TOTAL SYNTHESIS OF STREPTOMYCIN

Sir:

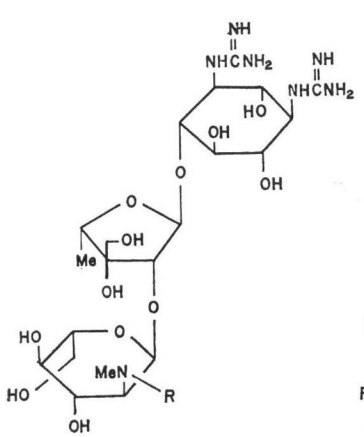
Streptomycin discovered by WAKSMAN and coworkers[1] in 1944 was the first useful *Streptomyces* antibiotic. Its structure was established[2] by 1948 except for the glycosidic linkage between streptose and streptidine which was again shown[3,4] to be α-L. However, the total synthesis of the molecule has not been achieved. We[5] have recently prepared by total synthesis dihydrostreptomycin (DSM) which is produced by hydrogenation[6,7] of streptomycin or by direct fermentation.[8] In this paper, we wish to report the conversion of DSM to streptomycin, thereby completing the first rational synthesis of streptomycin.

When DSM (**1**) trihydrochloride was treated with equimolecular quantities of benzyl chloroformate and sodium carbonate in aqueous acetone with cooling, benzyloxycarbonylation selectively occurred at the N-methyl group of the L-glucosamine portion, giving 2″-N-benzyloxycarbonyldihydrostreptomycin (**2**) dihydrochloride (77 %): $[\alpha]_D^{20}$—71° (*c* 1.5, H_2O); NMR (in D_2O): δ 3.10 (3H s, NCH_3), 7.57 (5H s, C_6H_5). Calcd. for $C_{29}H_{47}N_7O_{14}\cdot 2HCl\cdot H_2O$: C 43.07, H 6.36, N 12.12, Cl 8.77. Found: C 43.20, H 6.20, N 11.93, Cl 8.79.

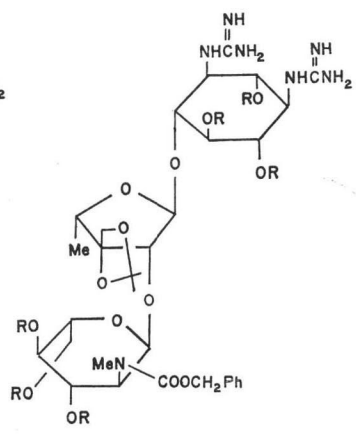
Treatment of **2** with excess 2,2-dimethoxypropane in the presence of a trace amount of *p*-toluenesulfonic acid gave a mixture of per-O-isopropylidenated products. However, on treatment with 20 % acetic acid in methanol at 50°C for 4.5 hours, the mono-isopropylidene derivative (**3**) was obtained; 49 %; $[\alpha]_D^{20}$-73° (*c* 1, H_2O); NMR (in D_2O): δ 1.25 (3H d, CCH_3), 1.27 and 1.37 (each 3H s, isopropylidene), 3.08 (3H s, NCH_3), 7.55 (5H s, C_6H_5). Calcd. for $C_{32}H_{51}N_7O_{14}\cdot 2HCl\cdot H_2O$: C 44.75, H 6.46, N 11.42, Cl 8.26. Found: C 44.93, H 6.18, N 11.10, Cl 8.06. It should be noted that the isopropylidene group in the dihydrostreptose portion is the most stable.

Acetylation of **3** with acetic anhydride in the presence of a catalytic amount of *p*-toluenesulfonic acid at 50°C for 70 hours gave the hexaacetyl derivative (**4**); 92%; $[\alpha]_D^{20}$-60° (*c* 1.4, acetone); NMR (in $CDCl_3$): δ 1.85～2.2 (18H unresolved, m, Ac). Calcd. for $C_{44}H_{63}N_7O_{20}\cdot 2HCl$: C 48.80, H 6.05, N 9.05, Cl 6.55. Found: C 48.66, H 6.05, N 8.76, Cl 6.50.

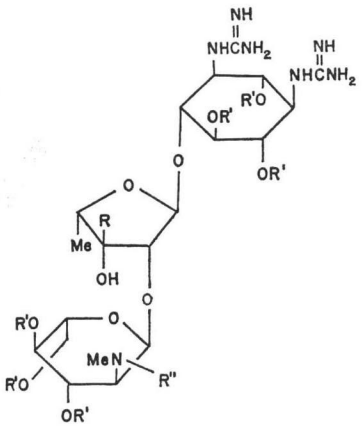
Selective hydrolysis of **4** with 75 % aqueous acetic acid at 55°C for 30 hours led to the compound (**5**), which has free primary and tertiary hydroxyl groups in the dihydrostreptose portion, 86 %; $[\alpha]_D^{20}$-66° (*c* 1.2, acetone); Rf 0.5 (TLC with Avicel, pyridine-ethyl acetate-ether-20% acetic acid (2:2:3:1), visualized by diacetyl). Calcd. for $C_{41}H_{59}N_7O_{20}\cdot 2HCl$: C 47.23, H 5.90, N 9.40, Cl 6.80. Found: C 46.90, H 5.80, N 9.11, Cl 6.99. The structure (**5**) was confirmed by the NMR spectrum (in pyridine-d_5-D_2O): δ 1.55 (3H d, CCH_3), 2.0～2.4 (18H m, Ac), 3.37 (3H s,



