13)

A soln prepared from 6.2 g of 11,[4] 10.2 g N - (2,2,2 - trichloroethoxycarbonyloxy)phthalimide and 120 ml $CHCl_3$ was stirred at room temp for 18 hr. The soln was shaken with a mixture of 5% $NaHCO_3$ aq and $CHCl_3$. The $CHCl_3$ layer was separated and evaporated to dryness to give 12 as a bright yellow oil. The oil was heated under reflux for 1.5 hr in a soln prepared from 100 ml MeOH, 20 ml water and 8.5 g $NaHCO_3$. Tlc examination of the soln showed the conversion of 12 to a new, slower moving product which was isolated by $CHCl_3$ extraction after dilution of the mixture with 5% $NaHCO_3$ aq. Evaporation of the $CHCl_3$ gave 10.3 g crude 13 as a glassy residue.

For an analytical sample, 12.0 g product prepared as described above was chromatographed on a column of 400 g silica gel prepared and eluted with a solvent system composed of 1,2-dichloroethane–methanol–conc. ammonium hydroxide (17.2:2.8:0.1) to yield 8.4 g pure 13: $[\alpha]_D^{25}+0.2°$ (*c* 1.0, MeOH): IR ($CDCl_3$) 3457, 3435, 3332, 1760 and 1717 cm^{-1}; PMR ($CDCl_3$) δ0.99 (d, C_6–CH_3, $J_{6',7'}$ = 6.6 Hz), 2.84 (s, $\underline{N}CH_3$), 3.46 (s, $\underline{O}CH_3$). (Found: C, 51.40; H, 5.49; N, 6.82; Cl, 12.41. Calc. for $C_{35}H_{43}N_4O_{12}Cl_3$: C, 51.38; H, 5.30; N, 6.85; Cl, 13.00%).

1,2' - *Di* - N - *benzyloxycarbonylfortimicin* B-4,5-*carbamate* (14)

A suspension prepared from 10.3 g crude 13, 150 ml glacial AcOH and 22 g Zn dust was stirred for 18 hr at room temp. The Zn was removed by filtration. The filtrate was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 5% $NaHCO_3$ aq and dried ($MgSO_4$). Evaporation of the $CHCl_3$ gave 7.20 g residue. A 12.0 g sample thus prepared was chromatographed on a column of 400 g silica gel prepared and eluted with a solvent system composed of 1,2-dichloroethane-methanol-conc. ammonium hydroxide (17.2:2.8:0.1) to give 8.35 g of 14: $[\alpha]_D^{25}+33.2°$ (*c* 1.0, MeOH); IR ($CDCl_3$) 3440, 1750 and 1704 cm^{-1}; PMR ($CDCl_3$) δ0.86 (d, C_6–CH_3, $J_{6',7'}$ = 6.2 Hz), 2.83 (s, $\underline{N}CH_3$), 3.43 (s, $\underline{O}CH_3$). (Found: C, 59.41; H, 6.74; N, 8.96. Calc. for $C_{32}H_{42}N_4O_{10}$: C, 59.80; H, 6.59; N, 8.72%).

1,2' - *Di* - N - *benzyloxycarbonyl* - 6' - N - *chlorofortimicin* B - 4,5 - *carbamate* (15)

A soln prepared from 2.56 g of 14, 1.56 g N-chlorosuccinimide and 100 ml CH_2Cl_2 was stirred at room temp for 1 hr. Evaporation of the CH_2Cl_2 gave crude 15: IR ($CDCl_3$) 3553, 3439, 1754 and 1713 cm^{-1}; PMR ($CDCl_3$) δ 0.902 (d, C_6–CH_3, $J_{6',7'}$ = 3.1 Hz, 2.86 (s, $\underline{N}CH_3$), 3.46 (s, $\underline{O}CH_3$).

Singlets at 2.63 and 2.81 were attributed to the presence of succinimide and N-chlorosuccinimide which could be completely removed by passage of a soln of 15 in MeOH through a column of AG2-X8 (OH) resin packed in MeOH giving the purified 15 the PMR spectrum of which was consistent with its structure.

