SPECTINOMYCIN MODIFICATION

V. THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF SPECTINOMYCIN ANALOGS WITH RING-EXPANDED SUGARS

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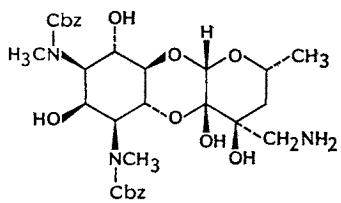
Tiffeneau-Demjanov rearrangement of 3′-(*R*)-*N*,*N*′-dibenzyloxycarbonyl-3′-aminomethyldihydrospectinomycin results in ring expansion affording the homologous analog with a seven-membered sugar ring. In stark constrast, attempted rearrangement of the 3′-*S*-isomer leads only to epoxide formation. Deprotection of the ring-expanded homolog gives homospectinomycin. The synthesis and biological activity of this interesting new member of the spectinomycin series and the derived dihydrohomospectinomycin is detailed in this paper.

In the previous paper in this series[1] we described an efficient synthesis of 3′-(*S*)- and 3′-(*R*)-*N*,*N*′-dibenzyloxycarbonyl-3′-aminomethyldihydrospectinomycin (**1** and **2**) *via* the intermediacy of spectinomycin 3′-cyanohydrins. These aminoalcohols, while of interest for preparation of derivatives of the primary amino group, have also provided us with an opportunity to explore the effect of ring-expansion of the sugar moiety of spectinomycin on bioactivity. Such homospectinomycins, with a seven-membered carbohydrate ring, are of special interest in light of the significant increase in activity seen with increasing sugar lipophilicity in other analog series.[2,3]

This paper describes the interesting results obtained on diazotization of **1** and **2**, and the successful preparation of homospectinomycin (**3**), the first spectinomycin analog containing a seven-membered sugar ring.

Results and Discussion

Vicinal amino alcohols such as **1** and **2** are the usual substrates for the Tiffeneau-Demjanov ring expansion,[4~6] a process which results in a ring-expanded ketone. At the onset of this work, it was not known which of the two possible rearranged products, **3** or **4** would result from ring expansion of either of the two epimeric aminoalcohols. The course of the Tiffeneau-Demjanov rearrangement and the mechanistically related homologation of ketones with diazomethane have been shown to have



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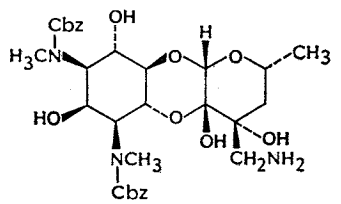

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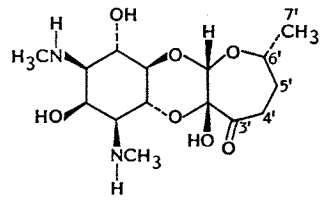

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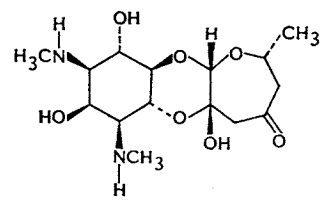

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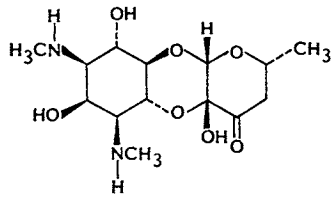

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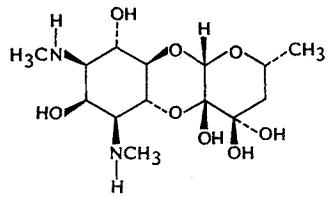

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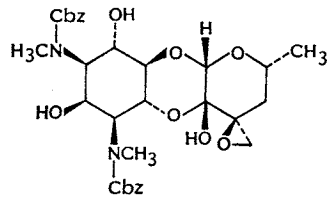

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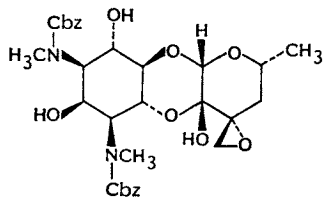

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a strong dependance on stereochemistry, reaction conditions, and the nature of the substituents on the migrating carbon atoms. On the basis of literature precedent, we can predict that in the absence of other overriding factors the more electron rich carbon atom, C-4′, should migrate generating isomer **3**. In addition to the desired rearrangement products, diazotization of the amine could also lead to ring closure by attack of the hydroxyl group to give an epoxide, or attack by the aqueous solvent to afford the corresponding diol. Both such competing reaction pathways are well documented in the literature.[7~9]

