SPECTINOMYCIN MODIFICATION. I

7-*EPI*-9-DEOXY-4(R)-DIHYDROSPECTINOMYCIN

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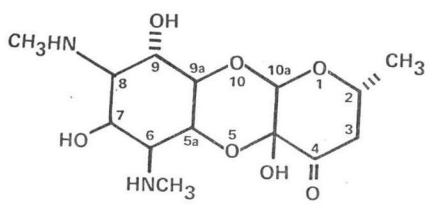
7-*Epi*-9-deoxy-4(R)-dihydrospectinomycin (**10**) has been prepared and its structure firmly established by complete analysis of its pmr spectrum. This analog of spectinomycin is devoid of antibiotic activity.

Spectinomycin (1) is a fused ring aminocyclitol antibiotic unique both in structure[1,2] and in biological activity. The bioactivity of spectinomycin resembles that of other aminocyclitol antibiotics only in that its antibacterial spectrum is broad and its mode of action is the inhibition of protein synthesis by an interaction with the 30S ribosomal subunit.[3] Spectinomycin's action is bacteriostatic rather than bactericidal and, although its spectrum is described as broad, *in vitro* potency is generally low. Resistance has, however, been observed in the laboratory and resistant strains carrying multiple R-factors inactivate spectinomycin by adenylylation, apparently at the 9-position.[4,5] Spectinomycin does not share the ototoxic and nephrotoxic properties of the aminoglycosidic aminocyclitols[6] and is, therefore, an important and interesting substrate for chemical modification. The bulk of the chemistry applied to aminocyclitol antibiotics has been directed toward the elimination or modification of sites within the molecule which are involved in the enzymatic inactivation of the antibiotic by R-factor mediated resistant organisms. Structure-activity relationships (SAR) among the aminocyclitol antibiotics, derived mainly from naturally occuring variations,[7] suggest that a streptamine or 2-deoxystreptamine moiety, while not sufficient for the antibiotic activity of the intact drug, is necessary in most instances. Stereochemistry at the 2-position of the aminocyclitol ring is also important since the semisynthetic hybrimycins B_1 and B_2, in which 2-*epi* streptamine has been incorporated, exhibit greatly reduced antibiotic activity.[8]

The major concern with spectinomycin and the object of our current work is the enhance-

Fig. 1.



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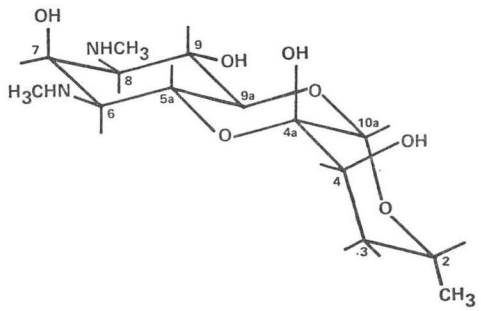

