SPECTINOMYCIN MODIFICATION. III

CHLORO-DEOXY ANALOGS

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9-*Epi*chloro-9-deoxy-4(R)-dihydrospectinomycin (**3**), 9-chloro-9-deoxy-4(R)-dihydrospectinomycin (**7**), 9-deoxy-8,9-epimino-4(R)-dihydrospectinomycin (**6**), and 9-*epi*chloro-9-deoxyspectinomycin (**10**) have been prepared and their structures established by proton magnetic resonance. These analogs are devoid of antibiotic activity.

The R-factor mediated inactivation of spectinomycin by adenylylation, apparently at the 9-position[1,2], prompted a study of the chemical modification of spectinomycin at C-9. While the preparation of 7-*epi*-9-deoxy-4(R)-dihydrospectinomycin has already been reported[3], its total lack of antibacterial activity precluded assessment of the significance of the 9-hydroxyl group. Subsequent preparation of the inactive 7-*epi*-spectinomycin and 7-*epi*-4(R)-dihydrospectinomycin analogs[4], however, established the importance of the stereochemistry if not the presence of the C-7 hydroxyl group. Although the direct C-9 deoxygenation of spectinomycin and 4(R)-dihydrospectinomycin was not achieved, a number of interesting analogs were prepared; *viz.* 9-*epi*chloro-9-deoxy-4(R)-dihydrospectinomycin (**3**), 9-chloro-9-deoxy-4(R)-dihydrospectinomycin (**7**), 9-deoxy-8,9-epimino-4(R)-dihydrospectinomycin (**6**), 8-*epi*chloro-8-des(methylamino)-9-*epi*(methylamino)-9-deoxy-4(R) dihydrospectinomycin (**8**) and 9-*epi*chloro-9-deoxyspectinomycin (**10**).

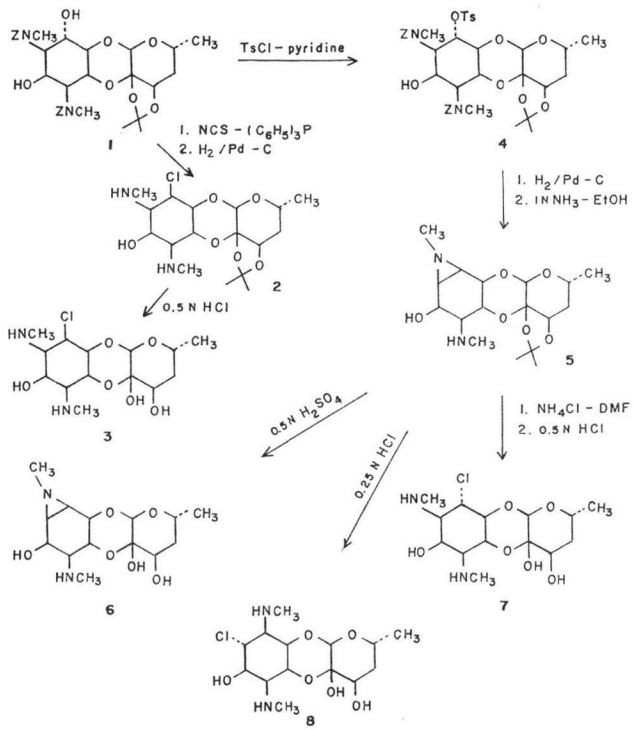
N,N′-Dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**1**)[4] was chlorinated at C-9 (Scheme 1), with inversion, by the method of BOSE and LAL[5] to yield, after removal of the carbobenzoxy blocks by the usual procedure, 9-*epi*chloro-9-deoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**2**). The PMR spectrum offers strong support for the proposed structure. The resonance of H-9 is observed at 4.58 ppm and exhibits two small couplings ($J=3.5$ Hz) requiring an equatorial orientation which thereby establishes the 9-*epi* configuration. The resonances of H-8 and H-9a also exhibit the small couplings arising as a consequence of C-9 epimerization.