Either of the two spectinomycin homologs would be of interest in helping refine our theories regarding structure activity relationships in this class of antibiotics. Spectinomycin (**5**) exists in aqueous solution as the ketone hydrate (**6**). Ketone **3** would be of interest since it retains the carbonyl group α to the 2′-hemiketal functionality as is present in spectinomycin. This arrangement is responsible for the highly electrophilic character at C-3′ which provides the driving force for the formation of the ketone hydrate. Ketone **4**, with its carbonyl group isolated from the hemiketal would not be expected to hydrate as readily.

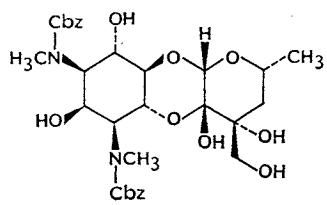
The first attempt at ring-expansion was done on 3′-(*S*)-*N*,*N*′-dibenzyloxycarbonyl-3′-aminomethyldihydrospectinomycin (**1**). Treatment of **1** with $NaNO_2$ in aqueous acetic acid led to the very rapid formation of a single new product in 95% yield. The crude product, which showed no impurities by TLC, was identified on the basis of direct comparison (TLC and ^{13}C NMR) as epoxide **7** which had

been prepared earlier *via* an alternative route.[10] No evidence was seen for the presence of any ring-expanded materials. The remaining 5% of the theoretical yield was recovered from the diazotization as unreacted starting material. Although epoxide formation is often a competing side reaction in the acid-catalyzed rearrangement of vicinal aminoalcohols, we are unaware of any other instances where this is the sole reaction pathway. KIRMSE and GRUBER[8] have reported a case where epoxide formation is a minor pathway in acidic medium but becomes the sole course of reaction under alkaline conditions. While not providing the desired homospectinomycin, this sequence represents the best synthesis of epoxide **7**, a versatile synthetic intermediate.

Although disappointed by the lack of rearrangement in the 3′-*S* series, the diazotization was attempted on **2**, in hopes of at least preparing the more important *R*-epoxide **8**. Treatment of amino-alcohol **2** with $NaNO_2$ in aqueous acetic acid resulted in a slower reaction than in the prior instance and the formation of three products in roughly equal amounts. After separation by chromatography, two of the components were identified as epoxide **8** and diol **9**. The third product was identified as the desired ring-expansion product **10**. A variety of reaction conditions were examined, but all resulted in nearly equal amounts of the three products.

N,N′-Dibenzyloxycarbonylhomospectinomycin (**10**) can be employed as the starting material for the synthesis of a family of analogs related to spectinomycin. For example, reduction of **10** with ethanolic sodium borohydride gives the corresponding dihydrohomospectinomycin derivative **11**. Deprotection of **10** and **11** by transfer hydrogenolysis[11] (palladium black - formic acid) gave homospectinomycin **3** and dihydrohomospectinomycin **12** which were isolated as the dihydrochloride salts.

The structure of **3** was assigned on the basis of ^1H and ^{13}C NMR spectra which ruled out the alternative rearrangement product **4**. Initially, we were reluctant to make this assignment due to the presence of a carbonyl carbon in the ^{13}C NMR spectrum and no indication of any ketone hydrate, the only form observed for spectinomycin. This led us to believe that the product might be **4**, even though this would not be the product predicted on the basis of the electronic nature of the potential migrating carbon atoms. Analysis of the 200 MHz ^1H NMR spectrum confirmed the assignment as **3**. Decoupling experiments readily established the connectivity of the carbon atoms labelled 4′,



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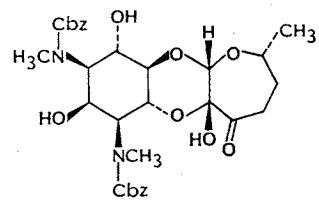

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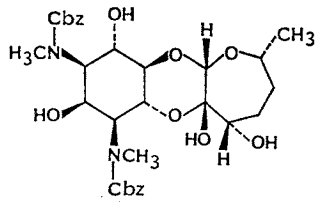

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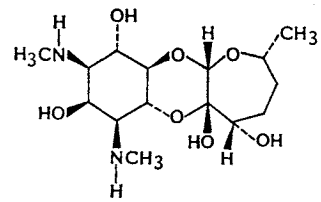

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5′, 6′ and 7′ in the figure. Irradiation of the 7′-methyl group allowed unambiguous identification of the 6′-H multiplet. In a similar fashion, irradiation of the latter resonance identified the complex multiplets for the C-5′ methylene protons. These protons were directly coupled to the C-4′ methylene protons with appropriate coupling constants for the vicinal C-4′ – C-5′ relationship. Values for those coupling constants discernable from the spectrum are cited in the experimental section.