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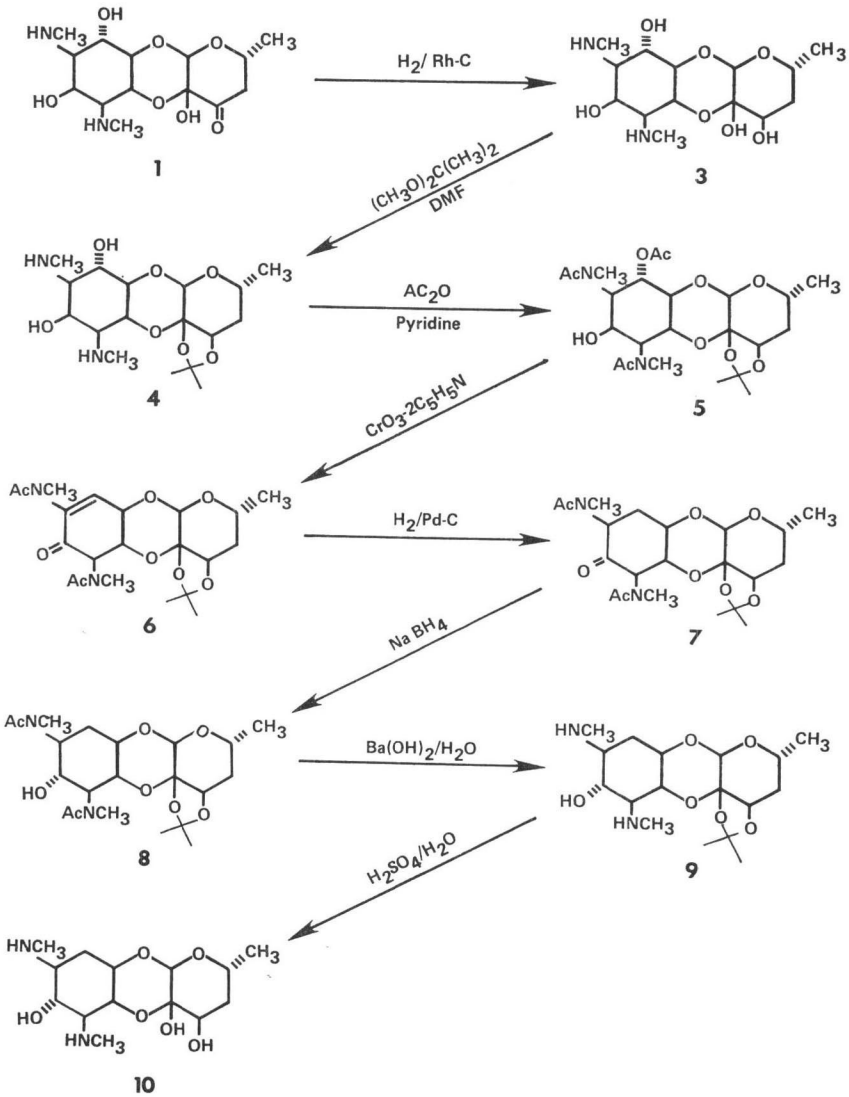
ment of antibiotic potency. Our general approach is based on the above SAR rationale and is centered on the chemical and stereochemical modification of spectinomycin at the 7-position, to enhance antibiotic potency and bactericidal action and at the 9-position, to reduce the probability of enzymatic inactivation. 7-*Epi*-9-deoxyspectinomycin and the corresponding 7-*epi*-9-deoxy-4(R)-dihydrospectinomycin (**10**) are evident analogs for meeting both of these objectives. The dihydro analog (**10**) is of interest because both C-4 dihydrospectinomycin epimers, although substantially less active than spectinomycin, exhibit both *in vitro* and *in vivo* antibiotic activity.[9] It was hoped that the activities were related and that activity in the dihydro-series could be extrapolated to the 4-oxo or spectinomycin series. This paper deals with the preparation of 7-*epi*-9-deoxy-4(R)-dihydrospectinomycin (**10**) as outlined in Scheme I.

The labile nature of the spectinomycin molecule precluded direct modification and a

Scheme 1



suitably blocked derivative was required to withstand the rigors of reactions leading to deoxygenation and epimerization. The most unstable feature of the molecule, the *alpha* keto *hemi* ketal functionality at 4a was protected by preparation of the 4(R)-dihydrospectinomycin-4, 4a-acetonide (**4**).

