SPECTINOMYCIN MODIFICATION. II

7-*EPI*-SPECTINOMYCIN

WILLIAM ROSENBROOK, Jr., RONALD E. CARNEY, RICHARD S. EGAN, RUTH S. STANASZEK, MOMIR CIROVIC, TOSHINARI NISHINAGA,* KENICHI MOCHIDA* and YASUKI MORI*

Abbott Laboratories, Division of Antibiotics and Natural Products, North Chicago, Illinois 60064, U.S.A.
*Tokyo Research Laboratory, Kyowa Hakko Kogyo Co., Ltd., Asahimachi, Machidashi, Tokyo, Japan

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7-*Epi*-spectinomycin (**9**) and 7-*epi*-4(R)-dihydrospectinomycin (**10**) have been prepared and their structures firmly established by proton magnetic resonance. Both of these spectinomycin analogs are devoid of antibiotic activity.

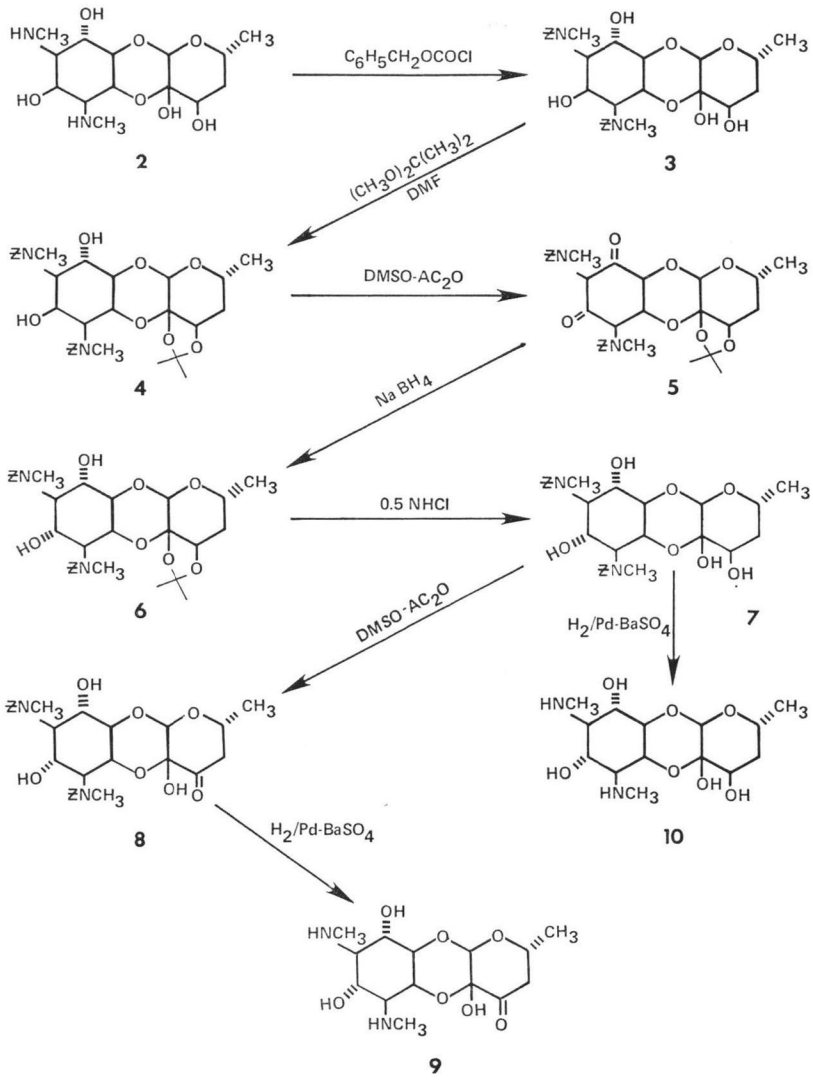
Spectinomycin (**1**), a fused ring aminocyclitol antibiotic, is of particular interest as a substrate for chemical modification because it does not share the ototoxic and nephrotoxic properties of the aminoglycosidic aminocyclitols. Consideration of current structure-activity relationships (SAR) among the aminocyclitol antibiotics suggested that the *axial* hydroxyl group at C-7 in the aminocyclitol moiety of spectinomycin might be responsible for the low activity of this antibiotic. This cyclitol moiety, actinamine, is an analog of 2-*epi*-streptamine, which was shown to cause a reduction of antibiotic activity in the semisynthetic neomycin analogs, hybrimycins B_1 and B_2.[1] Our primary concern with spectinomycin has been the enhancement of antibiotic activity by chemical and stereochemical modifications especially at the 7-position. The preparation and complete lack of antibiotic activity of 7-*epi*-9-deoxy-4(R)-dihydrospectinomycin has already been reported.[2] Because two additional variations are incorporated in this analog, evaluation of the SAR rationale for spectinomycin's low potency was precluded. We wish to report the preparation of 7-*epi*-spectinomycin (**9**) as outlined in Scheme 1.

