SPECTINOMYCIN MODIFICATION. I

CATALYTIC *N*-DEMETHYLATION OF SPECTINOMYCIN

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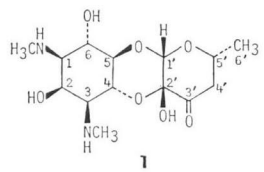
Treatment of an aqueous solution of spectinomycin dihydrochloride with oxygen and platinum black results in *N*-demethylation, affording the partially and fully *N*-demethylated spectinomycins in good yield. The 1: 1 mixture of mono-*N*-demethylspectinomycins has an over all reduced bioactivity relative to the parent, whereas *N*,*N*′-didemethylspectinomycin is nearly devoid of biological activity.

Spectinomycin (**1**) is an aminocyclitol antibiotic used clinically in the treatment of gonorrhea, especially for penicillin resistant strains. Spectinomycin has broad spectrum antibacterial activity and lacks the oto- and nephrotoxicity usually associated with the aminoglycosidic aminocyclitol antibiotics. The broad spectrum activity and lack of toxicity combine to make spectinomycin an attractive candidate for structural modifications aimed at enhancing its potency or further expanding its antibacterial spectrum.

As part of a program for the preparation of analogs of spectinomycin[1,2] we have examined a variety of methods for modification of the intact antibiotic, with the goal of preparing versatile intermediates that will allow access to a variety of analogs. The preparation of such intermediates from the natural product is rendered difficult by a) the high degree of functionality present in the molecule, b) the water solubility of spectinomycin, and c) the lability of the masked α-diketone system at carbons 2′ and 3′.* Exposure of spectinomycin to mild alkali results in rearrangement yielding inactive actinospectinoic acid (**2**).[3,4] This latter problem is of great importance since the C-3′ carbonyl group is required for optimal bioactivity. Reduction of this ketone to give the dihydrospectinomycins improves stability, but greatly reduces the bioactivity.[5] Reoxidation of such dihydro-compounds has proven difficult.



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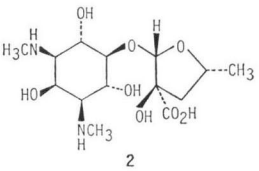

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Recent reports[6,7] have described the synthesis of spectinomycin analogs having modification in the aminocyclitol ring, generally involving oxidation, epimerization or removal of the C-2 or C-6 hydroxyl groups. These compounds, prepared *via* modification of protected 3′-*R*-dihydrospectinomycin, were found to be devoid of antibacterial activity, even in those cases where reoxidation to the C-3′ ketone was successful.

In light of these results, we were previously reluctant to explore modification of the actinamine ring of spectinomycin and have focused our attention on the sugar moiety.[2] A comparison of spectinomycin to the vast majority of aminocyclitol antibiotics, however, suggested that analogs lacking the *N*-methyl groups, which would more closely resemble the 2-deoxystreptamine containing antibiotics such as genta-

* For convenience, we have adopted the aminoglycoside numbering system for all of our spectinomycin analog work.

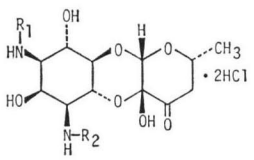
micin, might have improved bioactivity. It was also of interest to determine the effects on bioactivity of replacing the *N*-methyl groups with larger alkyl substituents. In this paper, we describe the successful application of methodology which allows the selective removal of the *N*-methyl groups of spectinomycin, affording biologically active *N*-demethylspectinomycin analogs. The use of these compounds as substrates for the preparation of *N*-alkyl spectinomycin analogs will be the subject of a separate report.

Results and Discussion

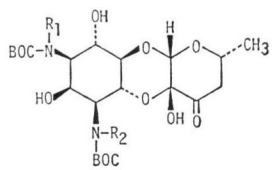
Our goal in this study was to develop methodology that would allow *N*-demethylation under mild conditions which would not further degrade the sensitive substrate. Ideally, the reaction would be conducted in aqueous solution on the parent base-labile 3′-ketone dihydrochloride salt to avoid the protecting group manipulations necessary to achieve organic solvent solubility and to avoid the problems of reoxidizing 3′-dihydro-substrates. These goals were successfully realized through the use of catalytic oxidative *N*-demethylation.

The transition metal catalyzed oxidation of alcohols is a technique that is widely used, especially in carbohydrate chemistry.[8] This methodology has been extended successfully to the oxidation of hydroxyl groups in nitrogen containing substrates, when the nitrogen atoms are protected. Little work has appeared, however, on the oxidation of amines by this method. DAVIS and ROSENBLATT[9] have described the oxidation of *N*-methyl groups in tertiary amines to the corresponding *N*-formyl groups under such catalytic conditions. More recently, the oxidative *N*-dealkylation of tertiary amines in the lincomycin series was reported by BIRKENMEYER and DOLAK.[10] Although no secondary amines were examined by the latter authors, the mildness of their methodology and the ability to work in an aqueous medium made this the logical choice for the attempted removal of the secondary *N*-methyl groups of spectinomycin.

