2-DEOXY-3-DEMETHOXYFORTIMICIN A

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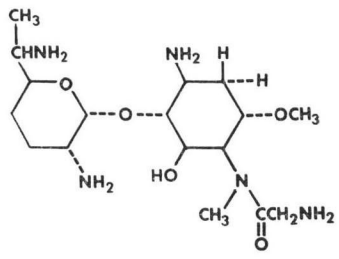
The preparation of 2-deoxy-3-demethoxyfortimicin A (**4**) from 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**5**) is described. The overall *in vitro* antibacterial activity of **4** against a variety of organisms was almost twice that of fortimicin A.

Chemical modifications of fortimicin A at the C_2 and C_3 positions have led to derivatives with good antibacterial activities. Among the most active fortimicin derivatives yet prepared are 2-deoxyfortimicin A (**1**)[1], 2-*epi*-fortimicin A (**2**)[2] and 3-*O*-demethylfortimicin A (**3a**)[3]. The present report concerns the preparation and antibacterial activity of 2-deoxy-3-demethoxyfortimicin A (**4**).

The starting point for the preparation of **4** was 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**5**)[3]. The latter **4** was originally prepared from 3-*O*-demethylfortimicin B (**6**)[3]. More recently an improved method was discovered for the preparation of both 3-*O*-demethylfortimicin B and 3-*O*-demethylfortimicin A by means of boron tribromide 3-*O*-demethylation[4]. In the latter process the isolation of 3-*O*-demethylfortimicin A was facilitated by the preparation of the 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl derivative **3b**. The latter was readily converted to 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B by mild, base-catalyzed hydrolysis.

The conversion of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**5**) to 2-deoxy-3-demethoxyfortimicin A (**4**) was accomplished as follows. Treatment of **5** with salicylaldehyde gave the 4,5-



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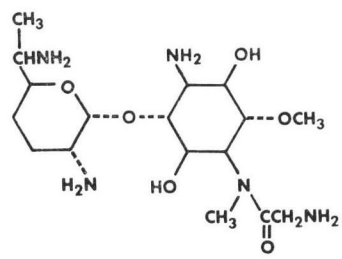

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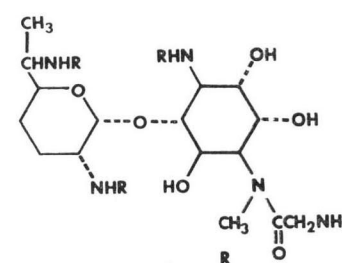
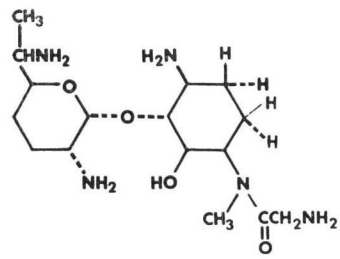


3a R = H
3b R = Z



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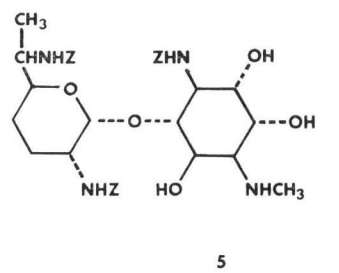

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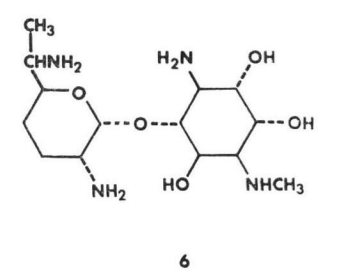

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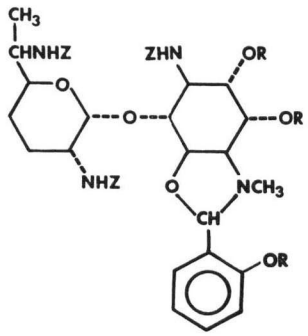
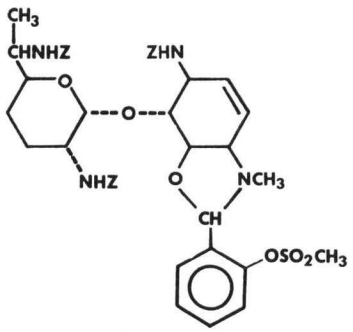