The amino functions of 4(R)-dihydrospectinomycin[2] (**2**) were protected from oxidation by formation of the N,N′-dicarbobenzoxy derivative (**3**). 4(R)-Dihydrospectinomycin is a convenient starting material, considerably more stable than spectinomycin itself, and is amenable to protection of its *alpha* hydroxy *hemi* ketal functionality *via* the 4,4a-acetonide. Also, the *axial* hydroxyl group of any 4(R)-dihydro-analog was anticipated to undergo ready oxidation back to the 4-oxo or spectinomycin series. Reaction of **3** with 2,2-dimethoxy propane readily afforded N,N′-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**4**).

Oxidation of **4** with dimethylsulfoxide—acetic anhydride gave 7,9-dioxo-N,N′-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**5**). **5** was used in the next step without further purification because of its labile nature. Treatment of the diketone (**5**) with sodium borohydride provided a stereospecific reduction at both sites to give the desired 7-*epi*-N,N′-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**6**). The acetonide block was then removed with dilute hydrochloric acid to give 7-*epi*-N,N′-dicarbobenzoxy-4(R)-dihydrospectinomycin (**7**).

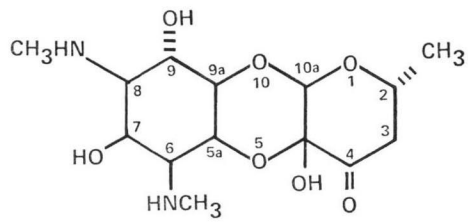
A selective oxidation of the C-4 *axial* hydroxyl group of **7** was accomplished by treatment

Scheme 1



with a dimethylsulfoxide - acetic anhydride mixture for a short period of time to yield the desired 4-oxo-analog, 7-*epi*-N,N′-dicarbobenzoxy spectinomycin (**8**).

Removal of the carbobenzoxy groups from **8** by catalytic hydrogenation was achieved only after exhaustive purification to yield 7-*epi*-spectinomycin (**9**). Partition column chromatography succeeded in the removal of catalyst poisons derived, apparently, from the DMSO oxidation step.



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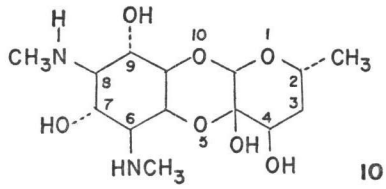
Evidence for the C-7 stereochemistry could not be obtained from the PMR spectra of carbobenzoxy-blocked intermediates **6**, **7** or **8** due to rotamer problems which cause broad and

ill-defined resonances. Extensive chemical shift overlap of the ring protons of **9** also precluded stereochemical confirmation. Removal of the carbobenzoxy groups from **7** by catalytic hydrogenolysis gave 7-*epi*-(4R)-dihydrospectinomycin (**10**) which proved to be suitable for PMR analysis. The 270 MHz PMR spectrum of **10** in dimethylsulfoxide-d_6 solution at 110°C is shown in Fig. 1 and analysis of the spectrum is presented in Table 1. Assignment of ring proton resonances was accomplished by use of spin-decoupling experiments; however, examination of the observed coupling constants reveals that only H-3 eq and H-4 exhibit small couplings. This then requires that all the protons in the spectinamine derived ring are now axial and confirms the C-7 stereochemistry regardless of the specific proton assignments.

Table 1. PMR Parameters of 7-*epi*-(4R)-dihydrospectinomycin (**10**) at 270 MHz.



