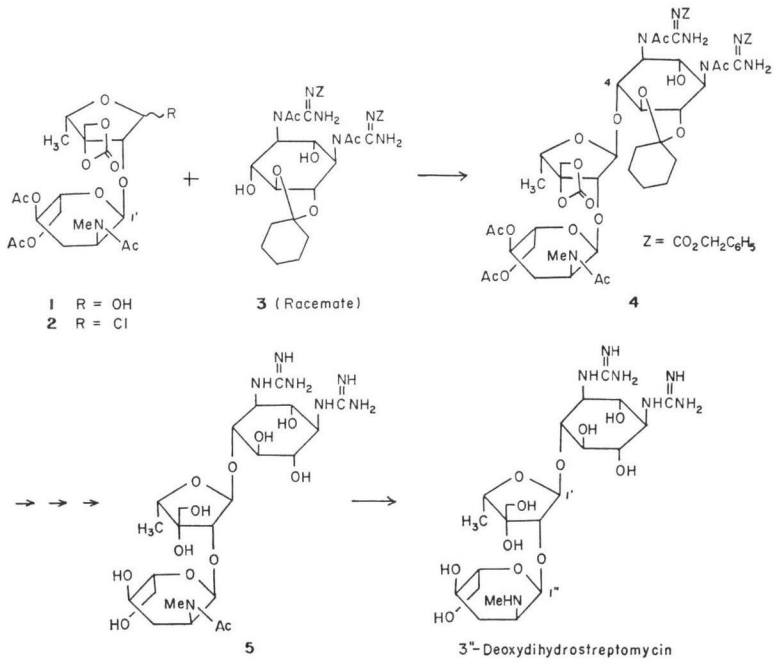
SYNTHESIS OF 3″-DEOXYDIHYDROSTREPTOMYCIN ACTIVE AGAINST RESISTANT BACTERIA

Sir:

Recent studies[1,2,3] on the mechanism of inactivation of aminoglycoside antibiotics by resistant bacteria have shown that naturally isolated drug-resistant organisms produce enzymes which phosphorylate, adenylylate, or acetylate the special positions of the drugs. In the case of streptomycin or dihydrostreptomycin, *E. coli* carrying R factor[4,5,6] and resistant *Pseudomonas*[7,8] produce intracellular enzymes which catalyze the reaction of streptomycins with ATP leading to 3″-O-adenylylated or 3″-O-phosphorylated derivatives. General methods to overcome the inactivation of aminoglycoside antibiotics have recently been established by us.[9] One of these is to remove the functional group to be attacked. If, therefore, the 3-hydroxyl group of 2-methylamino-2-deoxy-L-glucose moiety of streptomycin is removed, then streptomycin should be transformed to a derivative which does not undergo the reaction of the above enzymes. If the 3-hydroxyl group in question is not essential for the antibacterial activity of streptomycin, the derivative should inhibit the growth of resistant organisms. We now wish to report the synthesis and microbial activity of 3″-deoxydihydrostreptomycin.

2-O-(2-Acetamido-4,6-di-O-acetyl-2,3-dideoxy-N-methyl-α-L-glucopyranosyl)-3,3′-O-carbonyldihydrostreptose (**1**)*, which was prepared from dihydrostreptobiosamine *via* 16 steps as will be reported in another paper,[10] was dissolved in thionyl chloride and, after standing for 18 hours, the solution was concentrated to give a glycosyl chloride (**2**)** as a solid. The chloride was condensed (at room temperature, 65 hours) with di-N-acetyl-di-N-benzyloxycarbonyl-4,5-O-cyclohexylidenestreptidine[11] (racemate, **3**) in dichloromethane in the presence of mercuric cyanide and molecular sieves 4A. The mixture of the condensation products was separated by column chromatography on silica gel with benzene - methyl ethyl ketone (5: 3, gradually changed to 1: 1) as the developer to give three condensation products **4** (56 mg from 1 g of **1**), **4′** (620 mg) and **4″** (431 mg) in the order of elution. They all were confirmed by elementary analysis and NMR spectra to be the expected condensation products.