The acetonide block was removed from **2** with dilute hydrochloric acid to yield 9-*epi*chloro-4(R)-dihydrospectinomycin (**3**), which gave a mass spectrum with a protonated molecular ion at *m/e* 353. Immediate elimination of HCl precluded verification of the chlorine substituent location.

Reaction of **1**[4] with *p*-toluenesulfonyl chloride in pyridine (Scheme 1) afforded, preferentially, the 9-O-tosyl derivative (**4**). After removal of the carbobenzoxy groups, the intermediate 9-O-tosylate cyclised in ethanolic ammonia to give the 8,9-epimino compound (**5**). The assigned composition of **5** was established by high resolution mass spectrometry (Found M^+ *m/e* 356.1928, $C_{17}H_{28}N_2O_6$ requires 356.1947). Integral analysis of the PMR spectrum of **5** reveals the presence of two additional protons between 1.5 and 2.1 ppm and the absence of one proton downfield of 3.5 ppm. Only a single resonance is observed at approximately 2.3 ppm in the region where the resonances of both H-6 and H-8 generally

appear. These observations are consistent with the 8,9-epimino structure in which the high field resonances arise from H-8 and H-9 while H-6 retains its normal chemical shift and the downfield resonance generally associated with H-9 is lost. High pressure hydrogenation of the epimine (**5**) as well as its 6-acetyl derivative under a variety of conditions (*e.g.*, see Ref. 6) failed to give a ring opened analog.

Scheme 1.



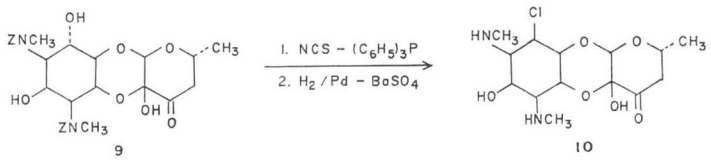
The epimine (**5**) was deblocked with dilute sulfuric acid to give 8,9 epimino-4(R)-dihydrospectinomycin (**6**).

Treatment of the epimine (**5**) with ammonium chloride in N,N-dimethyl-formamide[7] and deblocking by the usual procedure yielded the ring-opened 9-chloro-4(R)-dihydrospectinomycin (**7**). Although the resonance of H-9 could not be directly observed in the PMR of **7** due to extensive chemical shift overlap, the spectrum did offer corroboration of the C-9 stereochemistry. The resonances of H-6 and H-8 appear as overlapping doublet of doublets at 3.99 ppm. They each exhibit one small coupling ($J_{6,7} \sim J_{7,8} = 3$ Hz) and a second large coupling ($J_{5a,b} \simeq J_{8,9} \simeq$ 10 Hz) which establishes that C-9 has the normal stereochemistry. A small but distinct protonated molecular ion was observed in the mass spectrum of **7**. Fragmentation was characteristic of general structure, but non-specific regarding location of the chlorine substituent because of an initial loss of HCl.

Treatment of compound **5** with dilute hydrochloric acid (0.2 N) at room temperature followed by deblocking in the usual manner gave an unstable product tentatively identified as 8-*epi*chloro-8-des-(methylamino)-9-*epi*(methylamino)-9-deoxy-4(R)-dihydrospectinomycin (**8**). Due to the lability of the compound a satisfactory PMR spectrum was not obtained. The structural assignment is supported by the fact that it is chromatographically different (TLC) from **7**, the mass spectrum gives evidence for the presence of chlorine (*m/e* 352 and 354, M^+) and it has been reported[7] that aziridine rings may open in the opposite manner in the presence of hydrochloric acid.