1,2' - *Di* - N - *benzyloxycarbonyl* - 6' - *iminofortimicin* B - 4,5 - *carbamate* (16)

A soln of the crude 15, prepared as described,[12] in 200 ml 1% soln of triethylenediamine in anhyd EtOH, previously dried over 3A molecular sieves, was allowed to stand at room temp for 1.3 hr. The soln was shaken with a mixture of $CHCl_3$ and 5% $NaHCO_3$ aq. The $CHCl_3$ layer was separated and washed with 5% NaCl aq. The aqueous layer was washed with $CHCl_3$. The combined $CHCl_3$ solns were dried ($MgSO_4$) and evaporated to

dryness to give 3.0 g white solid. The solid was chromatographed on a column containing 50 ml anion exchange resin, AG2-X8 (OH^- form), prepared and eluted with MeOH to give 2.54 g crude **16**, free of N-chlorosuccinimide and succinimide. For an analytical sample, the crude **16** was chromatographed on a column of 250 g silica gel prepared and eluted with 1,2-dichloroethane-MeOH (18.5:1.5) to give 1.2 g of **16**: $[\alpha]_D^{23}+14.9°$ (*c* 1.0, MeOH); IR ($CDCl_3$) 3556, 3434, 1758, 1712 and 1616 cm^{-1}; PMR ($CDCl_3$) δ2.05 (s, C_6–CH_3), 2.86 (s, $\underline{N}CH_3$), 3.44 (s, $\underline{O}CH_3$). (Found: C, 57.09; H, 5.89: N, 8.01: Cl, 5.54. Calc. for $C_{32}H_{40}N_4O_{10}\cdot 0.35\,CHCl_3$: C, 56.92; H, 5.95; N, 8.21; Cl, 5.45%).

Later fractions contained mixtures of **16** contamined with small amounts of **17**.

1,2' - *Di* - N - *benzyloxycarbonyl* - 6' - *oxofortimicin B* - 4,5 - *carbamate* (**17**)

A soln prepared from 0.91 g crude **16**, 20 ml 0.4 N HCl and 60 ml THF was allowed to stand at room temp for 18 hr. The soln was shaken with a mixture of $CHCl_3$ and 5% $NaHCO_3$ aq. The $CHCl_3$ portion was separated and washed with water. The aqueous layer was washed with $CHCl_3$ and the combined $CHCl_3$ solns were dried ($MgSO_4$). Evaporation of the $CHCl_3$ gave 0.794 g solid which was chromatographed on a column (1.6 × 71 cm) of silica gel prepared and eluted with a solvent system consisting of 1,2-dichloroethane-MeOH (18.5:1.5). Fractions containing only the major product were taken to dryness to give 0.396 g of **17**: $[\alpha]_D^{23}+8.6°$ (*c* 1.0, MeOH); IR ($CDCl_3$) 3559, 3439, 1760 and 1713 cm^{-1}; PMR ($CDCl_3$) δ2.06 (s, C_6-CH_3), 2.86 (s, $\underline{N}CH_3$), 3.45 (s, $\underline{O}CH_3$). (Found: C, 59.81; H, 6.40; N, 6.62. Calc. for $C_{32}H_{39}N_3O_{11}$: C, 59.89; H, 6.13; N, 6.55%).

1,2 - *Di* - N - *benzyloxycarbonyl* - 6' - epi - *fortimicin B* - 4,5 - *carbamate* (**18**)

(a) A soln prepared from 0.645 g of **17**, 0.085 g sodium cyanoborohydride, 0.945 g ammonium acetate and 12.5 ml MeOH was stirred at room temp for 18 hr. The mixture was shaken with a mixture of $CHCl_3$ and 5% $NaHCO_3$ aq. The $CHCl_3$ portion was separated and washed with water. The aqueous layer was in turn washed with $CHCl_3$. The combined $CHCl_3$ solns were dried ($MgSO_4$) and taken to dryness to give 0.570 g white glass. The glass was chromatographed on a column of 40 g silica gel prepared and eluted with a solvent system consisting of CH_2Cl_2–EtOH–MeOH-conc. NH_4OH (18:1:1:0.1). The first fractions eluted gave 0.122 g of **14**, identical in all respects with a sample of authentic material.

Continued elution gave fractions containing 0.180 g of **18**: $[\alpha]_D^{23}+36.7°$ (*c* 1.0, MeOH); IR ($CDCl_3$) 3437, 1750 and 1702 cm^{-1}; PMR ($CDCl_3$) δ1.02 (d, C_6–CH_3, $J_{6',7'}=5.6$ Hz), 2.82 (s, $\underline{N}CH_3$), 3.44 (s, $\underline{O}CH_3$). (Found: C, 58.36; H, 6.68; N, 8.55. Calc. for $C_{32}H_{42}N_4O_{10}\cdot H_2O$: C, 58.17; H, 6.71; N, 8.48%).

