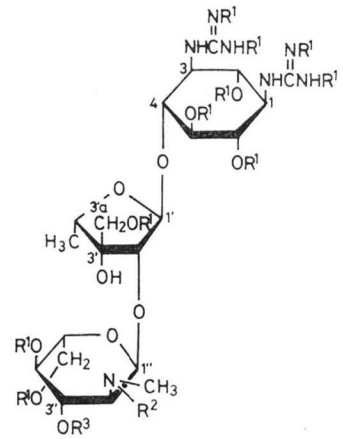
Communications to the editor

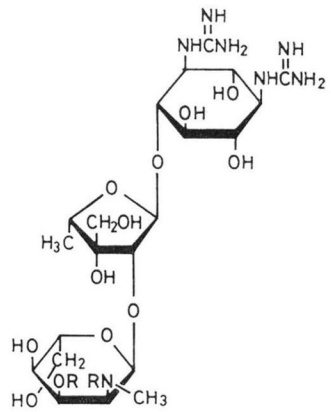
SYNTHESIS OF 3''-*EPI*-DIHYDROSTREPTOMYCIN ACTIVE AGAINST RESISTANT BACTERIA

Sir:

Streptomycin is inactivated by enzymes of resistant bacteria, adenylylating[1,2] or phosphorylating[3,4] the 3''- or the 6-hydroxyl group of the antibiotic. Previously synthesized 3''-deoxydihydrostreptomycin[5,6] had been shown to be active against resistant strains producing 3''-modifying enzymes. This paper describes the synthesis and the antibacterial activity of 3''-*epi*-dihydrostreptomycin (3''-*epi*-DHSM) in order to see the effect of epimerization of the 3''-hydroxyl group in dihydrostreptomycin (DHSM) against DHSM-resistant strains.

2''-*N*-Benzyloxycarbonyl-DHSM[7] monosulfate (**1**) was treated with acetic anhydride and sodium acetate (80°C, 18 hours) with vigorous stirring to give tetra-N^G-acetyl-2,5,6,3'a,3'',4'',6''-hepta-*O*-acetyl-2''-*N*-benzyloxycarbonyl-dihydrostreptomycin (**2**) (98%), $[\alpha]_D^{23}$ −72° (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$): δ 1.20 (3H d, CCH_3), 1.95 (6H), 1.99 (3H), 2.03 (3H), 2.09 (6H) and 2.19 (15 H) (each s, Ac), 2.87 (3H s, NCH_3). Hydrogenolysis (Pd black, H_2 was bubbled for 3 hours) of the *N*-protecting group of **2** in ethanol gave the de-*N*-benzyloxycarbonyl derivative (**3**) (98%), $[\alpha]_D^{23}$ −82° (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$): δ 1.20 (CCH_3), 1.97, 2.00, 2.08 (6H), 2.10, 2.12, 2.14, 2.16, 2.18, 2.21 and 2.22 (each s, 3H except for 2.08, Ac), 2.48 (3H s, NCH_3), 9.06 and 9.27 (each 1H d, *J* 9 Hz, N^GH); Found (Calcd. for $C_{48}H_{63}N_7O_{23}$): C 49.17 (49.37), H 6.09 (6.07), N 9.12 (9.37). To a solution of **3** in ethanol maintained at 25°C, carbon dioxide was introduced until neutral (pH ~7), and the solution was kept at the temperature for 35 hours. Concentration followed by chromatography of the residue gave the 3''-de-*O*-acetyl derivative (**4**) (43%), $[\alpha]_D^{23}$ −71° (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$): δ 2.0~2.3 (30H, Ac), 2.51 (3H s, NCH_3). Treatment of **4** with benzyl chloroformate gave the 2''-





	R^1	R^2	R^3
1	H	$CO_2CH_2C_6H_5$	H
2	Ac	$CO_2CH_2C_6H_5$	Ac
3	Ac	H	Ac
4	Ac	H	H
5	Ac	$CO_2CH_2C_6H_5$	H
6	Ac	$CO_2CH_2C_6H_5$	SO_2CH_3
7	H	$CO_2CH_2C_6H_5$	SO_2CH_3

	R, R
8	>C=O
3''-*epi*-DHSM	H, H

Table 1. Minimal inhibitory concentration (μg/ml) of 3''-*epi*-dihydrostreptomycin and dihydrostreptomycin.

	3''-*epi*-DHSM	DHSM		3''-*epi*-DHSM	DHSM
Staphylococcus aureus FDA 209P	3.12	3.12	*Escherichia coil* K-12 C600 R135[f]	3.12	50
〃 〃 Smith	3.12	1.56	*Mycobacterium smegmatis* ATCC 607	0.78	0.78
〃 〃 APO1[a]	12.5	6.25	*Klebsiella pneumoniae* PCI 602	3.12	3.12
〃 *epidermidis* 109[a]	>100	>100	〃 〃 22#3038[d,e]	25	>100
Micrococcus flavus FDA 16	6.25	3.12	*Shigella dysenteriae* JS 11910	6.25	6.25
〃 *luteus* PCI 1001	0.78	1.56	*Salmonella typhi* T-63	0.78	25
Bacillus subtilis PCI 219	0.78	0.78	*Proteus vulgaris* OX19	1.56	1.56
〃 〃 NRRL B-558	1.56	3.12	〃 *rettgeri* GN311	1.56	1.56
Corynebacterium bovis 1810	3.12	3.12	*Serratia marcescens*	12.5	6.25
Escherichia coli K-12	1.56	1.56	〃 sp SOU	6.25	>100
〃 〃 〃 R5[b]	6.25	>100	*Providencia* sp Pv16[g]	6.25	25
〃 〃 〃 J5 R11-2[c]	3.12	3.12	〃 sp 2991[g]	50	>100
〃 〃 〃 ML1629[c]	1.56	100	*Pseudomonas aeruginosa* A3	3.12	12.5
〃 〃 〃 ML1630	3.12	>100	〃 〃 No. 12	25	25
〃 〃 〃 ML1410	3.12	3.12	〃 〃 H9[c]	>100	>100
〃 〃 〃 〃 R81[c]	12.5	>100	〃 〃 TI-13[c]	50	>100
〃 〃 〃 LA290 R55[d]	3.12	3.12	〃 〃 GN315[b]	25	50
〃 〃 W677	0.78	0.78	〃 〃 99[f]	>100	>100
〃 〃 JR66/W677[d,e]	12.5	>100	〃 〃 B-13	50	>100