7a R = H
7b R = SO_2CH_3



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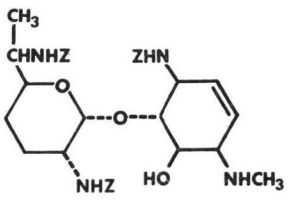

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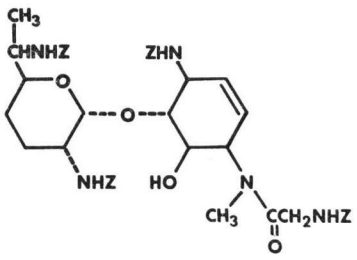

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oxazolidine derivative **7a**. The latter was converted to the trimethanesulfonate **7b** with methanesulfonyl chloride in pyridine. TIPSON-COHEN elimination of the C_2 and C_3 methanesulfonate groups of **7b** gave the olefin **8** which was converted to 1,2′,6′-tri-*N*-benzyloxycarbonyl-2,3-anhydro-3-demethoxyfortimicin B (**9**) by mild, acid-catalyzed hydrolysis. The latter **9** was converted to 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-2,3-anhydro-3-demethoxyfortimicin A (**10**) on treatment with *N*-(*N*-benzyloxycarbonylglycyloxy)-succinimide. Catalytic hydrogenation of **10** reduced the 2,3-double bond and removed the *N*-benzyloxy-

Table 1. *In vitro*, antimicrobial activities, MIC (μg/ml).*

Organism	Fortimicin A	2-Deoxy-3-demethoxy-fortimicin A	Organism	Fortimicin A	2-Deoxy-3-demethoxy-fortimicin A
S. aureus Smith	0.39	0.2	*P. aeruginosa* KY-8512	3.1	1.56
S. faecalis 10541	100	50	*P. aeruginosa* KY-8516	1.56	1.56
E. aerogenes 13048	3.1	1.56	*P. aeruginosa* 209	>100	100
E. coli JUHL	6.2	3.1	*P. aeruginosa* 27853	6.2	3.1
E. coli BL-3676 (Res)	25	12.5	*S. typhimurium* Ed. #9	6.2	1.56
E. coli 76-2	3.1	1.56	*S. marcescens* 4003	3.1	1.56
K. pneumoniae 10031	1.56	0.78	*S. sonnei* 9290	3.1	3.1
K. pneumoniae KY-4262	12.5	3.1	*P. rettgeri* U-6333	6.2	6.2
Providencia 1577	3.1	3.1	*P. vulgaris* JJ	6.2	3.1
P. aeruginosa BMH #10	0.78	0.39	*P. mirabilis* Fin. #9	3.1	1.56

* *In vitro* antibacterial activities were determined by serial, two-fold dilution method using MUELLER-HINTON agar. Compounds were assayed as disulfate salts. Activities are expressed as μg of free base per ml.

carbonyl protecting groups to give 2-deoxy-3-demethoxyfortimicin A (**4**).

The *in vitro* antibacterial activities of **4** and fortimicin A against a variety of organisms are recorded in Table 1. The overall activity of **4** was approximately twice that of fortimicin A, and like 3-*O*-demethylfortimicin A, **4** had enhanced activity against *Pseudomonas* organisms.

Experimental

General

Optical rotations were determined with a Perkin-Elmer Model 521 grating spectrometer. PMR spectra were determined at 100 MHz with a Varian Associates HA-100 spectrometer at ambient temperature. Chemical shifts in $CDCl_3$ are reported relative to internal TMS. Chemical shifts determined in D_2O are reported relative to internal sodium 3-trimethylsilylpropionate-2,2,3,3-d_4. Mass spectra were obtained with an A.E.I. MS-902 spectrometer operated at 70eV and 100~150°C with a direct probe insert. Silica gel for column chromatography was that of Merck (Darmstadt), 70~230 mesh. Ratios for chromatography solvents are expressed by volume. Solutions were dried with magnesium sulfate. Solvents were evaporated under diminished pressure on a rotary evaporator.