(b) A stirring, ice bath-cooled soln prepared from 5.41 g crude **16**, 120 ml MeOH and 24 ml water was treated portionwise with 5.8 g $NaBH_4$. After addition was complete, stirring was continued in the cold for 2 hr. The mixture was shaken with a mixture of $CHCl_3$ and 5% $NaHCO_3$ aq. The $CHCl_3$ layer was separated and washed with sat NaCl aq. The aqueous solns were washed with $CHCl_3$ and the combined $CHCl_3$ solns were dried ($MgSO_4$) and evaporated to give 4.91 g solid. The solid was chromatographed on a column of 400 g silica gel prepared and eluted with a solvent system composed of CH_2Cl_2–EtOH–MeOH-conc. NH_4OH (18:1:1:0.1). Initial fractions gave 0.276 g of **14** identical in all respects with an authentic sample.

Further elution gave fractions containing 2.73 g of **18** identical with the material obtained by procedure (a) above.

Reductive amination of 1,2' - *di* - N - *benzyloxycarbonyl* - 6' - *oxo* - *fortimicin* B - 4,5 - *carbamate* (**17**) *in the presence of* 1,2' - *di* - N - *benzyloxycarbonyl* - 6' - *imino* - *fortimicin* B - 4,5 - *carbamate* (**16**)

Recovery of the imine **16**. To a stirred soln of 0.400 g of **17**, 0.400 g of **16**, and 1.21 g ammonium acetate in 15.5 ml MeOH was added 0.120 g sodium cyanoborohydride. Stirring was continued at ambient temp for 19 hr. The product (0.756 g) was isolated by $CHCl_3$ extraction and chromatographed on a column of 40 g silica gel packed with a solvent system composed of CH_2Cl_2–MeOH (9:1). Elution with CH_2Cl_2–MeOH (9:1) gave 0.380 g pure **16**. Elution with CH_2Cl_2–MeOH-conc. NH_4OH (9:4:0.4) gave 0.281 g of a mixture of **14** and **18**.

Attempted reduction of 1,2' - *di* - N - *benzyloxycarbonyl* - 6' - *iminofortimicin* B - 4,5 - *carbamate* (**16**) *with sodium cyanoborohydride in methanol in the presence of ammonium acetate*

Recovery of the imine (**16**). To a stirred soln of 0.4525 g of **16**, 0.6857 g ammonium acetate and 8.75 ml MeOH was added 0.0677 g sodium cyanoborohydride. Stirring was continued for 18 hr. $CHCl_3$ extraction gave 0.4230 g of recovered **16**.

6'-epi-*Fortimicin* B (**5**)

A soln prepared from 17.8 g of **18** and 125 ml 6 N KOH and 250 ml EtOH, previously purged with N_2, was heated at 80° overnight under N_2. The mixture was cooled in an ice bath and adjusted to pH 7.0 with 2 N HCl. Evaporation of the solvent left a residue which was repeatedly co-distilled with anhyd EtOH to remove residual water. The residue was repeatedly triturated with MeOH. The MeOH washings were filtered and the filtrate was evaporated to dryness to give 11.9 g solid which was chromatographed on a column of 550 g silica gel. Elution with the lower phase of a solvent system prepared from CH_2Cl_2–MeOH-water-conc. NH_4OH (2:2:1:1) gave 8.1 g pure **5**: $[\alpha]_D^{23}+28.4°$ (*c* 1.0, MeOH); PMR (D_2O, pD 11.02) δ1.54 (d, C_6–CH_3, $J_{6',7'}=7.5$ Hz), 2.86 (s, $\underline{N}CH_3$), 3.94 (s, $\underline{O}CH_3$), 5.55 (d, $H_{1'}$, $J_{1',2'}=3.6$ Hz); mass spectrum, *m/e* 348.2391 (M^+). Calc. for $C_{15}H_{32}N_4O_5$ 348.2373; sugar fragment *m/e* 143.1190. Calc. for $C_7H_{15}N_2O$ 143.1184; aminocyclitol fragment *m/e* 207.1350. Calc. for $C_8H_{19}N_2O_4$ 207.1345.

1,2',6' - *Tri* - N - *benzyloxycarbonyl* - 6' - epi - *fortimicin* B (**19**)

A stirring, ice bath-cooled soln prepared from 8.88 g of **5**, 260 ml MeOH and 130 ml water was treated with 21.0 g N - (benzyloxycarbonyloxy)succinimide. Stirring was continued in the cold for 3 hr and then at room temp for 18 hr. The mixture was added to 11 5% $NaHCO_3$ aq and extracted with $CHCl_3$. The $CHCl_3$ extract was dried ($MgSO_4$) and evaporated to dryness to give 20.7 g residue. The residue was chromatographed on a column of 750 g silica gel prepared and eluted with a mixture of 1,2-dichloroethane-EtOH-conc. NH_4OH (18:2:0.1) to give 13.8 g of **19**: $[\alpha]_D^{23}+24.7°$ (*c* 1.0, MeOH); IR ($CDCl_3$) 3554, 3434, 3334 and 1708 cm^{-1}; PMR ($CDCl_3$) δ1.02 (d, C_6–CH_3, $J_{6',7'}=6.4$ Hz), 2.34 (s, $\underline{N}CH_3$), 3.42 (s, $\underline{O}CH_3$). (Found: C, 62.13; H, 6.77; N, 7.37. Calc. for $C_{39}H_{50}N_4O_{11}$: C, 62.39; H, 6.71; N, 7.46%).