N,N′-Dicarbobenzoxyspectinomycin (**9**) was likewise chlorinated at C-9[5] (Scheme 2) to yield, after deblocking, 9-*epi*chlorospectinomycin (**10**). Although a complete analysis of the ring proton resonances of **9** was not possible, support for the proposed C-9 stereochemistry was supplied by the 4.34 ppm resonance of H-8 which appeared as a triplet (J = 3.5 Hz). The magnitudes of the couplings require that both H-7 and H-9 have equatorial orientations. The couplings exhibited by the 4.01 ppm resonance of H-6 ($J_{5a,6} = 10$ Hz, $J_{6,7} = 3$ Hz) are unchanged from the corresponding couplings in spec-

Scheme 2.



9 10

tinomycin.

Attempts to prepare the bromo-analog of **9** in the same manner using N-bromosuccinimide failed as did subsequent attempts to catalytically remove the chloro-group.

All of the spectinomycin analogs described above, **3**, **6**, **7**, **8** and **10**, are devoid of antibiotic activity as measured by the agar dilution method on pH 8 nutrient agar at 500 μg/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.

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.

9-*Epi*chloro-4(R)-dihydrospectinomycin-4,4a-acetonide (**2**)

To a stirred solution of N-chlorosuccinimide (7.7 g, 58 m mole) in 300 ml THF was added, dropwise, a solution of triphenylphosphine (15.1 g, 58 m mole) in 360 ml THF. N,N'-Dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**1**)[4] (15.4 g, 24 m mole) in 300 ml THF was then added to the reagent and the mixture stirred at room temperature overnight. The reaction mixture was evaporated to a residue and pure product was isolated by silica gel column chromatography (*n*-hexane - ethylacetate, 1: 1) as a white glass (11.2 g, 70% of theory). This material was dissolved in 1 liter of ethanol with 5.5 g of 5% Pd/C and treated with hydrogen at 3 atm for 5 hours. The reaction mixture was filtered, the filtrate evaporated to a residue and "9-*epi*chloro-acetonide" (**2**) was isolated by silica gel column chromatography (chloroform - methanol - conc.ammonia, 90: 10: 1) as a colorless foam (5.9 g, 89% of theory): PMR ($CDCl_3$) δ 1.27 (d, 2-CH_3); 1.48 (s, acetonide - CH_3) 1.6~2.1 (m, 3-CH_2); 2.43, 2.47 (s, N-CH_3); ~2.5 (m, H-6); 2.70 (t, $J_{7,8}=J_{8,9}=3.5$ Hz, H-8); ~3.9 (m, H-2); 3.98 (dd, $J_{5a,9a}=10.0$ Hz, $J_{9,9a}=3.5$ Hz, H-9a); 4.14 (t, $J_{6,7}=J_{7,8}=3.5$ Hz, H-7); 4.14 (t, $J_{3a,4}=J_{3e,4}=3.0$ Hz); 4.23 (t, $J_{5a,6}=J_{5a,9a}=10.0$ Hz); 4.58 (t, $J_{8,9}=J_{9,9a}=3.5$ Hz); 4.70 (s, H-10a).

9-*Epi*chloro-4(R)-dihydrospectinomycin (**3**)

The acetonide (**2**) was dissolved in 50 ml of 0.5 N hydrochloric acid and the solution was allowed to stand at room temperature for 72 hours. The reaction mixture was evaporated to a residue and pure 9-*epi*chloro-4(R)-dihydrospectinomycin (**3**) was isolated by silica gel column chromatography (chloroform - methanol - conc.ammonia, 10: 10: 1) as a colorless foam (4.7 g, 90% of theory): $[\alpha]_D^{23}$ 60°; PMR (D_2O) δ1.70 (d, 2-CH_3); 2.1~2.5 (m, 3-CH_2); 2.82, 2.84 (s, N-CH_3); 3.10 (m, H-6); 3.37 (t, $J_{7,8}=J_{8,9}=3.5$ Hz, H-8); 4.29 (t, $J_{3a,4}=J_{3e,4}=3.0$ Hz, H-4); 4.5~4.9 (m, H-2, 5a, 7, 9a); 5.05 (t, $J_{8,9}=J_{9,9a}=3.5$ Hz, H-9); 5.35 (s, H-10a);
MS *m/e* 353 and 355 $(M+H)^+$