1,2′,6′-Tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**5**)

A solution prepared from 20.0 g of 1,2′,6′,2″-tetra-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin A (**3b**), 40 ml of 5% aqueous sodium bicarbonate, and 600 ml of methanol was heated under reflux for 4 hours. The solution was cooled and shaken with a mixture of chloroform and 5% aqueous sodium bicarbonate. The chloroform solution was separated and dried ($MgSO_4$). Evaporation of solvent left 16.0 g of **5** (100% yield) identical with that prepared as described previously[3].

1,2′,6′-Tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-4-*N*,5-*O*-salicylidenefortimicin B (**7a**)

A solution of 16.0 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethylfortimicin B (**5**), 2.9 ml of salicylaldehyde and 320 ml of methanol was heated under reflux for 1 hour. The major portion of the methanol was evaporated under reduced pressure, and residual methanol was removed by co-distillation with chloroform under reduced pressure leaving 19.4 g of **7a** (106% yield): δ ($CDCl_3$) 0.98 (d, 6′-CH_3, $J_{6',7'}$=7.0 Hz), 2.25 (NCH_3).

1,2′,6′-Tri-*N*-benzyloxycarbonyl-2,3-di-*O*-methanesulfonyl-3-*O*-demethyl-4-*N*,5-*O*-(2-*O*-methanesulfonyl)salicylidenefortimicin B (**7b**)

To a magnetically stirred solution of 19.4 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-3-*O*-demethyl-4-*N*,5-*O*-salicylidene fortimicin B (**7a**) in 180 ml of pyridine, cooled in an ice bath, was added 21.3 ml of methanesulfonyl chloride. After the addition was complete, stirring was continued with cooling for 0.5 hour and then at room temperature overnight. The resulting solution was shaken with a mixture of chloroform and 5% aqueous sodium bicarbonate. The chloroform solution was separated and dried. The chloroform was evaporated and residual pyridine was removed by co-distillation with toluene leaving 24.8 g of **7b** (100% yield): ($CDCl_3$) 1.03 (d, 6′-CH_3, $J_{6',7'}$=7.3 Hz); 2.28 (NCH_3); 2.94, 3.09, 3.21 (OSO_2CH_3's).

1,2′,6′-Tri-*N*-benzyloxycarbonyl-2,3-anhydro-3-demethoxy-4-*N*,5-*O*-(2-*O*-methanesulfonyl)salicylidenefortimicin B (**8**)

A magnetically stirred suspension of 2.02 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2,3-di-*O*-methanesulfonyl-3-*O*-demethyl-4-*N*,5-*O*-(2-*O*-methanesulfonyl)salicylidenefortimicin B (**7b**), 21.8 g of sodium iodide, 11.0 g of zinc dust, and 86 ml of dimethylformamide was heated at 100°C overnight. The suspension was cooled to room temperature and diluted with 500 ml of chloroform. The insoluble solid present was removed by filtration. The filtrate was washed with 500 ml of 5% aqueous sodium thiosulfate and 250 ml of 5% aqueous sodium bicarbonate. The resulting chloroform solution was dried and the chloroform was evaporated. Residual dimethylformamide was removed by co-distillation with toluene under reduced pressure leaving 1.49 g of crude **8** which was used as such in the next reaction: δ ($CDCl_3$) 1.01 (d, 6′-CH_3, $J_{6',7'}$=6.5 Hz); 2.15 (NCH_3); 2.98 (OSO_2CH_3).

1,2′,6′-Tri-*N*-benzyloxycarbonyl-2,3-anhydro-3-demethoxyfortimicin B (**9**)

A solution of 1.49 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2,3-anhydro-3-*O*-demethyl-4-*N*,5-*O*-(2-*O*-methanesulfonyl)salicylidenefortimicin B (**8**), 0.75 g of GIRARD's reagent T, 0.70 ml of acetic acid, and 36 ml of methanol was heated under reflux for 3.5 hours. The resulting solution was cooled and shaken with a mixture of chloroform and 5% aqueous sodium bicarbonate. The chloroform solution was separated and dried. Evaporation of the chloroform under reduced pressure left 0.938 g of a light yellow glass.