Treatment of an aqueous solution of spectinomycin dihydrochloride with oxygen in the presence of a platinum catalyst results in the removal of the *N*-methyl groups, affording mixtures of the mono- and didemethylated products **3a**～**c**. There is no apparent kinetic preference for selective removal of either of the methyl groups and the monodemethylated spectinomycins are present as a 1 : 1 mixture of **3b** and **3c**. The presence of both isomers is readily determined by analysis of ^{13}C NMR spectra (*vide infra*). Variation of the reaction conditions allows adjustment of the ratio of recovered starting material to the mono- and didemethylated products. Little or no reaction occurs at room temperature. Warming to 50°C, however, provides a convenient reaction rate, while heating to reflux results in significant decomposition. Catalyst poisoning occurs as the reaction proceeds and therefore, the catalyst is best added in portions. Poisoning of the catalyst limits the reaction so that complete conversion to didemethylspectinomycin is impractical. The use of somewhat large quantities of catalyst is not a serious drawback in as much as the platinum is recovered quantitatively for recycling.



3 **a** $R_1=R_2=H$
b $R_1=CH_3$, $R_2=H$
c $R_1=H$, $R_2=CH_3$



4 **a** $R_1=R_2=H$
b $R_1=CH_3$, $R_2=H$
c $R_1=H$, $R_2=CH_3$
d $R_1=R_2=CH_3$

Competitive oxidation of the hydroxyl groups and cleavage of the ring carbon-nitrogen bonds are not serious problems. Reaction for extended periods at elevated temperatures, however, does result in

the production of small amounts of colored by-products with a characteristic phenolic odor, suggesting that some oxidation does occur in the actinamine ring. The base lability of spectinomycins causes problems in separating the product mixture into its component parts. The facile rearrangement of spectinomycin free base dictates that separation be done on salts or *N*-protected derivatives. Several attempts at purification of the hydrochloride salts *via* ion exchange, carbon, cellulose or silica gel chromatography were unsuccessful. Purification was finally achieved by conversion of the mixture to either the benzyloxycarbonyl or *tert*-butoxycarbonyl derivatives. Treatment of the crude reaction mixture with sodium bicarbonate and di-*tert*-butyldicarbonate in aqueous *tert*-butyl alcohol gives the desired *t*-BOC derivatives **4a**~**d**. Chromatography on silica gel serves to separate the mixture. The *t*-BOC derivatives of residual starting material (**4d**) and of *N,N'*-didemethylspectinomycin (**4a**) are obtained in pure form by this process. The chromatography is not sufficient to separate the isomeric monodemethyl derivatives **4b** and **4c** and they are treated as the mixture. Treatment of the *t*-BOC derivatives with gaseous HCl in dichloromethane affords the deprotected products in quantitative yield.

Analysis of the product mixture and the purified products by ^{13}C NMR (Table 1) established that the desired *N*-demethylation had been achieved, with no other alteration of the parent structure. Didemethylspectinomycin exhibited the expected upfield shifts for C-1 and C-3, and downfield shift of C-2 as well as the absence of the N-CH_3 signals. The remainder of the spectrum, notably that portion due to the sugar moiety, was superimposable with that of spectinomycin. The isomeric mixture of monodemethylspectinomycins had a pair of signals for both C-1 and C-3, with chemical shifts corresponding to those found for $-NHCH_3$ and $-NH_2$ bearing carbons. Both types of *N*-methyl groups were also observed. All of these lines were reduced in intensity relative to the remainder of the spectrum by approximately 50%. The signal for C-2 was observed midway between that of spectinomycin and didemethylspectinomycin as expected. Again, no other changes in the spectrum were apparent.

Antimicrobial Activities

The results of *in vitro* antibacterial testing of the *N*-demethylated spectinomycin analogs *vs.* a variety of Gram-positive and Gram-negative bacteria are presented in Table 2.

Removal of both *N*-methyl groups as in **4a** results in a dramatic decrease in bioactivity, although weak activity can still be demonstrated. The isomeric mixture of mono-*N*-demethylspectinomycins (**4b** & **4c**) has an altered spectrum with activity equal to the parent against some organisms, but greatly reduced activity in other cases. Since the monodemethylspectinomycins were tested as a mixture, the data do not reveal whether the two isomers are equally active or if the bulk of the activity resides in one isomer. The presence of the *N*-methyl groups in spectinomycin clearly enhances activity relative to the *N*-demethylated analogs. The use of the *N*-demethylspectinomycins as substrates for the

Table 1. Selected ^{13}C NMR chemical shifts*.