As previously reported,[1] catalytic hydrogenation and sodium borohydride reduce spectinomycin to dihydrospectinomycin. Reduction of spectinomycin dihydrochloride with borohydride in methanol was observed to proceed stereospecifically to the 4(S)-dihydrospectinomycin epimer as opposed to a mixture of the two epimers as recently reported.[9] While catalytic hydrogenation gives a mixture of the two epimers, conditions were discovered using a rhodium on carbon catalyst in which up to 95% of the mixture is constituted by the 4(R)-dihydrospectinomycin epimer (**3**). Mixtures of the 4(R) and 4(S) epimers could be separated and quantitated by a gas chromatographic procedure (unpublished data, R. J. MAURITZ) which was quite useful in optimizing the catalytic hydrogenation. The stereochemistry of the two epimers was established largely by analysis of their pmr spectra. Severe chemical shift overlap prevented a complete analysis of the spectra; however, the resonance arising from H-4 and its coupling pattern with the C-3 methylene protons were clearly visible for both isomers. A recent X-ray study[2] determined the stereochemistry and conformation of the dideoxyhexopyranose portion of spectinomycin dihydrobromide pentahydrate and thereby permits the use of the H-4 coupling pattern to assign C-4 stereochemistry. Cognizant of the fact that H-4 represents the X-proton of an ABX spin system, the magnitude of the sum of the vicinal couplings can be measured with assurance and indicates the stereochemical relationship between the coupled protons. The measured splittings in one isomer were found to be $J_{3ax,4}=J_{3eq,4}=2.5$; Sum=5.0 Hz which is indicative of an equatorial orientation of H-4 and thereby designates the C-4 stereochemistry as 4(R) (**2**). The second isomer revealed $J_{3ax,4}=11.8$; $J_{3eq,4}=5.3$; Sum=17.1 which requires an axial H-4 and 4(S) stereochemistry.

Reaction of an epimeric mixture of the dihydrospectinomycins with 2, 2-dimethoxy propane gave, as expected, only the *cis* product, 4(R)-dihydrospectinomycin-4, 4a-acetonide (**4**). The resonance of H-4 in the pmr spectrum of the acetonide exhibits $J_{3ax,4}=J_{3eq,4}=2.0$; Sum=4.0 Hz consistent with the assigned 4(R) stereochemistry.

Acetylation of the acetonide (**4**) in pyridine gave the N, N′-9-*O*-triacetyl-4(R)-dihydrospectinomycin-4, 4a-acetonide (5) in which the amino functions are protected from oxidation and the 9-hydroxyl group is converted to a leaving group. Treatment of **4** with a modified COLLINS reagent[10] gave the α, β-unsaturated 7-oxo analog (**6**). Although somewhat hampered by the presence of at least three rotamers, the pmr of **6** offers evidence for the position of the double bond and ketone function. Spin decoupling experiments involving the predominant rotamer indicate that the C-9 vinyl proton at 6.81 ppm has a small (2.0 Hz) coupling with a multiplet at 5.07 ppm which can be assigned to H-9a. The couplings of both H-9a and H-6 with the 4.32 ppm resonance of H-5a are found to be large (8.3 and 12.8 Hz respectively). The resonance of H-6 is at low field (5.62 ppm) as a consequence of its proximity to the C-7 ketone carbonyl and exhibits only a single large vicinal coupling. Taken together, these observations require that the C-6 through C-9a stereochemistry is unchanged, and fix the position of the α, β-unsaturated ketone.

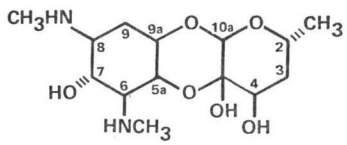
Catalytic hydrogenation of **6** selectively reduced the $\Delta^{8,9}$ double bond with concomitant regeneration of the natural stereochemistry at C-8 to give 7-oxo-9-deoxy-N, N′-diacetyl-4(R)-dihydrospectinomycin-4, 4a-acetonide (**7**). The pmr spectrum revealed the absence of the vinyl proton observed with **6**; however, the chemical shift of H-6 remained at low field (5.41 ppm) indicating that the C-7 ketone remained unaffected.

Sodium borohydride reduction of the 7-oxo analog (**7**) in 2-propanol proceeded in a stereospecific manner to give only the unnatural epimer at C-7, 7-*epi*-9-deoxy-N, N′-diacetyl-4(R)-dihydrospectinomycin-4, 4a-acetonide (**8**).

The acetyl blocks were removed from **8** by treatment with barium hydroxide to give 7-*epi*-9-deoxy-4(R)-dihydrospectinomycin-4, 4a-acetonide (**9**). Removal of the acetonide block from **9** was accomplished by treatment with dilute sulfuric acid to give the final product, 7-*epi*-9-deoxy-4(R)-dihydrospectinomycin sulfate (**10**).