1 (DSM): R = H
2: R = $COOCH_2Ph$

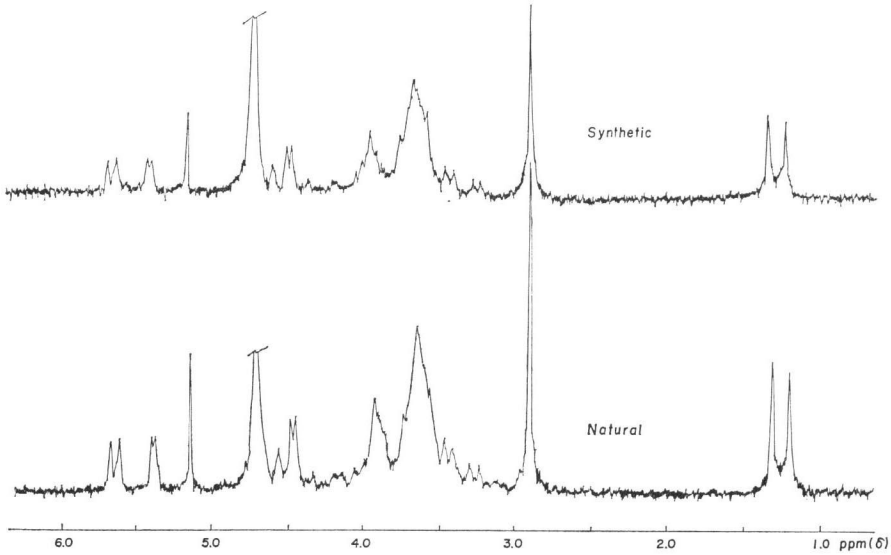


3: R = H
4: R = Ac



5: R = CH_2OH, R′ = Ac, R″ = $COOCH_2Ph$
6: R = CHO, R′ = H, R″ = $COOCH_2Ph$
7 (Streptomycin): R = CHO, R′ = R″ = H

Fig. 1. NMR spectra of natural and synthetic streptomycin in D_2O (DSS 0 ppm), 60 MHz.



NCH_3), 7.53 (5H s, C_6H_5).

Compound **5** was then converted into the aldehyde derivative (**6**) by PFITZNER-MOFFATT oxidation with dimethyl sulfoxide, dicyclohexylcarbodiimide, trifluoroacetic acid, and pyridine at room temperature for 1.5 hours. The desired compound from the reaction product was difficult to isolate, so the crude product was deacetylated with methanolic ammonia and chromatographed on Dowex 1 ×2 (OH form) with water to afford the 2″-N-benzyloxycarbonylstreptomycin (**6**) (18 %); dihydrochloride dihydrate: $[\alpha]_D^{28}$-70° (*c* 1, H_2O); NMR (in D_2O): δ 1.27 (3H d, CCH_3), 3.10 (3H s, NCH_3), 7.60 (5H s, C_6H_5). Calcd. for $C_{29}H_{45}N_7O_{14}\cdot 2HCl\cdot 2H_2O$: C 42.23, H 6.23, N 11.89, Cl 8.60. Found: C 42.21, H 6.09, N 11.63, Cl 8.73. Oxidation with ruthenium tetroxide was unsuccessful.

At this point, we were able to establish the identity with natural material which was prepared from natural streptomycin by benzy-

Table 1. Antibacterial spectra of natural and synthetic streptomycin

Test organisms*	MIC (mcg/ml)	
	Natural	Synthetic
Staphylococcus aureus FDA 209P	3.12	1.56
〃 〃 〃 SM ST f.	>100	>100
Bacillus subtilis NRRL B. 558	12.5	6.25
〃 PCI 219	0.39	0.2
Bacillus agri	>100	>100
Escherichia coli K-12	1.56	1.56
〃 〃 ML 1629	100	100
〃 〃 ML 1410	1.56	1.56
〃 〃 W 677	1.56	3.12
〃 〃 JR 66/W 677	>100	>100
Pseudomonas aeruginosa A3	25	25
Mycobacterium smegmatis ATCC 607**	0.39	0.39

* Agar dilution streak method (nutrient agar, 37°C, 17 hours).
** 48 hours.

loxycarbonylation. The specific rotation, IR and NMR spectra and chromatographic behavior of the synthetic and natural specimens were identical.

Finally, catalytic hydrogenolysis of the synthetic **6** with palladium black in aqueous solution acidified with acetic acid produced streptomycin. It should be noted that, in this case, pretreatment of the aqueous solution of **6** with a small amount of RANEY nickel improved the yield of streptomycin. By chromatography on Dowex 1×2 (Cl form), we readily obtained pure streptomycin (75 %); trihydrochloride: $[\alpha]_D^{25}$ −82° (*c* 1, H_2O) (lit[9] −86.7°, (*c* 1, H_2O)). The synthetic streptomycin trihydrochloride was identical with the natural specimen with respect to the IR and NMR spectra, thin-layer and paper chromatographic behavior, and antibacterial spectra. In the NMR spectra (Fig. 1), we can observe the proton singlet at δ 5.15 which is due to the unusual C-3 formyl group and consisdered to be a hydrated form.[10] The antibacterial spectra (Table 1) are essentially identical, showing the same characteristic features of activity against both sensitive and resistant organisms.

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