# SPECTINOMYCIN MODIFICATION. IV

## 7-DEOXY-4(R)-DIHYDROSPECTINOMYCIN

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 Company, Ltd., 3-6-6 Asahimachi, Machidashi, Tokyo, Japan

(Received for publication March 22, 1978)

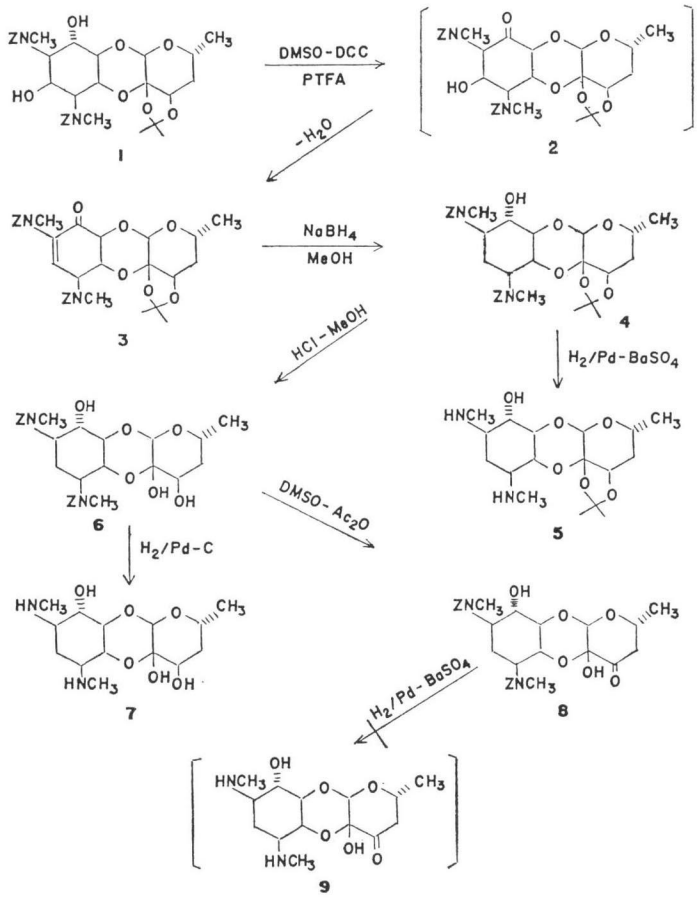
7-Deoxy-4(R)-dihydrospectinomycin (**7**) has been prepared and its structure firmly established by proton magnetic resonance and high resolution mass spectrometry. This spectinomycin analog is devoid of antibiotic activity.

The fused ring aminocyclitol antibiotic, spectinomycin, is of particular interest as a substrate for chemical modification because it does not share the ototoxic and nephrotoxic properties of the aminoglycosidic aminocyclitol antibiotics. The major concern with spectinomycin is the enhancement of antibiotic potency and bactericidal action. Consideration of current structure-activity relationships 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] While not sufficient for the antibiotic activity of the intact drug, a 2-deoxystreptamine or streptamine moiety is necessary in most instances. 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*-spectinomycin,[2] 7-*epi*-4(R)-dihydrospectinomycin[2] and 7-*epi*-9-deoxy-4(R)-dihydrospectinomycin[3] has been reported. While these results suggest that the structure-activity relationship derived from the various aminocyclitol antibiotics is not applicable to spectinomycin, the 7-deoxy analogs of spectinomycin and 4(R)-dihydrospectinomycin remain of interest as analogs of 2-deoxystreptamine. We wish to report the preparation of 7-deoxy-4(R)-dihydrospectinomycin (**7**) as outlined in Scheme 1.

N,N'-Dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide[2] (**1**) was selectively oxidized in high yield by the PFITZNER-MOFFATT technique[4] to give the 9-oxo-analog (**2**). Complete characterization of **2** was precluded by the ready elimination of water to give the $\alpha,\beta$-unsaturated ketone (**3**). The pmr spectrum of **3** shows a resonance at 6.48 ppm which is due to a vinyl proton.

Treatment of the unsaturated ketone (**3**) with sodium borohydride provided a stereospecific reduction with regeneration of the natural stereochemistry at both C-8 and C-9 to give 7-deoxy-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**4**). Removal of the carbobenzoxy groups from **4** by catalytic hydrogenation yielded 7-deoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**5**), an intermediate whose structure and stereochemistry could be assigned from the 270 MHz pmr spectrum.

Scheme 1.