In light of the complete hydration of the parent six-membered ring compound, the lack of hydration of ketone **3** deserves comment. Examination of molecular models provides a possible explanation for this observation. In saturated seven-membered rings there is no conformation that is free of both transannular and Pitzer strain.[12] The ketone form of **3**, with its sp^2 carbon atom can adopt a conformation without Pitzer strain, which has a pseudoequatorial 6′-methyl group and only one transannular interaction between hydrogen atoms at C-1′ and C-4′. The corresponding ketone hydrate, however, with an sp^3 carbon at C-3′ has numerous additional steric problems arising from the geminal hydroxyl groups. These interactions are not present in the parent spectinomycin ketone hydrate. The magnitude of the steric interactions in **3** is apparently sufficient to favor the ketone over the ketone hydrate form, at least to the limits of detectability by ^{13}C NMR.

Biological Evaluation

The results of *in vitro* antimicrobial testing for **3** and **12** are shown in Table 1. Homospectinomycin is equal to the parent *versus* some of the organisms tested, but is significantly less active against others. The dihydro-analog **12** is, as expected,[13] much less active than the ketone. *In vivo*, homospectinomycin had approximately one-third the activity of the parent. It was nontoxic in mice at 100 mg/kg, the highest dose tested.

These results are of interest in light of our knowledge of the structure-activity relationships of the various C-3′ modified spectinomycin analogs. The most potent members of the spectinomycin family have two heteroatom substituents (hydroxyl or amino) capable of hydrogen bonding at or near carbon 3′.[10] The geminal hydroxyl groups of the spectinomycin ketone hydrate being representative. Homospectinomycin, however, is shown by ^{13}C NMR to exist primarily if not exclusively in the depicted ketone form. Whether or not the actual bioactive form of **3** is a small equilibrium amount of hydrate is unknown. The present results do show a tolerance of the spectinomycin binding site for the expanded ring which has different spatial requirements than the parent.

Table 1. MIC (μg/ml)[a].

Organisms	**5**	**3**	**12**
Staphylococcus aureus UC 76	10	20	80
Streptococcus faecalis UC 694	40	160	>160
Escherichia coli UC 45	5	10	40
Klebsiella pneumoniae UC 58	2.5	5	40
Pseudomonas aeruginosa UC 95	20	80	>160
Proteus vulgaris UC 93	10	80	>160
P. mirabilis UC 6671	10	40	160
Shigella flexneri UC 143	10	10	40
Salmonella typhi UC 215	5	5	40
Serratia marcescens UC 131	5	20	80

[a] All compounds tested as the dihydrochloride salt.

Experimental

^{13}C NMR spectra were recorded on a Varian CFT-20 or FT80A spectrometer in the indicated solvent using $(CH_3)_4Si$ or CH_3CN (for D_2O solutions) as internal standards. Chemical shifts are reported in ppm downfield from $(CH_3)_4Si$. ^1H NMR spectra were recorded on a Varian EM 390 spectrometer in the indicated solvent with external reference for D_2O solutions. Mass spectra and optical rotations were measured by the Physical and Analytical Chemistry Unit of The Upjohn Company.

The MIC *versus* various bacteria was determined by a microplate broth dilution technique. Serial 2-fold dilutions of the antibiotic were prepared in 50 μl of modified brain-heart infusion medium[14] in the wells of a microplate. Each well was then inoculated with 50 μl of standardized cell suspension to yield a final concentration of *ca.* 10^5 viable cells per ml of drug supplemented medium. The microplates were incubated at 37°C for 20 hours and the MIC was read as the lowest concentration of drug that inhibited visible growth of the organism.