1,2',6',2'' - *Tetra* - N - *benzyloxycarbonyl* - 6' - epi - *fortimicin* B (**20**)

A soln prepared from 12.8 g of **19**, 5.75 g N - (benzyloxycarbonylglycyloxy)succinimide and 450 ml THF was stirred for 24 hr at room temp. An additional 0.85 g N - (benzyloxycarbonylglycyloxy)succinimide was added and stirring was continued for 18 hr. The THF was evaporated and the resulting residue was taken up in $CHCl_3$. The $CHCl_3$ soln was washed with 5% $NaHCO_3$ aq and dried ($MgSO_4$). Evaporation of the $CHCl_3$ gave 17.1 g solid which was chromatographed on a column of 750 g silica gel prepared and eluted with EtOAc. Fractions containing the major component were evaporated to give 11.8 g of **20**: $[\alpha]_D^{23}+74.1°$ (*c* 1.0, MeOH); IR ($CDCl_3$) 3552, 3415, 1713 and 1637 cm^{-1}; PMR ($CDCl_3$) (rotamers) δ1.78 (d, C_6–CH_3, $J_{6',7'}=3.9$ Hz), 2.82, 2.98 ($\underline{N}CH_3$, major, minor respectively), 3.26 (s, $\underline{O}CH_3$). (Found: C, 62.32; H, 6.30; N, 7.34. Calc. for $C_{69}H_{59}N_5O_{14}$: C, 62.48; H, 6.31; N, 7.43%).

6'-epi-*Fortimicin* A (**4**)

A soln prepared from 2.83 g of **20** in 240 ml 0.2 N HCl in MeOH was hydrogenated under 3 atmospheres H_2 for 4 hr in the presence of 2.8 g 5% Pd–C. The catalyst was removed by filtration and the filtrate was evaporated to leave a solid. Excess HCl was removed by repeated codistillation with MeOH to give 1.70 g of **4** isolated as the tetrahydrochloride salt: $[\alpha]_D^{23}+77.7°$ (*c* 1.0, MeOH); IR (KBr) 1646 cm^{-1}; PMR (D_2O, pD 2.39) δ1.78 (d, C_6–CH_3, $J_{6',7'}=7.8$ Hz), 3.60 (s, $\underline{N}CH_3$), 3.96 (s, $\underline{O}CH_3$); mass

spectrum, *m/e* 405.2596 ($M^{+\cdot}$). Calc. for $C_{17}H_{35}N_5O_6$ 405.2587; sugar fragment *m/e* 264.1553. Calc. for $C_{10}H_{22}N_3O_5$ 264.1559; aminocyclitol fragment *m/e* 143.1209. Calc. for $C_7H_{15}N_2O$ 143.1184.

The disulfate salt of **4** was prepared by passing a water soln of 6.61 g 6'-*epi*-fortimicin A tetrahydrochloride through a column (2.5 × 32 cm) of an anion exchange resin, AG1-X2 (SO_4 = form). Fractions containing the antibiotic were taken to dryness to give 7.0 g of **4** as the disulfate salt: $[\alpha]_D^{23}$ +72.4° (*c* 1.0, water); IR (KBr) 1646 cm^{-1}; PMR (D_2O, pD 4.2) δ1.75 (d, C_6–CH_3, $J_{6',7'}$ = 6.9 Hz), 3.58 (s, NCH_3), 3.94 (s, OCH_3); mass spectrum, meas. 405.2596 ($M^{+\cdot}$) Calc. for $C_{17}H_{35}N_5O_6$ 405.2587. (Found: C, 31.67; H, 6.65; N, 11.47. Calc. for $C_{17}H_{39}N_5O_{14}S_2 \cdot 2H_2O$: C, 32.01; H, 6.80; N, 10.98%).

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[12]If the N-chloroamine (**15**) freed of N-chlorosuccinimide and succinimide by passage through an AG2-X8 (OH) column was used a mixture of ketone **17** and imine **15** was obtained in which the ketone predominated.