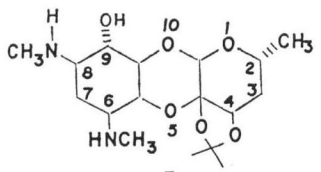



The spectrum revealed additional methylene resonances present at high field (Table 1). These resonances were affected when H-6 and H-8 were irradiated, thereby proving the methylene group is at C-7. The large magnitudes of the $J_{5a,6}$; $J_{8,9}$; $J_{9,9a}$ and $J_{9a,5a}$ coupling constants (Table 1) indicate that the interacting protons are all *axial* and thereby specify that the normal spectinomycin stereochemistry has been maintained at three centers.

The acetonide block was removed from **4** by treatment with dilute hydrochloric acid to give the carbobenzoxy protected dihydrospectinomycin analog (**6**). Removal of the carbobenzoxy groups from **6** by catalytic hydrogenation gave 7-deoxy-4(R)-dihydrospectinomycin (**7**) which also proved to be suitable for pmr analysis at 270 MHz. The spectrum showed the absence of the acetonide group and, as in the case of **5**, the additional multiplicity of H-6 and H-8 (Table 2) indicated the presence of a 7-$CH_2$ group. The actinamine ring coupling constants remained unchanged (Table 2).

The selective oxidation of the C-4 *axial* hydroxyl group of **6** by the dimethylsulfoxide-acetic

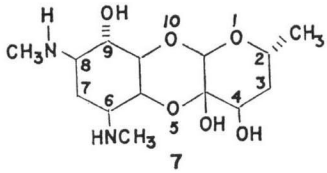
Table 1. PMR parameters of 7-deoxy-4(R)-dihydrospectinomycin-4,4a acetonide (**5**) at 270 MHz in $CDCl_3$



**5**

| Chemical shifts (ppm) | | Coupling constants | |
|---|---|---|---|
| H-2 | 3.89 | $J_{2,3\,ax}$ | 13.2 |
| H-3 ax | 1.76 | $J_{2,3\,eq}$ | 2.3 |
| H-3 eq | 1.97 | $J_{3\,ax,3\,eq}$ | 14.8 |
| H-4 | 4.17 | $J_{3\,ax,4}$ | 4.5 |
| H-5a | 3.69 | $J_{3\,eq,4}$ | 1.3 |
| H-6 | 2.57 | $J_{5a,6}$ | 9.0 |
| H-7 ax | 1.04 | $J_{6,7\,ax}$ | 11.8 |
| H-7 eq | 2.27 | $J_{6,7\,eq}$ | 3.9 |
| H-8 | 2.43 | $J_{7\,ax,7\,eq}$ | 13.0 |
| H-9 | 3.44 | $J_{7\,ax,8}$ | 12.6 |
| H-9a | 3.78 | $J_{7\,eq,8}$ | 3.9 |
| H-10a | 4.66 | $J_{8,9}$ | 9.0 |
| 2-$CH_3$ | 1.28 | $J_{9,9a}$ | 9.0 |
| N-$CH_3$ | 2.42, 2.43 | $J_{9a,5a}$ | 9.0 |
| C-$(CH_3)_2$ | 1.48 | | |

Table 2. PMR parameters of 7-deoxy-4(R)-dihydrospectinomycin (**7**) at 270 MHz in $D_2O$



**7**

| Chemical shifts (ppm) | | Coupling constants | |
|---|---|---|---|
| H-2 | 4.39 | $J_{2,3\,ax}$ | 13.0 |
| $CH_2$-3 | ~2.05 | $J_{2,3\,eq}$ | ~3.0 |
| H-4 | 4.11 | $J_{2,CH_3}$ | 6.5 |
| H-5a | 4.09 | $J_{3\,ax,3\,eq}$ | — |
| H-6 | 3.57 | $J_{3\,ax,4}$ | ~4.0 |
| H-7 | ~2.05 | $J_{3\,eq,4}$ | ~4.0 |
| H-8 | 3.80 | $J_{5a,6}$ | ~10.0 |
| H-9 | 4.41 | $J_{6,7\,eq}$ | ~4.2 |
| H-9a | 4.25 | $J_{6,7\,ax}$ | ~11.5 |
| H-10a | 5.14 | $J_{7\,ax,7\,eq}$ | ~12.0 |
| 2-$CH_3$ | 1.47 | $J_{7\,ax,8}$ | 12.0 |
| $(N\text{-}CH_3)_2$ | 3.02 | $J_{7\,eq,8}$ | ~4.0 |
| | | $J_{8,9}$ | 10.5 |
| | | $J_{9,9a}$ | 10.5 |
| | | $J_{9a,5a}$ | 10.5 |

anhydride procedure used for the preparation of 7-*epi*-spectinomycin[2] gave the desired 4-oxo-analog, 7-deoxy-N,N′-dicarbobenzoxyspectinomycin (**8**). The blocked deoxyspectinomycin analog (**8**) was subjected to partition column chromatography in an effort to remove catalyst poisons derived from the DMSO oxidation step. All attempts to remove the carbobenzoxy groups from **8** by catalytic hydrogenation, however, failed to give the desired 7-deoxyspectinomycin (**9**). The lability of the spectinomycin molecule precluded more vigorous deblocking procedures.