Diazotization of *N,N'*-Dibenzyloxycarbonyl-3'-(*S*)-3'-(aminomethyl)dihydrospectinomycin

In 14 ml of AcOH - H_2O (1 : 1) is dissolved 761 mg (1.2 mmol) of *N,N'*-dibenzyloxycarbonyl-3'-(*S*)-3'-(aminomethyl)dihydrospectinomycin. To this solution is added 416 mg (6.0 mmol) of $NaNO_2$. An immediate evolution of gas is observed. The reaction is stirred for 5 minutes and concentrated *in vacuo*. The residue is taken up in 30 ml of EtOAc and washed with H_2O (2×20 ml). The combined washes are backwashed with 20 ml of EtOAc. The combined EtOAc extracts are washed with 30 ml brine and dried over $MgSO_4$. Removal of the solvent affords 703 mg of a white solid, whose ^{13}C NMR and TLC mobility are identical with *N,N'*-dibenzyloxycarbonyl 3'-methylene spectinomycin oxide (**7**) prepared *via* epoxidation. The yield of epoxide is 95%. The aqueous washes are made alkaline with concentrated NH_4OH. Extraction with EtOAc and normal workup as described above affords 37 mg of unreacted amine.

N,N'-Dibenzyloxycarbonylhomospectinomycin (**10**)

In 125 ml of H_2O - AcOH (1 : 1) is dissolved 5.0 g (7.92 mmol) of *N,N'*-dibenzyloxycarbonyl-3'-(*R*)-aminomethyldihydrospectinomycin. To this solution is then added 2.7 g (39.6 mmol) of $NaNO_2$. An immediate evolution of N_2 is observed and the reaction is allowed to stir for 40 minutes. The solution is then poured into H_2O (250 ml) and extracted with EtOAc (2×100 ml). The combined extracts are then washed with saturated $NaHCO_3$ (2×100 ml) and 10% aqueous NH_4OH (1×100 ml). The combined washes are backwashed with EtOAc. The combined organics are washed with brine and dried over $MgSO_4$. Removal of the solvent *in vacuo* affords 5.07 g of a white solid. The product is taken up in $CHCl_3$ and chromatographed on 300 g of silica, slurry packed in $CHCl_3$. The column is eluted as follows: 1 liter 1% MeOH - $CHCl_3$, 5 liters 2% MeOH - $CHCl_3$, 1 liter 3% MeOH - $CHCl_3$, 4 liters 5% MeOH - $CHCl_3$, 2 liters 10% MeOH - $CHCl_3$. Each 50-ml fraction was analyzed by TLC. Pure fractions were pooled and concentrated *in vacuo* to afford the final product. In elution volume 2.7~3.95 liters, 1.133 g of *N,N'*-dibenzyloxycarbonylhomospectinomycin (**10**) is obtained; ^{13}C NMR (acetone-d_6) δ 189.0, 138.0, 129.1, 128.3, 99.1, 94.6, 78.0, 74.8, 67.2, 66.5, 65.8, 65.7, 37.1, 36.4, 31.5, 22.2; exact mass was calcd for $C_{40}H_{62}N_2O_{11}Si_{13}$ (tri-TMS) 830.3661, found 830.3653. Also recovered from the column were 1.4 g of epoxide (**8**) and 0.95 g of diol (**9**).

Homospectinomycin Dihydrochloride

To a solution of 100 mg (0.16 mmol) of **10** in 3 ml of MeOH was added 100 mg of palladium black and 86 μl (1.6 mmol) of formic acid. The mixture was stirred 10 minutes at room temperature, filtered and concentrated *in vacuo*. The residue was dissolved in 2 ml of H_2O, treated with 0.35 ml (0.35 mmol) of 1 N HCl and lyophilized to afford 65 mg (0.16 mmol, 100%) of product as a white solid: ^{13}C NMR (D_2O, CH_3CN internal reference) δ 189.9, 98.2, 94.2, 81.6, 70.9, 66.9, 62.3, 60.7, 59.3, 39.3, 37.5, 32.0, 31.5, 21.8; ^1H NMR (200 MHz, D_2O, 50°) δ 5.40 (1H, s, 1'-H), 4.34 (1H, d of d, $J_{3,4}$= 10 Hz, $J_{4,5}$=10 Hz, 4-H), 4.24 (1H, m, 6'-H), 3.99 (1H, dd, $J_{1,6}$=10 Hz, $J_{5,6}$=10 Hz, 6-H), 3.77 (1H, dd, $J_{4,5}$=10 Hz, $J_{5,6}$=10 Hz, 5-H), 3.52 (1H, dd, $J_{2,3}$=2 Hz, $J_{3,4}$=10 Hz, 3-H), 3.36 (1H, dd, $J_{1,2}$=