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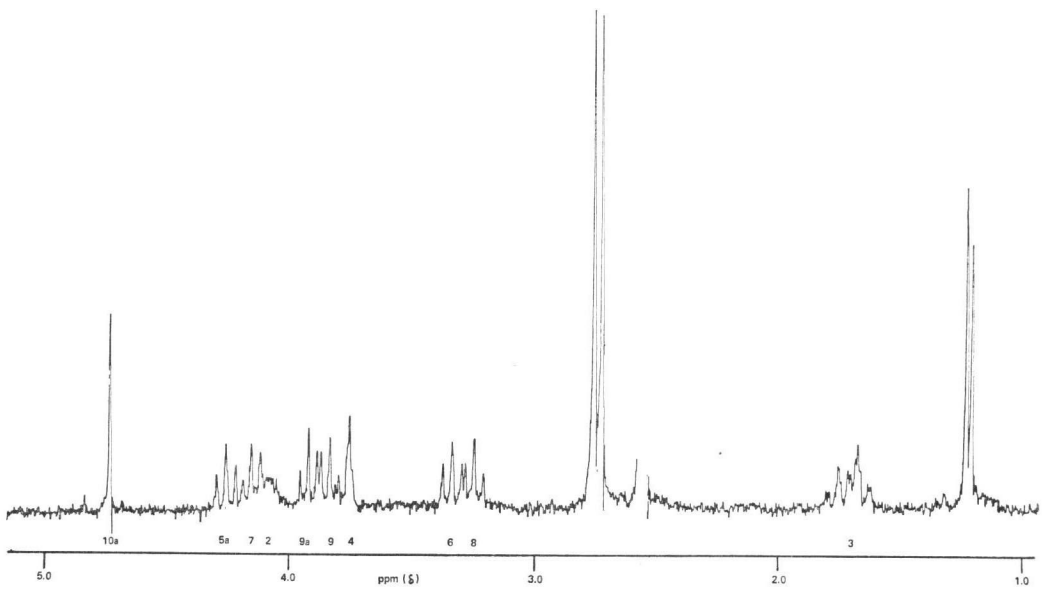
Chemical shifts (ppm)*δ		Coupling constants (Hz)**	
H-2	4.01	$J_{2,3ax}$	12.0
H-3ax	1.69	$J_{2,3eq}$	2.5
H-3eq	1.58	J_{2,CH_3}	6.0
H-4	3.69	$J_{3ax,3eq}$	12.0
H-5a	4.19	$J_{3ax,4}$	2.5
H-6	3.26	$J_{3eq,4}$	2.5
H-7	4.09	$J_{5a,6}$	10.0
H-8	3.17	$J_{6,7}$	10.0
H-9	3.76	$J_{7,8}$	10.0
H-9a	3.85	$J_{8,9}$	10.0
H-10a	4.66	$J_{9,9a}$	10.0
2-CH_3	1.15	$J_{9a,5a}$	10.0
N-CH_3	2.70, 2.66		

* measured from internal TMS
** directly measured from spectra, accuracy ±0.5 Hz

Both 7-*epi*-spectinomycin (**9**) and its 4(R)-dihydro-analog (**10**) are devoid of antibiotic activity as measured by the agar dilution method on pH 8 nutrient agar at 500 μg/ml. This result suggests that the structure-activity relationships derived from the various other aminocyclitol antibiotics are not applicable to spectinomycin.

Fig. 1.



Experimental Section

PMR spectra were measured on a Varian Associates HA-100 spectrometer in deuterated solvents. Chemical shifts are reported in ppm downfield from internal TMS (in D_2O, TMS in external capillary) and coupling constants are reported in Hz. The PMR spectrum of **10** was also determined at 270 MHz on a Bruker HX-270 spectrometer in d_6-dimethylsulfoxide at 110°. Mass spectra were obtained on an A.E.I. MS-902 spectrometer at 70 eV and 100~150°C using the direct insertion probe. IR spectra were determined with *d*-chloroform solutions or KBr pellets using a Perkin-Elmer Model 521 grating spectrometer. Optical rotations were determined with 2 % solutions in water at pH 7 with a Hilger and Watts polarimeter.

Microanalytical results are reported for those products which could be prepared free of solvent and carbonates formed by atmospheric exposure.