Anal. Calcd. for $C_{14}H_{25}N_2O_6Cl$: C, 47.62; H, 7.14; N, 7.94; O, 27.21; Cl, 10.05
Found: C, 47.56; H, 7.42; N, 7.76; O, 26.96; Cl, 10.06

9-O-Tosyl-N,N'-dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**4**)

N,N'-Dicarbobenzoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**1**) (12.8 g, 19 m mole) was dissolved in 250 ml dry pyridine (1 CaH_2), cooled to $<10°C$ and *p*-toluensulfonyl chloride (5.1 g, 1.5 equiv.) was added with stirring. The mixture was allowed to warm to room temperature and then heated at 70~76°C (bath) for 44 hours. An additional 1.7 g of reagent was added and the reaction was maintained at 70~76°C for an additional 60 hours. The reaction mixture was evaporated to a residue *in vacuo* and pure product (**4**) was isolated by silica gel column chromatography (ethyl acetate - *iso*octane, 1: 1) as a white foam (11 g, 70% of theory):
MS *m/e* 796 (M^+)

Anal. Calcd. for $C_{40}H_{48}N_2O_{13}S$: C, 60.29; H, 6.07; N, 3.52; O, 26.10; S, 4.02
Found: C, 60.17; H, 6.30; N, 3.37; O, 25.87; S, 3.85

8,9-Epimino-4(R)-dihydrospectinomycin-4,4a-acetonide (**5**)

The tosylate (**4**) (16.8 g, 21 m mole) was dissolved in 880 ml of ethanol with 8 g of 5% Pd/C and treated with 3 atm of hydrogen for 20 hours. The reaction mixture was evaporated to a residue *in vacuo* and the intermediate tosyl acetonide was isolated by ion-exchange chromatography (CG 50, NH_4^+ form) as a white foam (7.6 g, 68% of theory). Cyclization of the latter compound (4 g, 7.6 m mole) was induced by dissolution and reflux in 300 ml/M ethanolic ammonia for 6 hours. The reaction mixture was evaporated to a residue *in vacuo*, the product (**5**) isolated by silica gel column chromatography (chloroform - methanol - ammonia, 93: 7: 1) and crystallized from anhydrous diethyl ether: (1.5 g, 56% of theory). PMR ($CDCl_3$) δ 1.27 (d, 2–CH_3); 1.43, 1.45 (s, acetonide - CH_3); 1.5~2.1 (m, 3–CH_2, H–8, H–9); ~2.3 (m, H–6); 3.7~4.3 (m, H–2, 4, 5a, 7, 9a); 4.65 (s, H–10a):
MS *m/e* 356 (M^+)

Anal. Calcd. for $C_{17}H_{28}N_2O_6$: C, 57.29; H, 7.92; N, 7.86; O, 26.94
Found: C, 57.09; H, 8.07; N, 7.67

8,9-Epimino-4(R)-dihydrospectinomycin (**6**)

The blocked epimine (**5**) (1 g, 2.8 m mole) was dissolved in 50 ml 0.5 N sulfuric acid. After 24 hours at room temperature the reaction mixture was adjusted to pH 7 with 5 N sodium hydroxide and evaporated to a residue *in vacuo*. The product (**6**) was crystallized from methanol (800 mg, 88% of theory); $[\alpha]_D^{23}$ 79°; PMR (D_2O) δ 1.68 (d, 2–CH_3); 1.9~2.4 (m, 3–CH_2); ~2.7 (m, H–8, H–9); 2.79, 3.18 (s, N–CH_3); 3.61 (m, H–6); 4.27 (m, H–4); 5.35 (s, H–10a):
MS *m/e* 316 (M^+).