Treatment of each of **4**, **4′** and **4″** with ammonia saturated in methanol removed the O-acetyl, carbonate, and N-acetyl (at the guanidino group) groups simultaneously as described in a previous



* The characteristics of **1** were as follows: $[\alpha]_D^{20} - 127°$ (*c* 1, chloroform); IR (KBr): 1820 (carbonate), 1750 (ester), 1640 (amide I) cm^{-1} ~1550 cm^{-1} (amide II) was not observed; NMR ($CDCl_3$): δ 1.30 and 1.40 (totally 3H doublets in the ratio of ~3: 1, J=6 Hz, CCH_3; appearance of two doublets will be assigned to the anomeric mixture of **1**), 2.06 (6H s, OAc), 2.13 (3H s, NAc), 2.95 (3H s, $AcNCH_3$), 5.39 (1H d, J=4 Hz, H-1′). The signals ascribable to 3-deoxy protons of L-glucosamine moiety were overlapped with other signals. Found: C, 49.67; H, 6.27; N, 2.51%. Calcd for $C_{20}H_{29}NO_{12}\cdot 1/2H_2O$: C, 49.58; H, 6.24; N 2.89%.

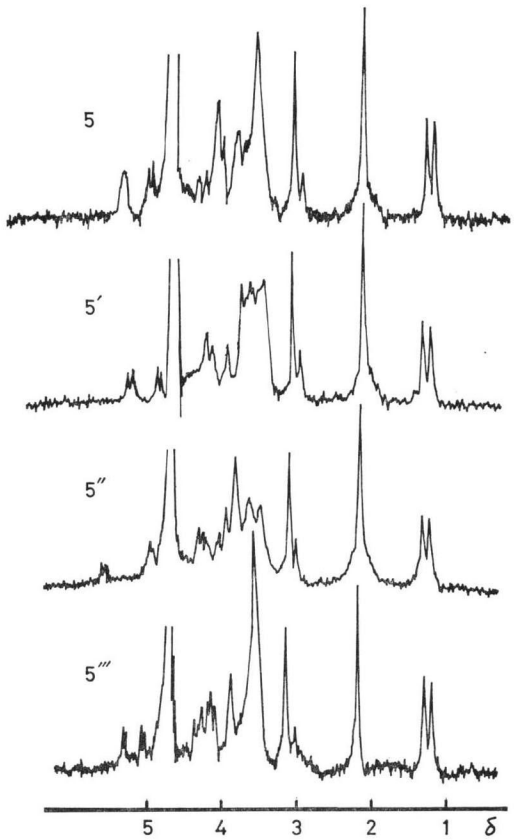
** In the NMR spectrum of **2** in $CDCl_3$. a slightly broadened singlet assignable to H-1 appeared at δ 6.2.

paper from the synthesis of dihydrostreptomycin.[12] Successive treatment of the products with palladium and hydrogen in aqueous dioxane and with 50% acetic acid (55°C, 2.5 hours) removed the benzyloxycarbonyl and cyclohexylidene groups, respectively. Purification of the resulting products by column chromatography with Amberlite CG 50 (NH_4 form) resin and ammonium carbonate as the developer with gradient increase from 0.5 to 8% gave the N-acetyl (at N-methyl group) compounds (**5**,**5**′ and **5**″), which were then transformed to the corresponding dihydrochlorides by passing through a column of Dowex 1×2 (Cl form) with water. Compound **5**: 21 mg, Rf 0.54 (tlc with microcrystalline cellulose powder "Avicel" (Funakoshi Co.) with pyridine - water - ethyl acetate - acetic acid, 5 : 3 : 2 : 1), $[\alpha]_D^{25}-64°$ (*c* 1, water); found: C, 39.53; H, 6.38; N, 13.67%; Calcd for $C_{23}H_{43}N_7O_{12}\cdot 2HCl\cdot H_2O$: C, 39.43; H, 6.76; N, 14.00%. Compound **5**′: 210 mg, Rf 0.46. Compound **5**″: 86 mg, Rf 0.49. For reference, we prepared N-acetyldihydrostreptomycin(**5**‴) by acetylation of dihydrostreptomycin with Ac_2O-Na_2CO_3 in aqueous acetone and its Rf value was confirmed to be 0.43.