3 Hz, $J_{1,6}$=10 Hz, 1-H), 3.05 (1H, ddd, $J_{4'A,4'B}$=12 Hz, $J_{4'A,5'A}$=12 Hz, $J_{4'A,5'B}$=3 Hz, 4'A-H), 2.89 (3H, s, NCH_3), 2.88 (3H, s, NCH_3), 2.52 (1H, ddd, $J_{4'A,4'B}$=12 Hz, $J_{4'B,5'A}$=3 Hz, $J_{4'B,5'B}$=6 Hz, 4'A-H), 2.19 (1H, m, $J_{5'A,5'B}$=14 Hz, 5'B-H), 1.69 (1H, m, $J_{5'A,5'B}$=14 Hz, $J_{5'A,6'}$=12 Hz, 5'A-H), 1.25 (3H, d, $J_{6',7'}$=7 Hz, 7'-CH_3); MS exact mass for M^+ of pentakistrimethylsilyl ether, $C_{30}H_{66}N_2O_7Si_5$ requires: 706.3716, found: 706.3739.

N,N'-Dibenzyloxycarbonylhomodihydrospectinomycin (**11**)

In 2 ml of absolute ethanol is dissolved 520 mg (0.85 mmol) of *N,N'*-dibenzyloxycarbonylhomospectinomycin. To this solution is then added 8.0 mg (0.21 mmol) of $NaBH_4$. The reaction is stirred for 2 hours and concentrated *in vacuo*. The residue is then partitioned between EtOAc and water. The water is acidified (pH 2) with 1 N aqueous hydrochloric acid and the EtOAc is separated. The EtOAc is combined with a second EtOAc extract, of the aqueous solution, washed with brine and dried over magnesium sulfate. After filtering, the solvent is removed *in vacuo* to afford 536 mg of a white solid. The product is taken up in $CHCl_3$ and chromatographed on 70 g of silica, slurry-packed in $CHCl_3$. The column is eluted with 1% MeOH in $CHCl_3$ (1.5 liters), followed by 2% MeOH in $CHCl_3$. The desired product is found by TLC analysis of each 40 ml fraction. This afforded 218 mg of a white solid. This product is further purified by reverse-phase chromatography using a C-18 packing. The column is eluted with acetonitrile - water (60 : 40) and the eluent is monitored at 257 nm. Each 20-ml fraction is analyzed by analytical HPLC and pure fractions are combined, concentrated *in vacuo* and extracted with EtOAc. After normal workup there is recovered 109 mg of the *N,N'*-dibenzyloxycarbonylhomodihydrospectinomycin as a white solid: ^{13}C NMR (acetone-d_6) δ 138.5, 129.2, 128.5, 100.4, 96.4, 79.9, 75.8, 75.3, 75.0, 74.8, 67.3, 66.7, 65.0, 61.1, 60.9, 60.1, 58.0, 57.6, 38.1, 31.5, 30.8, 22.6; exact mass calcd for $C_{43}H_{72}N_2O_{11}Si_4$ (tetra-TMS): 904.4213, found: 904.4222.

Homodihydrospectinomycin Dihydrochloride

In 2 ml of MeOH is dissolved 104 mg (0.168 mmol) of *N,N'*-dibenzyloxycarbonylhomodihydrospectinomycin. To this solution is added 100 mg of palladium black followed by 66 μl (1.68 mmol) of formic acid. The reaction is stirred for 20 minutes, filtered and concentrated *in vacuo* to afford 78 mg of a white solid. The product is taken up in water and treated with 1 ml of 1 N aqueous HCl. The solution is frozen and lyophilized to afford 73 mg (100%) of the homodihydrospectinomycin dihydrochloride as a white solid: ^{13}C NMR (acetone-d_6) δ 99.5, 97.0, 82.4, 76.6, 71.5, 67.1, 66.0, 62.4, 60.7, 59.7, 37.4, 31.9, 31.6, 29.8, 22.3; exact mass calcd for $C_{27}H_{60}N_2O_7Si_4$ (tetra-TMS): 636.3477, found: 636.3422.

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