Resistance mechanism: [a] AAD(4'), [b] AAC(6'), [c] APH(3')-I, [d] AAD(2''), [e] APH(3')-II, [f] AAC(3), [g] AAC(2').

N-benzyloxycarbonyl-3''-hydroxy derivative (**5**) (91%), $[\alpha]_D^{23}$ −71° (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$): δ 3.02 (3H s, NCH_3). Sulfonylation of **5** with methanesulfonyl chloride in pyridine gave the 3''-*O*-methylsulfonyl derivative (**6**) (91%), $[\alpha]_D^{23}$ −72° (*c* 1, chloroform); ^{1}H NMR ($CDCl_3$): δ 2.73 (3H s, Ms), 3.00 (3H s, NCH_3); Found (Calcd. for $C_{50}H_{69}N_7O_{26}S$): C 49.09 (49.38), H 5.64 (5.72), N 7.76 (8.06), S 2.73 (2.64). Treatment of **6** with 0.2 M sodium methylate in methanol (room temperature, 1.5 hours) gave the de-N^G, *O*-acetyl derivative (**7**) (92%), $[\alpha]_D^{23}$ −66° (*c* 0.5, water); ^{1}H NMR (D_2O): δ 1.20 (CCH_3), 3.01 and 3.06 (each s, 3H in total, NCH_3), 3.16 (3H s, Ms). Attempted removal of the benzyloxycarbonyl group of **7** by hydrogenolysis (atm. pressure of H_2, Pd black, room temperature, 30 minutes) unexpectedly gave 2''-*N*,3''-*O*-carbonyl-3''-*epi*-dihydrostreptomycin (**8**) as the major product (~63%) (IR: 1740 cm^{-1}), which, without purification, was hydrolyzed with aqueous barium hydroxide to cleave the *cis*-fused cyclic carbamate. The crude mixture obtained was chromatographed on a column of Amberlite CG-50 resin (NH_4^+ form) using a gradient of 1~9% anmmonium carbonate to give the desired 3''-*epi*-DHSM as the 3/2 carbonate (36%), $[\alpha]_D^{23}$ −80° (*c* 1, water). The structure was proved by comparison with the identical compound prepared by an unambiguous multistep method[8]. Identity was established by chromatographic mobilities, ^{1}H [D_2O, pD ~9, δ 2.81 (t, H-2''), 5.08 (d, H-1''); $J_{1'',2''}=J_{2'',3''}=$ 4 Hz] and ^{13}C NMR spectra and antibacterial activity.

3''-*Epi*-dihydrostreptomycin showed strong activity (Table 1) against sensitive and resistant bacteria for DHSM, and its activity seems to be comparable with that of 3''-deoxydihydrostreptomycin[5]. It should be stressed that this is a novel example that epimerization of the hydroxyl group give birth to a potent derivative.

TSUTOMU TSUCHIYA
SHUICHI SAKAMOTO
TETSURO YAMASAKI
SUMIO UMEZAWA

Institute of Bioorganic Chemistry,
1614 Ida, Nakahara-ku, Kawasaki 211
Japan

HAMAO UMEZAWA

Institute of Microbial Chemistry,
Kamiosaki, Shinagawa-ku, Tokyo 141,
Japan

(Received February 22, 1982)

References

1) 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
TAKASAWA, S.; R. UTAHARA, M. OKANISHI, K. MAEDA & H. UMEZAWA: Studies on adenylylstreptomycin, a product of streptomycin inactivation by *E. coli* carrying the R-factor. J. Antibiotics 21: 477~484, 1968

2) SUZUKI, I.; N. TAKAHASHI, S. SHIRATO, H. KAWABE & S. MITSUHASHI: Adenylylation of streptomycin by *Staphylococcus aureus*: a new streptomycin adenylyltransferase. *In* Microbial Drug Resistance, *Ed.* S. MITSUHASHI & H. HASHIMOTO, pp. 463~473, Univ. Tokyo Press, 1975

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

4) MILLER, A. L. & J. B. WALKER: Enzymatic phosphorylation of streptomycin by extracts of streptomycin-producing strains of *Streptomyces*. J. Bacteriol. 99: 401~405, 1969

5) SANO, H.; T. TSUCHIYA, S. KOBAYASHI, M. HAMADA, S. UMEZAWA & H. UMEZAWA: Synthesis of 3″-deoxydihydrostreptomycin active against resistant bacteria. J. Antibiotics 29: 978~980, 1976

6) USUI, T.; T. TSUCHIYA, H. UMEZAWA & S. UMEZAWA: Transformation of dihydrostreptomycin into 3″-deoxydihydrostreptomycin. Bull. Chem. Soc. Jpn. 54: 781~786, 1981

7) UMEZAWA, S.; Y. TAKAHASHI, T. USUI & T. TSUCHIYA: Total synthesis of streptomycin. J. Antibiotics 27: 997~999, 1974

8) TSUCHIYA, T. & T. SHITARA: Synthesis of 3″-epidihydrostreptomycin. Carbohydr. Res., 1982, under submission.