The structure and stereochemistry of **10** were assigned from the 270 MHz pmr spectrum of its free base measured in pyridine-d_5 solution at 110°C. A first order spectrum in which every chemical shift and coupling constant could be measured resulted from this combination of high field and favorable solvent shifts (Table 1, Fig. 2). The resonances of

Table 1. PMR Parameters of 7-*epi*-9-Deoxy-4(R)-dihydrospectinomycin free base (**10**) in pyridine-d_5 at 110°C



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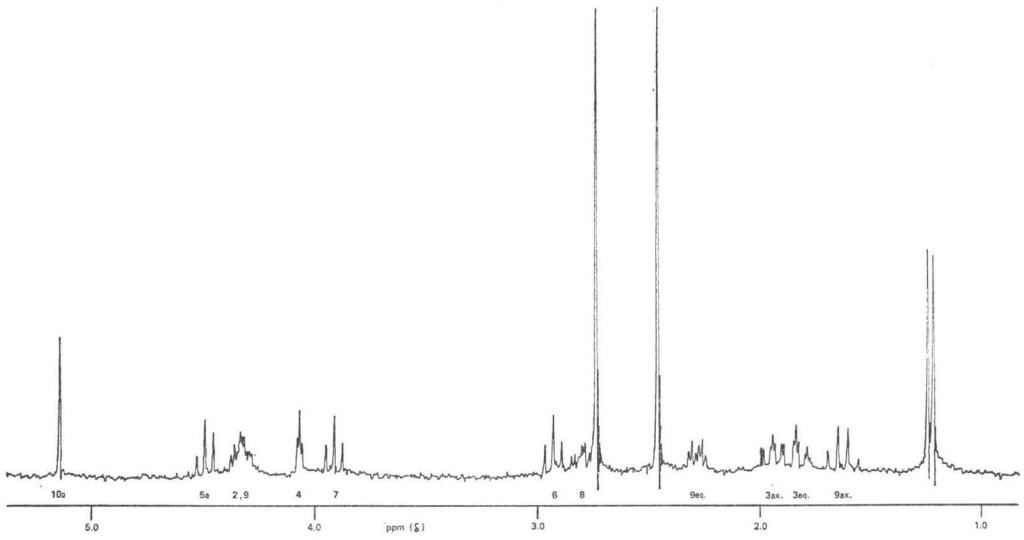
Chemical shifts ppm δ		Coupling constants Hz	
H-2	~4.4	J_{2,CH_3}	6.0
H-3 axial	1.98	$J_{2,3eq}$	2.4
H-3 equatorial	1.85	$J_{2,3ax}$	11.1
H-4	4.11	$J_{3eq,3ax}$	13.5
H-5a	4.54	$J_{3eq,4}$	2.6
H-6	2.97	$J_{3ax,4}$	2.6
H-7	3.97	$J_{5a,6}$	9.6
H-8	2.84	$J_{6,7}$	9.6
H-9 axial	1.68	$J_{7,8}$	9.6
H-9 equatorial	2.33	$J_{8,9ax}$	12.0
H-9a	4.38	$J_{8,eq9}$	4.4
H-10a	5.19	$J_{9ax,9eq}$	12.0
2-CH_3	1.27	$J_{9ax,9a}$	12.0
N-CH_3	2.78	$J_{9eq,9a}$	4.4
N-CH_3	2.51	$J_{9a,5a}$	9.6

Fig. 2.



H-6 and H-8 can be readily distinguished from other ring protons by their unique chemical shifts which result from deshielding by nitrogen. Spin decoupling experiments performed at 100 MHz clearly reveal that both protons have identical large couplings with H-7 establishing that the compound has a 7-*epi*-configuration. The vicinal couplings of every aminocyclitol ring proton (with the exception of the equatorial C-9 methylene proton) are large, indicating that each proton is axial and therefore no other center has epimerized. The small couplings exhibited by H-4 confirm the 4(R) stereochemistry at that center.