A sample of 1.25 g of product prepared as described above was chromatographed on a column of 120 g of silica gel packed and eluted with a solvent system composed of dichloromethane - methanol - concentrated ammonium hydroxide (18.5: 1.5: 0.1) to yield 0.512 g of **9** (29% yield based on **7b**): $[\alpha]_D^{28}$ +52° (*c* 1.0, CH_3OH); δ ($CDCl_3$) 1.01 (d, 6′-CH_3, $J_{6',7'}$=6.4 Hz),

Anal. Calcd for $C_{38}H_{46}N_4O_9$: C 64.94, H 6.60, N 7.97.
Found: C 64.91, H 6.66, N 7.95.

1,2′,6′,2″-Tetra-*N*-benzyloxycarbonyl-2,3-anhydro-3-demethoxyfortimicin A (**10**)

A magnetically stirred solution of 0.490 g of 1,2′,6′-tri-*N*-benzyloxycarbonyl-2,3-anhydro-3-demethoxyfortimicin B (**9**), 0.235 g of *N*-(*N*-benzyloxycarbonylglycyloxy)succinimide, and 25 ml of tetrahydrofuran was kept at room temperature overnight. The resulting solution was shaken with a mixture of 200 ml of chloroform and 400 ml of 5% aqueous sodium bicarbonate. The chloroform solution was separated and dried. Evaporation of chloroform left 0.623 g of tan glass. The latter (0.600 g) was chromatographed on a column of 70 g of silica gel packed and eluted with a solvent system composed of ethyl acetate - hexane (4: 1) to yield 0.500 g of **10** (75% yield): $[\alpha]_D^{28}$ +67° (*c* 1.0, CH_3OH), $\tilde{\nu}_{max}$ ($CDCl_3$) 3440, 3330, 1712, 1644, 1605 cm^{-1}; δ ($CDCl_3$) 0.99 (d, 6′-CH_3, $J_{6',7'}$=6.1 Hz), 2.92 (NCH_3).

Anal. Calcd. for $C_{48}H_{55}N_5O_{12}$: C 64.49, H 6.20, N 7.83.
Found: C 64.29, H 6.23, N 7.69.

2-Deoxy-3-demethoxyfortimicin A (**4**)

1,2′,6′,2″-Tetra-*N*-benzyloxycarbonyl-2,3-anhydro-3-demethoxyfortimicin A (0.416 g, **10**) in 19 ml of 0.2 N hydrochloric acid in methanol and 6 ml of methanol was catalytically hydrogenated under 3 atm of hydrogen for 17 hours in the presence of 0.42 g of 5% Pd/C. The methanol was evaporated under reduced pressure and residual hydrochloric acid was removed by codistillation with methanol under reduced pressure to leave 0.272 g of **4** as the tetrahydrochloride. The latter (0.252 g) was converted to the disulfate salt (0.228 g, 89% yield) with AG1-X2 (SO_4^{2-}) resin: $[\alpha]_D^{27}$ +95° (*c* 1.0, H_2O); $\tilde{\nu}_{max}$ (-4HCl) (KBr), 1640 cm^{-1}, δ (D_2O) 1.33 (d, 6′-CH_3, $J_{6',7'}$=6.5 Hz), 3.05 (NCH_3), 5.36 (d, $H_{1'}$, $J_{1',2'}$=3.0 Hz), *m/z* (-4HCl) $M^{+\cdot}$, calculated for $C_{16}H_{34}N_5O_4$ 360.2607, measured 360.2607; diaminosugar, calculated for $C_7H_{15}N_2O$ 143.1184, measured 143.1189; (cyclitol-H_2O) calculated for $C_9H_{16}N_3O_2$ 198.1242, measured 198.1228.

Anal. Calcd. for $C_{16}H_{33}N_5O_4 \cdot 2H_2SO_4 \cdot 3.5\ H_2O$: C 31.06, H 7.17, N 11.32, S 10.36.
Found: C 31.03, H 6.81, N 11.22, S 11.03.

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