N,N′-Dicarbobenzoxy-4(R)-dihydrospectinomycin (**3**)

4(R)-Dihydrospectinomycin dihydrochloride (**2**)[2] (47 g, 0.12 moles, 74 % 4(R) epimer by glc) was dissolved in 500 ml 10 % aqueous sodium bicarbonate solution, cooled to 5~10°C (ice bath) and 300 ml of an acetone solution containing 28.2 ml (0.22 mole) of carbobenzoxy chloride was added slowly with stirring. The reaction mixture was removed from the ice bath and after standing two hours at room temperature the acetone was removed by evaporation *in vacuo* at 45°C (bath). The precipitated product (**3**) was collected by filtration, (51 g). IR 1683 cm^{-1}; PMR ($CDCl_3$) δ 1.19 (d, 2-CH_3); 3.00, 3.04 (S, N-CH_3's); 4.84 (S, H-10a); 5.11 (S, Z-CH_2); 7.30 (S, Z-Arom).

Anal. Calcd. for $C_{30}H_{33}N_2O_{11}$: C, 60.45; H, 6.37; N, 4.66; O, 29.20.
Found: C, 60.07; H, 6.34; N, 4.55; O, 29.10.

N,N′-Dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**4**)

N,N′-Dicarbobenzoxy-dihydrospectinomycin (**3**) (51 g, 0.63 moles *axial* epimer) was dissolved in 195 ml dimethylformamide (A.R.) with 3.4 g *p*-toluenesulfonic acid (dried *in vacuo* at 80°C for 3 hours) was treated with 700 ml 2,2-dimethoxy propane (5.7 mole) and the mixture stirred overnight at room temperature. IRA 400 (OH^-), 100 ml, in anhydrous ethanol was added to remove the sulfonic acid. The resin was removed by filtration, the filtrate taken to a syrup *in vacuo* and pure product (**4**) was isolated by silica gel column chromatography (*n*-hexane-ethyl acetate, 3:7) as a white glass (40 g, 102 % of theory): PMR ($CDCl_3$) δ 1.27 (d, 2-CH_3); 1.44, 1.48 (S, C-CH_3's); 3.07, 3.08 (S, N-CH_3's); 4.67 (S, H-10a); 5.14 (S, Z-CH_2); 7.33 (S, Z-Arom). MS, *m/e* 642 (M^+).

Anal. Calcd. for $C_{33}H_{42}N_2O_{11}$: C, 61.67; H, 6.59; N, 4.36; O, 27.38.
Found: C, 61.37; H, 6.82; N, 4.24; O, 27.11.

7,9-Dioxo-N,N′-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**5**)

N,N′-Dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**4**) (6.43 g, 10 m mole) was dissolved in 60 ml dimethylsulfoxide and 60 ml acetic anhydride. After 16 hours at room temperature, the reaction mixture was poured in 1,500 ml of ice water with vigorous stirring. After decantation the gummy mass was taken up in 400 ml chloroform, washed three times with 500 ml each of water, the chloroform phase dried over anhyd. $MgSO_4$, filtered and evaporated to a 6.2 g residue. Further purification of **5** was not attempted: IR 1760, 1690 cm^{-1}.

7-*Epi*-N,N′-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**6**)

The crude diketone (**5**) (3.2 g, 5 m mole) was dissolved in 100 ml of a 1:1 mixture of chloroform and methanol, cooled in an ice bath to 5~10°C, and treated with 190 mg of sodium borohydride (5 mmole). After 30 minutes the solvent was removed by evaporation *in vacuo* at 45°C (bath) and pure product (**6**) was isolated by silica gel column chromatography ($CHCl_3$-acetone, 3:1) as a white glass (1.64 g, 51 % of theory): IR 3590, 3430, 1695 cm^{-1}; PMR ($CDCl_3$) δ 1.24 (d, 2-CH_3); 1.40, 1.45 (S, C-CH_3's); 2.90, 2.95 (S, N-CH_3's); 4.62 (S, H-10a); 5.15 (S, Z-CH_2); 7.34 (S, Z-Arom). MS, *m/e* 643 (M^++1), 642 (M^+).