9-Chloro-4(R)-dihydrospectinomycin (**7**)

The blocked epimine (**5**) (3.0 g, 8.5 m mole) was dissolved in 150 ml of N,N-dimethylformamide and treated with ammonium chloride (0.96 g, 2 equiv.) under reflux for $2^1/_2$ hours. The reaction mixture was evaporated to a residue *in vacuo* and pure product was isolated by silica gel column chromatography (chloroform - methanol - ammonia, 93: 7: 1) as a white foam (930 mg, 28% of theory). The acetonide block was removed from the latter material by treatment with 48 ml of 0.5 N hydrochloric acid at room temperature for 24 hours. The reaction mixture was adjusted to pH 7 with 5 N sodium hydroxide, decolorized with Darco G-60, and evaporated to a residue *in vacuo*. Pure product (**8**) was isolated by silica gel column chromatography (chloroform - methanol - ammonia, 10: 10: 1) as a colorless foam (716 mg, 86% of theory): $[\alpha]_D^{23}$ 44°; IR (virtually identical with that of 4(R)-dihydrospectinomycin); PMR (D_2O) δ 1.72 (d, 2–CH_3); 3.28, 3.29 (s, N–CH_3); 3.99 (dd, H–6, 8); 4.37 (t, H–4); 4.5~5.0 (m, H–2, 5a, 9, 9a); 5.24 (t, H–7); 5.42 (s, H–10a);
MS *m/e* 353 and 355 $(M+H)^+$

Anal. Calcd. for $C_{14}H_{25}N_2O_6Cl$: C, 47.65; H, 7.17; N, 7.94
Found: C, 47.65; H, 7.06; N, 7.62

N,N'-Dicarbobenzoxy spectinomycin (**9**)

Spectinomycin dihydrochloride pentahydrate (100 g, 0.22 moles) was dissolved in 1 liter 10% aqueous sodium bicarbonate solution, cooled to 5~10°C (ice bath) and 600 ml of an acetone solution containing 56 ml (0.4 mole) of carbobenzoxy chloride was added slowly with stirring. The reaction

mixture was removed from the ice bath after 2 hours and after standing at room temperature overnight the acetone was removed by evaporation *in vacuo* at 45°C (bath). The aqueous phase was extracted with chloroform, the extracts were combined, dried, and evaporated to a glass (76 g, 63% of theory): IR 1745, 1682 cm^{-1}:
MS *m/e* 600 (M^+)

Anal. Calcd. for $C_{30}H_{36}N_2O_{11}$: C, 59.99; H, 6.01; N, 4.67; O, 29.33
Found: C, 59.67; H, 6.02; N, 4.60; O, 29.38

9-*Epi*chloro-9-deoxy-spectinomycin (**10**)

To a stirred solution of N-chlorosuccinimide (1.9 g, 14 m mole) in 75 ml tetrahydrofuran (THF) was added, dropwise, a solution of triphenylphosphine (3.8 g, 14 m mole) in 90 ml of THF. N,N'-Dicarbobenzoxyspectinomycin (**9**) (3.6 g, 6 m mole) in 75 ml THF was then added to the reagent and the mixture stirred overnight at room temperature. The reaction mixture was evaporated to a residue and pure product was isolated by silica gel column chromatography (*n*-hexane - ethyl acetate, 3: 7) as a white glass (470 mg, 12% of theory). This material was dissolved in 250 ml ethanol with 565 mg of 10% Pd-$BaSO_4$ and treated with hydrogen at 3 atm for 5 hours. The reaction mixture was filtered and the filtrate was evaporated to a white foam (**10**) (260 mg, 81% of theory): $[\alpha]_D^{23}$ 21°; IR (virtually identical with that of spectinomycin); PMR (D_2O) δ 1.72 (d, 2–CH_3); 3.28, 3.35 (s, N–CH_3); 5.38 (s, H–10a); MS, *m/e* 351 and 353 $(M+H)^+$.

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