Comparison of the NMR spectra of **5**, **5**′ and **5**″ with that of **5**‴ (Chart 1) showed that only the spectra of **5** bore resemblance to **5**‴, suggesting that **5** is N-acetyl-3″-deoxydihydrostreptomycin.

The final step, the removal of the acetyl group of **5** was effected with aqueous barium hydroxide (5.5%, 60°C, 2 hours), which was used to avoid the hydrolysis of the guanidino groups (to urea). In spite of this mild conditions, however, 3″-deoxydihydrostreptomycin was obtained in a low yield (~18%). It should be noted here that compound **5**′ and **5**″ resisted the deacetylation, whereas the deacetylation of **5**‴ gave dihydrostreptomycin quantitatively, indicating a remarkable influence of the 3″-deoxy function on the hydrolysis. The crude deacetylation product from **5** was purified by column chromatography with Amberlite CG 50 resin (NH_4 form, developed with 0.5~10% $(NH_4)_2CO_3$) to afford 3″-deoxydihydrostreptomycin as a carbonate, $[\alpha]_D^{20}-85°$ (*c* 0.15, water), $Rf_{\text{dihydrostreptomycin}}$ 1.1 (tlc with "Avicel" with pyridine - water - ethyl acetate - acetic acid, 5: 3: 2: 1); NMR (D_2O): δ 1.20 (3H d, J=6 Hz, CCH_3), 2.30 (3H s, NCH_3), 5.04 (1H d, J=3.5 Hz, H-1″), 5.3 (1H d, J=2 Hz, H-1′); Positive for SAKAGUCHI and diacetyl reagents.

Chart 1. NMR spectra of N-acetyl-3″-deoxydihydrostreptomycin (**5**) and its isomers (**5**′ and **5**″) and N-acetyldihydrostreptomycin (**5**‴) in D_2O



The synthesized 3″-deoxydihydrostreptomycin exhibited antibacterial activities for both usual and resistant strains as shown in Table 1. This is the first successful modification in the streptomycin series. This shows that the dehydroxylation procedure applied so far to kanamycins and other aminoglycoside antibiotics having 2-deoxystreptamine moiety is also applicable to antibiotics having streptidine moiety.

Deacetylation of **5**′ and **5**″ in a similar manner as described above gave the corresponding 3″-deoxydihydrostreptomycin isomers respectively. These, however, had no antibacterial activity.

Acknowledgement

The authors are indebted to Dr. H. NAGANAWA of Institute of Microbial Chemistry for carrying out NMR spectra.

Table 1. Antibacterial spectrum of 3″-deoxydihydrostreptomycin (DODSM) compared with dihydrostreptomycin (DSM).

Test organisms*	Minimal inhibitory concentration (mcg/ml)	
	DO-DSM	DSM
Staphylococcus aureus FDA 209P	3.12	3.12
Staphylococcus aureus MS 8800***	>25.0	>25.0
Sarcina lutea PCI 1001	0.78	1.56
Bacillus subtilis NRRL B-558	0.78	0.39
Bacillus agri	>25.0	>25.0
Klebsiella pneumoniae PCI 602	3.12	3.12
Escherichia coli NIHJ	1.56	3.12
Escherichia coli NIHJ SMf	>25.0	>25.0
Escherichia coli K-12	1.56	1.56
Escherichia coli K-12 ML 1629	1.56	>25.0
Escherichia coli K-12 ML 1410	3.12	3.12
Escherichia coli K-12 W 677	0.78	0.78
Escherichia coli K-12 JR66/W 677	12.5	>25.0
Escherichia freundii GN 346	12.5	>25.0
Salmonella typhi T-63	0.78	25.0
Shigella dysenteriae JS 11910	3.12	3.12
Enterobacter sp. A 21238	>25.0	>25.0
Alkaligenes sp. A 21383	>25.0	>25.0
Pseudomonas aeruginosa A 3	12.5	12.5
Pseudomonas aeruginosa No. 12	>25.0	>25.0
Pseudomonas aeruginosa H 9	>25.0	>25.0
Pseudomonas aeruginosa TI-13	>25.0	>25.0
Pseudomonas aeruginosa GN 315	>25.0	>25.0
Pseudomonas aeruginosa A 21509	>25.0	>25.0
Pseudomonas maltophilia A 21550	>25.0	>25.0
Proteus rettgeri GN 311	6.25	3.12
Proteus rettgeri GN 466	6.25	3.12
Proteus mirabilis IFM OM-9	12.5	25.0
Serratia marcescens	12.5	12.5
Mycobacterium smegmatis ATCC 607**	0.39	0.39