7-*Epi*-9-deoxy-4(R)-dihydrospectinomycin (**10**) is devoid of antibiotic activity as measured by an agar dilution method on pH 8 nutrient agar at 500 μg/ml. 4(R)- and 4(S)-Dihydrospectinomycin, although active, possess antibiotic spectra different from that of spectinomycin. This observation, coupled with the fact that 7-*epi*-9-deoxy-4(R)-dihydrospectinomycin (**10**) incorporates changes at two positions, hinders evaluation of the SAR rationale for the low antibiotic potency of spectinomycin.

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 spectrum of **10** was also determined at 270 MHz on a Bruker HX-270 spectrometer in pyridine-d_5 at 110°C.

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.

4(R)-Dihydrospectinomycin dihydrochloride (**3**).

Spectinomycin dihydrochloride pentahydrate (**1**) (50 g, 0.1 mole) was dissolved in 500 ml of a mixture of methanol-water-acetic acid (90:5:5, v/v) and treated with 3 atm of hydrogen over 15 g of 5% Rh-C for 3 hours. The catalyst was removed by filtration and the filtrate evaporated *in vacuo* at 40°C (bath) to a 47 g residue [75% (**3**) by glc and pmr]. Yields of the 4(R) epimer ranged from 80~95%: $[\alpha]_D^{23}$ 31°; pmr (D_2O); δ1.26 (d, 2-CH_3, J_{2,CH_3}=6.0 Hz); 2.82, 2.83 (s, *N*-CH_3); 4.92 (s, H-10a); (DMSO-d_6) δ1.15 (d, 2-CH_3, J_{2,CH_3}=6.0 Hz); 2.67 (s, 2× *N*-CH_3); 4.67 (s, H-10a); MS *m/e* 335 (M^+ +1), 334 (M^+), 316 (M^+-H_2O).

4(S)-Dihydrospectinomycin dihydrochloride.

Prepared by the method of H. HOEKSEMA and P. F. WILEY, U.S. Pat. 3,165,533. Further purification of this material was accomplished by Sephadex LH-20 column chromatography (methanol): $[\alpha]_D^{23}$ 28°; pmr (D_2O, measured from internal TSP), δ1.28 (d, 2-CH_3, J_{2,CH_3}=6.0 Hz); 2.86, 2.88 (s, *N*-CH_3); 4.79 (s, H-10a); (DMSO-d_6) δ1.16 (d, 2-CH_3, J_{2,CH_3}=6.0 Hz); 2.64 (s, 2×*N*-CH_3); 4.49 (s, H-10a); MS *m/e* 335 (M^+ +1), 334(M^+), 316 (M^+-H_2O).

4(R)-Dihydrospectinomycin-4,4a-acetonide (**4**).

4(R)-Dihydrospectinomycin (**3**) (100 g, 65% R by glc, 0.16 mole) dissolved in 700 ml DMF (AR) with 9.0 g *p*-toluenesulfonic acid (dried *in vacuo* at 80°C for 3 hours) was treated with 2, 2-dimethoxy propane (700 ml, 5.7 moles) and the mixture stirred overnight at room temperature. The solvent was removed *in vacuo* at 55°C (bath). The residue was taken up in 1 liter of deionized water adjusted to pH 6~7 with conc.NH_4OH (~3 ml) filtered and applied to a 1,600 ml column (4.5 × 100 cm) of Amberlite CG-50 ion-exchange resin (NH_4^+ form). The

column was washed with water and eluted with 0.25 M NH_4OH to yield 47 g of product (**4**) as a white glass from chloroform solution (77 % of theory): pmr ($CDCl_3$) δ 1.25 (s, 2-CH_3, J_{2,CH_3}=6.0 Hz); 1.46 (s, C-CH_3); 2.49, 2.56 (s, N-CH_3); 4.62 (s, H-10a); MS *m/e* 375 ($M^+ +1$), 374 (M^+).