Compound	C-1	C-2	C-3	NCH_3's
Spectinomycin (**1**)	63.0	61.2	60.0	32.3 / 31.9
N,N'-Didemethylspectinomycin (**4a**)	55.6	66.0	52.6	Absent
Isomeric monodemethyl-spectinomycins (**4b**/**4c**)	62.5 / 55.6	63.2	59.5 / 52.6	31.9 / 31.4

* Spectra recorded on the dihydrochloride salts in D_2O.

Table 2. Minimum inhibitory concentration.

Organism	UC® #	Spectinomycin (**1**) (μg/ml)	Mono-*N*-demethyl-spectinomycins (**4b** and **4c**) (μg/ml)	Di-*N*-demethyl-spectinomycin (**4a**) (μg/ml)
Staphylococcus aureus	76	7.8	15.6	250
Streptococcus faecalis	694	31.2	125	>1000
Escherichia coli	45	7.8	62.5	>1000
Klebsiella pneumoniae	58	2.0	15.6	125
Pseudomonas aeruginosa	95	31.2	62.5	250
Proteus vulgaris	93	7.8	15.6	125
Proteus mirabilis	6671	3.9	15.6	1000
Serratia marcescens	131	3.9	3.9	31.2
Shigella flexneri	143	3.9	15.6	250
Salmonella typhi	215	3.9	62.5	>1000

* Compounds tested as dihydrochloride salts.

introduction of *N*-alkyl groups other than methyl, and the bioactivity of these analogs will be described in a subsequent report.

Experimental

^{13}C NMR spectra were recorded on a Varian CFT-20 or FT80A spectrometer in the indicated solvents using Me_4Si or CH_3CN (for D_2O solutions) as an internal standard. Chemical shifts are recorded in parts per million downfield from Me_4Si. TLC analyses were carried out on Analtech plates coated with silica gel G and containing a UV phosphor. Mass spectra, optical rotations and melting points were measured by the Physical and Analytical Chemistry Unit at The Upjohn Company.

Oxidative *N*-Demethylation of Spectinomycin

Selection for the Mono-demethylated Products **3b** and **3c**: Platinum oxide (6.0 g, 26.4 mmole) was reduced in 20 ml of deionized water for 3 hours at 2.8 kg/cm^2 of hydrogen on the Parr hydrogenation apparatus. Spectinomycin dihydrochloride pentahydrate (20.0 g, 40.4 mmole), was dissolved in 200 ml of water, the platinum catalyst was added and the solution was warmed to 60°C. The suspension was rapidly stirred and oxygen gas was passed through the solution *via* a gas dispersion tube for 17 hours. The catalyst was removed by filtration and an aliquot was lyophilized for analysis. The silylated sample (1: 1 DMF/HMDS, 50°C, 30 minutes) showed three major peaks by gas chromatography (3% OV-17, 80 cm, 150~250°C at 5°C/minute) corresponding to spectinomycin (**1**), the isomeric monodemethylated compounds **3b** and **3c**, and the didemethylated **3a** in a ratio of 25: 52: 14, respectively.

The volume was reduced to *ca.* 100 ml and the solution was cooled to 0°C. $NaHCO_3$ (9.0 g, 107 mmole) was added, the mixture was stirred 10 minutes and a solution of 19.2 g (88.0 mmole) of di-*tert*-butyldicarbonate in 120 ml of *tert*-butanol was added. The mixture was warmed to room temperature and stirred 21.5 hours. The butanol was removed *in vacuo*, 100 ml of water was added and the mixture was extracted with 500 ml of EtOAc. The EtOAc was washed with 100 ml of water, 100 ml of brine, dried over $MgSO_4$ and concentrated *in vacuo* to give 16.8 g of yellow glass. Chromatography on 600 g of silica gel (MeOH - $CHCl_3$ gradient 2→4%) gave the following fractions, pooled on the basis of TLC (10% MeOH - $CHCl_3$): 0.36 g of **4d**; 1.94 g of a mixture of **4d** and the isomers **4b** and **4c**; 3.14 g of the isomeric mixture **4b** and **4c**; 3.48 g of a mixture of **4a** and the isomers **4b** and **4c**; 1.49 g of **4a**. The isomeric mono-demethyl compounds **4b** and **4c** are not resolved by this method.