Anal. Calcd. for $C_{33}H_{42}N_2O_{11}$: C, 61.67; H, 6.59; N, 4.35; O, 27.38.
Found: C, 61.35; H, 6.76; N, 3.96; O, 26.99.

7-*Epi*-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin (**7**)

7-*Epi*-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**6**) (2.0 g, 3.1 mmole) was hydrolyzed in 100 ml methanol with 100 ml 1 M hydrochloric acid under reflux for 2 hours. The reaction mixture was evaporated *in vacuo* to a residue and pure **7** was isolated by silica gel column chromatography ($CHCl_3$-MeOH, 4:1) as a white glass (0.82 g, 43 % of theory): PMR ($CDCl_3$) δ 1.17 (d, 2-CH_3); 2.90 (S, N-CH_3); 4.82 (S, H-10a); 5.09 (S, Z-CH_2); 7.29 (S, Z-Arom).

Anal. Calcd. for $C_{30}H_{33}N_2O_{11}$: C, 59.79; H, 6.36; N, 4.65; O, 29.21.
Found: C, 59.63; H, 6.75; N, 4.26; O, 29.28.

7-*Epi*-N,N'-dicarbobenzoxy spectinomycin (**8**)

7-*Epi*-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin (**7**) (6.46 g, 10.7 mmole) was dissolved in 65 ml dimethylsulfoxide and 65 ml acetic anhydride and the mixture stirred for $3^1/_2$ hours at room temperature. The reaction mixture was then poured into 1.5 liters of ice water with vigorous stirring. The aqueous phase was then extracted with chloroform and the crude product isolated as a white glass (5.8 g). Purification was obtained by silica gel column chromatography (CH_2Cl_2-MeOH, 9:1) followed by partition column chromatography on acid washed Celite (*iso*octane-ethyl acetate-methanol-water; 4:6:5:5, v/v) to yield 0.88 g of **8** (14 % of theory): IR 1740, 1690 cm^{-1}; PMR ($CDCl_3$) δ 1.36 (d, 2-CH_3); 2.95 (S, N-CH_3); 4.66 (S, H-10a); 5.12 (S, Z-CH_2); 7.31 (S, Z-Arom). MS, m/e 600 (M^+).

Anal. Calcd. for $C_{30}H_{36}N_2O_{11}$: C, 59.99; H, 6.04; N, 4.67; O, 29.30.
Found: C, 59.60; H, 6.11; N, 4.40; O, 29.68.

7-*Epi*-spectinomycin dihydrochloride (**9**)

7-*Epi*-N,N'-dicarbobenzoxy spectinomycin (**8**) (200 mg, 3.2 mmole) was dissolved in 100 ml ethanol with 200 mg of 10 % Pd-$BaSO_4$ and treated with hydrogen at 3 atm. for $5^1/_2$ hours. The reaction mixture was filtered, a drop of conc. HCl added to the filtrate, and the filtrate evaporated to a white glass (134 mg, 103 % of theory): $[\alpha]_D^{23}$ 12°; IR [virtually identical with that of spectinomycin (**1**)]; PMR (D_2O) δ 1.72 (d, 2-CH_3); 3.26, 3.28 (S, N-CH_3); 5.36 (S, H-10a). MS, *m/e* 333 (M^++1), 332 (M^+).

Anal. Calcd. for $C_{14}H_{24}N_2O_7 \cdot 2HCl$: C, 41.49; H, 6.47; N, 6.91.
Found: C, 41.04; H, 6.88; N, 6.49.

7-*Epi*-4(R)-dihydrospectinomycin dihydrochloride (**10**)

7-*Epi*-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin (**7**) (320 mg, 0.5 mmole) was dissolved in 100 ml methanol-water (1:1) with two drops conc. HCl and 300 mg 5 % Pd-C and treated with 3 atm. of hydrogen for six hours. The reaction mixture was filtered and the filtrate evaporated to a white glass (200 mg, 98 % of theory): $[\alpha]_D^{23}$ 19°; IR [virtually identical with that of 4(R)-dihydrospectinomycin (**2**)]; PMR (D_2O) δ 1.67 (d, 2-CH_3); 3.21, 3.23 (S, N-CH_3); 5.36 (S, H-10a). MS, *m/e* 335 (M^++1), 334 (M^+).

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