Anal. Calcd for $C_{17}H_{30}N_2O_7$: C, 54.52; H, 8.07; N, 7.48; O, 29.91.
Found: C, 54.13; H, 7.88; N, 7.17; O, 29.50.

N, N'-9-Triacetyl-4(R)-dihydrospectinomycin-4,4a-acetonide (**5**).

To a solution of 4(R)-dihydrospectinomycin-4, 4a-acetonide (**4**) (1 g, 2.2 moles) in 20 ml of pyridine (AR) cooled to ~5°C was added, dropwise, a solution of acetic anhydride (0.85 ml, 9 mmole) in 10 ml of pyridine. After warming to room temperature the mixture was allowed to stand overnight. The mixture was evaporated to a residue and pure product (**5**) was isolated by silica gel column chromatography ($CHCl_3$-MeOH, 9:1) as a white glass (**5**, 710 mg, 63 % of theory): ir 1760, 1650 cm^{-1}; pmr ($CDCl_3$) δ 1.25 (d, 2-CH_3, J_{2,CH_3}=6.0), 1.46 (s, C-CH_3); 2.05, 2.08, 2.11 (s, $COCH_3$); 3.04, 3.12 (s, NCH_3); 4.59 (s, H-10a) (values for major rotamer only). MS *m/e* 500 (M^+).

Anal. Calcd. for $C_{23}H_{36}N_2O_{10}$: C, 55.19; H, 7.25; N, 5.60; O, 31.37.
Found: C, 55.04; H, 7.33; N, 5.61; O, 31.11.

7-Oxo-Δ8,9-N, N'-diacetyl-4(R)-dihydrospectinomycin-4,4a-acetonide (**6**).

N, N', 9-Triacetyl-4(R)-dihydrospectinomycin-4, 4a-acetonide (**5**) (4.15 g, 8.3 mmoles) was added to a solution of chromium trioxide dipyridine complex (0.1 mole) prepared *in situ*[11] in 100 ml methylene chloride. After 30 minutes the reaction mixture was evaporated *in vacuo* and the residue triturated exhaustively with diethyl ether, and the triturates evaporated to a 2.3 g crude residue. The product (**6**) was isolated by silica gel column chromatography ($CHCl_3$-MeOH, 93:7) as a white glass (1.2 g, 33 % of theory): ir 1710, 1650 cm^{-1}; uv 330 nm ($\varepsilon \cong 50$); pmr ($CDCl_3$), three rotamers present; δ 1.31 (d, 2-CH_3, J_{2,CH_3}=6.0); 1.44, 1.47, 1-49 (s, C-CH_3); 1.90, 1.98, 2.09, 2.22 (s, $COCH_3$); 2.79, 2.87, 3.00, 3.02, 3.05, 3.25 (s, N-CH_3); 4.69, 4.72 (s, H-10a). MS *m/e* 438 (M^+).

Anal. Calcd. for $C_{21}H_{31}N_2O_8$: C, 57.39; H, 7.11; N, 6.38; O, 29.13.
Found: C, 56.75; H, 7.25; N, 6.12; O, 28.90.

7-Oxo-9-deoxy-N, N'-diacetyl-4(R)-dihydrospectinomycin-4,4a-acetonide (**7**).

7-Oxo-Δ8,9-N, N'-diacetyl-4(R)-dihydrospectinomycin-4, 4a-acetonide (**6**) (350 mg, 0.8 mmole) was dissolved in 100 ml absolute ethanol and treated with 3 atm. of hydrogen over 0.5 g of 5 % Pd-C for 3 hours. The reaction mixture was filtered and evaporated to a residue *in vacuo*. Pure product (**7**) was isolated by silica gel chromatography ($CHCl_3$-MeOH, 93:7) as a white glass (300 mg, 82 % of theory): ir 1733, 1650 cm^{-1}; pmr ($CDCl_3$) two rotamers present, δ 1.30 (d, 2-CH_3, J_{2,CH_3}=6.0 Hz); 1.45, 1.47 (s, C-CH_3); 2.09, 2.14, 2.17 (s, $COCH_3$); 2.89, 2.92, 2.95, 3.29 (s, N-CH_3); 4.63 (s, H-10a). MS *m/e* 440 (M^+).