7-Deoxy-4(R)-dihydrospectinomycin (**7**) is devoid of antibiotic activity as measured by the agar dilution method on pH 8 nutrient agar at 500 $\mu$g/ml.

## 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 unless otherwise stated) and coupling constants are reported in Hz. The pmr spectra of **5** and **7** were also determined at 270 MHz on a Bruker HX-270 spectrometer in deuterochloroform and deuterium oxide, respectively, at ambient temperatures. 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 chloroform-d 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.

*Δ*7,8-Anhydro-9-oxo-N,N′-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**3**)

To a mixture of dimethylsulfoxide (12 ml), dry benzene (12 ml), dicyclohexyl carbodiimide (2.5 g,

12 mmole) and pyridinium trifluoroacetate (390 mg, 2.0 mmole) was added 2.6 g (4.0 mmole) of N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide[2] (**1**) and the mixture stirred at 50°C for 4 hours. The reaction mixture was then poured into 500 ml of ethyl acetate and 500 ml of water with stirring, the insoluble dicyclohexyl urea was removed by filtration, and the ethyl acetate phase was washed well with water and dried. The ethyl acetate solution was reduced in volume to about 50 ml, cooled in an ice bath, dicyclohexyl urea again removed, and the filtrate evaporated to give 3.5 g of the crude 9-oxo-intermediate (**2**) [IR 3600, 3450, 1757 $cm^{-1}$; silica gel/chloroform-ethyl acetate (5: 1) TLC Rf-value 0.54; PMR ($CDCl_3$) $\delta$ 1.25 (d, 2-$CH_3$); 1.25, 1.44 (s, C-$CH_3$'s); 3.01, 3.11 (s, N-$CH_3$'s); 4.74 (s, H-10a); 5.14, 5.17 (s, Z-$CH_2$'s); 7.34 (s, Z-arom.)]. Dehydration of **2** occurred during silica gel column chromatography (chloroform - ethyl acetate, 5: 1) to give **3**, $\Delta$7,8-anhydro-9-oxo-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide as a colorless foam (2.3 g, 92% of theory): TLC Rf-value 0.59 (silica gel/chloroform - ethyl acetate, 5: 1); IR 1710, 1660 $cm^{-1}$; PMR ($CDCl_3$) $\delta$ 1.27 (d, 2-$CH_3$); 1.38, 1.44 (s, C-$CH_3$'s); 2.77, 2.98, 3.05 (s, N-$CH_3$'s, Rotamers); 4.73 (s, H-10a); 6.48 (m, vinyl-7); 7.30, 7.35 (s, Z-arom.); MS, *m/e* 622.2539 ($M^+$): 622.2526 calcd. for $C_{33}H_{38}N_2O_{10}$; *Anal.* Calcd. for $C_{33}H_{32}N_2O_{10}$: C, 63.65; H, 6.15; N, 4.50. Found: C, 63.64; H, 6.31; N, 4.25.

7-Deoxy-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**4**)

The $\alpha,\beta$-unsaturated ketone (**3**) (1.72 g, 2.7 mmole) was dissolved in methanol (134 ml) and treated with sodium borohydride (103 mg, 2.7 mmole) with stirring at room temperature for 2 hours. Acetone (10 ml) was added to consume excess reagent and the mixture was evaporated to a residue. Pure product (**4**) was isolated by silica gel column chromatography (methylene chloride - ethanol, 98: 2) as a colorless foam (960 mg, 56% of theory): IR 3580, 3430 $cm^{-1}$; PMR ($CDCl_3$) $\delta$ 1.27 (d, 2-$CH_3$); 1.42, 1.46 (s, C-$CH_3$'s); 2.86 (s, N-$CH_3$'s); 4.64 (s, H-10a); 5.13, 5.15 (s, Z-$CH_2$'s); 7.34 (s, Z-arom.): MS, *m/e* 626 ($M^+$); *Anal.* Calcd. for $C_{33}H_{42}N_2O_{10}$: C, 63.24; H, 6.76; N, 4.47. Found: C, 63.12; H, 6.53; N, 4.65.