For the isomeric mixture of *N,N'*-di-*tert*-butoxycarbonyl-mono-*N*-demethylspectinomycins **4b** and **4c**: ^{13}C NMR (CD_3COCD_3) δ 191.5, 157, 97.4, 97.1, 92.0, 79.6, 79.2, 74.8, 74.6, 72.7, 71.1, 70.0, 69.2, 68.2, 67.0, 66.0, 60.1, 57.1, 56.8, 54.3, 45.6, 28.9, 28.6, 21.6; IR ($CHCl_3$) 3500, 3000, 1730, 1680, 1490,

1440, 1380, 1350, 1240, 1150, 1120, 1050, 870 cm^{-1}; $[\alpha]_D$ −12° (*c* 0.916, $CHCl_3$); mp 153~180°C (decomposition); MS (for tristrimethylsilylether) $C_{32}H_{62}N_2O_{11}Si_3$ requires 734.3661, found 734.3665.

For *N,N'*-di-*tert*-butoxycarbonyl-*N,N'*-didemethylspectinomycin **4a**: ^{13}C NMR (CD_3COCD_3) δ 156.7, 97.1, 91.9, 79.1, 74.1, 71.1, 69.3, 68.2, 68.0, 56.7, 53.4, 45.7, 28.6, 21.6; IR ($CHCl_3$) 3500, 3000, 1690, 1500, 1445, 1380, 1360, 1240, 1160, 1130, 1060, 1040, 975, 870 cm^{-1}; $[\alpha]_D$ −15° (*c* 0.893, $CHCl_3$); mp 160~180°C (decomposition); MS (for tristrimethylsilylether) $C_{31}H_{60}N_2O_{11}Si_3$ requires 720.3505, found 720.3481.

Selection for the Didemethylated Product **3a**: A total of 12 g of PtO_2 was prereduced as described above. Spectinomycin dihydrochloride pentahydrate (20.0 g, 40.4 mmole) was dissolved in 250 ml of deionized water and heated to 50°C with rapid stirring. Oxygen was bubbled through the solution and the catalyst was added in portions over five days. After four additional days, the catalyst was removed by filtration, the filtrate was treated with 2 g of Darco decolorizing carbon, filtered and lyophilized to afford 15.2 g of pale yellow solid. Analysis by GC as above showed the presence of the isomeric monodemethylated compounds **3b** and **3c** and the fully demethylated product **3a** in a ratio of 19 to 43. Purification of the mixture was accomplished in the manner described previously.

Deprotection: Preparation of *N,N'*-Didemethylspectinomycin Dihydrochloride (**3a**)

N,N'-Di-*tert*-butoxycarbonyl-*N,N'*-didemethylspectinomycin (**4a**, 1.50 g, 3.0 mmole) was dissolved in 300 ml of CH_2Cl_2 and cooled with an ice bath. Gaseous HCl was bubbled through the solution for 60 seconds and the mixture was stirred 1 hour at 0°C. Removal of solvent *in vacuo* gave 1.14 g (3.0 mmole, 100%) of product **3a** as a white solid: mp 186~210°C (decomposition); ^{13}C NMR (D_2O, CH_3CN internal standard) δ 94.6, 92.7, 71.0, 69.3, 67.2, 66.8, 66.0, 55.6, 52.6, 42.6, 20.8; IR (KBr) 3400, 2900, 1600, 1500, 1370, 1150, 1050, 1020, 960 cm^{-1}; $[\alpha]_D$ +15° (*c* 0.726, H_2O); MS (for pentakistrimethylsilyl ether) $C_{27}H_{60}N_2O_7Si_5$ requires 664.3247, found 664.3294.

Deprotection: Preparation of the Isomeric Mixture of Mono-*N*-demethylspectinomycin Dihydrochlorides (**3b** and **3c**)

A solution of 200 mg (0.39 mmole) of the **4b**~**4c** mixture in 40 ml of CH_2Cl_2 was cooled with an ice bath and gaseous HCl was passed into the solution for 30 seconds. The mixture was stirred 40 minutes at 0°C and concentrated *in vacuo* to give 150 mg (0.38 mmole, 98%) of the mixture of **3b** and **3c** as a white powder: mp 186~210°C (decomposition); ^{13}C NMR (D_2O, CH_3CN internal standard) δ 94.5, 92.7, 70.8, 69.3, 67.0, 66.7, 63.2, 62.5, 59.5, 55.6, 52.6, 42.5, 31.9, 31.4, 20.7; IR (KBr) 3500, 3000, 1750, 1600, 1530, 1460, 1380, 1050 cm^{-1}; $[\alpha]_D$ +8° (*c* 0.93, H_2O); MS (for pentakistrimethylsilyl ether) $C_{28}H_{62}N_2O_7Si_5$ requires 678.3403, found 678.3420.

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) *vs.* various bacteria was determined by a microplate broth dilution technique. Serial twofold dilutions of the antibiotic were prepared in 50 μl of modified brain-heart infusion broth medium[11] in the wells of a microplate. Each well was then inoculated with 50 μl of a standardized cell suspension to yield a final concentration of ~10^5 viable cells per milliliter 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.

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