Anal. Calcd. for $C_{21}H_{32}N_2O_8$: C, 57.26: H, 7.32; N, 6.36; O, 29.06.
Found: C, 56.88; H, 7.21; N, 5.98; O, 28.71.

7-*Epi*-9-deoxy-N, N'-diacetyl-4(R)-dihydrospectinomycin-4,4a-acetonide (**8**).

7-Oxo-9-deoxy-N,N'-diacetyl-4(R)-dihydrospectinomycin-4,4a-acetonide (**7**) (250 mg, 0.57 mmole) was dissolved in 10 ml of 2-propanol and treated with sodium borohydride in 2-propanol (2.85 ml, 0.1 M). After 1 hour acetone was added to consume excess borohydride and the mixture was evaporated *in vacuo* to a residue. Pure product (**8**) was isolated by silica gel column chromatography ($CHCl_3$-MeOH, 95:5) as a white glass (180 mg, 72 % of theory): ir 3680, 3330, 1640 cm^{-1}; pmr ($CDCl_3$) two rotamers are present; δ 1.28 (d, 2-CH_3, J_{2,CH_3}=6.0 Hz); 1.47, 1.49 (s, C-CH_3); 2.08, 2.10, 2.13, 2.16 (s, $COCH_3$); 2.98, 3.05, 3.14, 3.18 (s, N-CH_3); 4.60, 4.63 (s, H-10a). MS *m/e* 443 ($M^+ +1$), 442 (M^+).

Anal. Calcd. for $C_{21}H_{34}N_2O_8$: C, 57.00; H, 7.74; N, 6.33; O, 28.93.
Found: C, 56.70; H, 7.34.

7-*Epi*-9-deoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**9**).

7-*Epi*-9-deoxy-N,N'-diacetyl-4(R)-dihydrospectinomycin-4,4a-acetonide (**8**) was dissolved in 25 ml 0.2 M barium hydroxide (satd.) and heated at 95°C for 8 hours. The reaction mixture was adjusted to pH 7 with dilute hydrochloric acid and evaporated *in vacuo* to a residue. The pure product (**9**) was isolated by silica gel column chromatography ($CHCl_3$-MeOH-conc.NH_4OH; 9:10:1) as a white glass (60 mg, 15 % of theory): pmr ($CDCl_3$) δ 1.29 (d, 2-CH_3, $J_{2\text{-}CH_3}$=6.0 Hz); 1.49 (s, C-CH_3); 2.47, 2.51 (s, N-CH_3); 4.61 (s, H-10a). MS *m/e* 359 (M^+ +1), 358 (M^+).

Anal. Calcd. for $C_{17}H_{30}N_2O_6$: C, 56.96; H, 8.44; N, 7.82; O, 26.78.
Found: C, 56.35; H, 8.52; N, 7.47; O, 26.32.

7-*Epi*-9-deoxy-4(R)-dihydrospectinomycin (**10**).

7-*Epi*-9-deoxy-4(R)-dihydrospectinomycin-4,4a-acetonide (**9**) (300 mg, 0.84 mmole) was dissolved in 30 ml 0.5 M sulfuric acid at room temperature. After 24 hours the reaction mixture was adjusted to pH 7 with 1 M sodium hydroxide and evaporated to a residue *in vacuo*. The residue was triturated with 50 ml of methanol, the triturates filtered, and 7-*epi*-9-deoxy-4(R)-dihydrospectinomycin (**10**) precipitated as the sulfate salt by the addition of acetone (160 mg, 46 % of theory): $[\alpha]_D^{28}$ 69°; ir (KBr) virtually identical with that of 3; pmr (D_2O) δ 1.68 (d, 2-CH_3 J_{2,CH_3}=6.0 Hz); 3.18 (s, N-CH_3); 5.32 (s, H-10a). MS *m/e* 319 (M^+ +1); 318 (M^+), 300 (M^+-H_2O).

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