7-Deoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**5**)

The blocked acetonide (**4**) (290 mg, 0.46 mmole) was dissolved in 100 ml of absolute ethanol with 400 mg of 10% Pd-$BaSO_4$ and treated with hydrogen at 3 atm. for 3 hours. The mixture was filtered, a drop of conc. HCl added to the filtrate, and the filtrate evaporated to a residue. Pure product (**6**) was isolated by silica gel column chromatography (chloroform - methanol - ammonia, 50: 50: 1) as a colorless foam (48 mg, 26% of theory): PMR (see Table 1): MS, *m/e* 358.2106 ($M^+$), 358.2104 calcd. for $C_{17}H_{30}N_2O_6$.

7-Deoxy-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin (**6**)

The acetonide (**4**) (1.6 g, 2.6 mmole) was dissolved MeOH (75 ml) and 1 N hydrochloric acid (75 ml) and the mixture refluxed for 1.5 hours. The solvent was removed *in vacuo* with several additions of absolute ethanol. Pure product (**6**) was isolated by silica gel column chromatography (chloroform - methanol, 5: 1) as a colorless foam (0.97 g, 64% of theory): PMR ($CDCl_3$) $\delta$ 1.26 (d, 2-$CH_3$): 2.82, 2.86 (s, N-$CH_3$'s); 4.85 (s, H-10a); 5.12 (s, Z-$CH_3$'s); 7.33 (s, Z-arom.); *Anal.* Calcd. for $C_{30}H_{38}N_2O_{10}$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.31; H, 6.51; N, 4.42.

7-Deoxy-4(R)-dihydrospectinomycin (**7**)

7-Deoxy-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin (**6**) (200 mg, 0.34 mmole) was dissolved in 100 ml of a mixture of methanol and water (1: 1) with 200 mg of 5% Pd-C and treated with 3 atm. of hydrogen for 3 hours. The mixture was filtered, a drop of conc.HCl was added to the filtrate, and the filtrate evaporated to give the dihydrochloride of **7** as a colorless foam (135 mg, 100% of theory): $[\alpha]_D^{22}$ 21°; IR (virtually identical with that of 4(R)-dihydrospectinomycin): PMR (see Table 2): MS, *m/e* 318.1768 ($M^+$), 318.1791 calcd. for $C_{14}H_{26}N_2O_6$.

7-Deoxy-N,N'-dicarbobenzoxyspectinomycin (**8**)

7-Deoxy-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin (**6**) (2.0 g, 3.5 mmole) was dissolved in 20 ml of dimethyl sulfoxide and 20 ml of acetic anhydride and the mixture stirred at room temperature. After 4 hours 500 ml of ice water was added to the reaction mixture and the products extracted with chloroform. Pure ketone (**8**) was isolated by silica gel column chromatography (sequential with 1, 2

and 3% methanol in methylene chloride) as a colorless foam (360 mg, 18% of theory): IR 3600, 3500, 1739 $cm^{-1}$; PMR ($CDCl_3$) $\delta$ 1.40 (d, 2-$CH_3$); 2.78, 2.88 (s, N-$CH_3$'s); 4.66 (s, H-10a); 5.10, 5.14 (s, Z-$CH_2$'s); 7.34 (s, Z-arom.); MS, *m/e* 584.2354 ($M^+$), 584.2370 calcd. for $C_{30}H_{36}N_2O_{10}$.

Acknowledgements

The authors are indebted to Mr. ROBERT DYKSTRA of the University of Chicago for the 270 MHz pmr data. We also wish to thank Mr. D. A. DUNNIGAN for the hydrogenations, Ms. S. L. MUELLER for the mass spectra and especially Prof. L. MITSCHER of the University of Kansas, Lawrence, for many helpful and stimulating discussions.

## References

1) SHIER, W. T.; K. L. RINEHART, Jr. & D. GOTTLIEB: Preparation of four new antibiotics from a mutant of *Streptomyces fradiae*. Proc. Natl. Acad. Sci., U.S. 63: 198~204, 1969
2) ROSENBROOK, WM., Jr.; R. E. CARNEY, R. S. EGAN, R. S. STANASZEK, M. CIROVIC, T. NISHINAGA, K. MOCHIDA & Y. MORI: Spectinomycin modification. II. 7-*Epi*-Spectinomycin. J. Antibiotics 28: 960~964, 1975
3) ROSENBROOK, WM., Jr. & R. E. CARNEY: Spectinomycin modification. I. 7-*epi*-9-Deoxy-4(R)-dihydrospectinomycin. J. Antibiotics 28: 953~959, 1975
4) PFITZNER, K. E. & J. G. MOFFATT: Sulfoxide-carbodiimide reactions. II. Scope of the oxidation reaction. J. Am. Chem. Soc. 87: 5670~5678, 1965