* Agar dilution streak method (nutrient agar, 37°C, 18 hours)

** 48 hours

*** PC, TC, SM, EM-Resistant strain

HIROSHI SANO
TSUTOMU TSUCHIYA
SHIGERU KOBAYASHI*
MASA HAMADA
SUMIO UMEZAWA
HAMAO UMEZAWA

Institute of Bioorganic Chemistry, 1614 Ida, Nakahara-ku, Kawasaki, Japan

* Department of Applied Chemistry, Faculty of Engineering, Keio University, Hiyoshi, Yokohama, Japan

(Received June 25, 1976)

References

1) UMEZAWA, H.; M. OKANISHI, S. KONDO, K. HAMANA, R. UTAHARA, K. MAEDA & S. MITSUHASHI: Phosphorylative inactivation of aminoglycosidic antibiotics by *E. coli* carrying R factor. Science 157: 1559~1561, 1967

2) UMEZAWA, H.: Mechanism of inactivation of aminoglycosidic antibiotics by enzymes of resistant organisms of clinical origin. Progress in Antimicrobial and Anticancer Chemotherapy. Vol. 2: pp. 567~571, University of Tokyo Press, 1970

3) UMEZAWA, H.: Biochemical mechanism of resistance to aminoglycosidic antibiotics. Advan. Carbohyd. Chem. Biochem. 30: 183~225, 1974

4) UMEZAWA, H.; S. TAKASAWA, M. OKANISHI & R. UTAHARA: Adenylylstreptomycin, a product of streptomycin inactivated by *E. coli* carrying R factor. J. Antibiotics 21: 81~82, 1968

5) TAKASAWA, S.; R. UTAHARA, M. OKANISHI, K. MAEDA & H. UMEZAWA: Studies on adenylylstreptomycin, a product of streptomycin inactivated by *E. coli* carrying the R factor. J. Antibiotics 21: 477~484, 1968

6) OZANNE, B.; R. BENVENISTE, D. TIPPER & J. DAVIES: Aminoglycoside antibiotics: Inactivation by phosphorylation in *E. coli* carrying R factor. J. Bact. 100: 1144~1146, 1969

7) DOI, O.; M. OGURA, N. TANAKA & H. UMEZAWA: Inactivation of kanamycin, neomycin, and streptomycin by enzymes obtained in cells of *Pseudomonas aeruginosa*. Appl. Microbiol. 16: 1276~1281, 1968

8) KAWABE, H.; F. KOBAYASHI, M. YAMAGUCHI, R. UTAHARA & S. MITSUHASHI: 3″-Phosphoryldihydrostreptomycin produced by the inactivating enzymes of *Pseudomonas aeruginosa*. J. Antibiotics 24: 651~652, 1971

9) UMEZAWA, S.: Structures and synthesis of aminoglycoside antibiotics. Advan. Carbohyd. Chem. Biochem. 30: 111~182, 1974

10) Details will be reported in Bull. Chem. Soc. Japan.

11) UMEZAWA, S.; Y. TAKAHASHI & T. TSUCHIYA: Synthesis of di-N-acetyl-di-N-benzyloxycarbonyl-O-cyclohexylidenestreptidine. Bull. Chem. Soc. Japan 48: 560~562, 1975

12) UMEZAWA, S.; T. YAMASAKI, Y. KUBOTA & T. TSUCHIYA: Total synthesis of dihydrostreptomycin. Bull. Chem. Soc. Japan 48: 563~569, 1975