Communications to the editor

SYNTHESIS OF 3′,4′-DIDEOXY-NEAMINE ACTIVE AGAINST KANAMYCIN-RESISTANT *E. COLI* AND *P. AERUGINOSA*

Sir:

H. UMEZAWA *et al.*[1,2,3] have clarified the mechanism of amino glycoside resistance of *Escherichia coli* K 12 carrying the R factor and of resistant *Pseudomonas*: these organisms produce an enzyme transferring phosphate from ATP to the 3-hydroxyl group of 6-amino-6-deoxy-D-glucose, 2,6-diamino-2,6-dideoxy-D-glucose or D-glucosamine moiety of deoxystreptamine-containing antibiotics, and as the result of the phosphorylation, antibiotics such as kanamycins, paromomycins and neomycins are inactivated. Based on this mechanism of resistance, the authors synthesized 3′-deoxykanamycin[4], 3′,4′-dideoxykanamycin B[5], and 3′,4′-dideoxyvistamycin*,[6], all of which were active against the resistant organisms. In this paper we report the synthesis of 4-O-(2,6-diamino-2,3,4,6-tetradeoxy-α-D-glucopyranosyl)-2-deoxystreptamine, which is also active against the resistant organisms.

The four amino groups of neamine were protected by carbomethoxy chloride in aqueous acetone (1:1) to give tetra-N-carbomethoxyneamine (**I**) in a yield of 87%, which was then treated with cyclohexanone dimethylketal in DMF in the presence of *p*-toluenesulfonic acid at 50°C under reduced pressure (20 Torr). Within 20 minutes a product [**II**, Rf 0.40 on TLC with silica gel and developed with chloroform-ethanol (8:1)] was produced, accompanied by two minor products having Rf 0.46 (**III**) and 0.90 (**IV**). On prolonged reaction, the initial major product (**II**) disappeared and the products **III** and **IV** became predominant. By addition of a small amount of methanol, the product **IV** was converted to **III**. From periodate oxidation followed by acidic hydrolysis and detection of deoxystreptamine by paperchromatography, **II**, **III** and **IV** were proved to be 3′,4′-O-cyclohexylidene, 5,6-O-cyclohexylidene and 3′,4′:5,6-di-O-cyclohexylidene derivative of **I**, respectively. This was also confirmed by their NMR spectra. Therefore it was concluded that **III** is more thermodynamically stable than **II**. Compound **III** was obtained from **I** in a yield of 70%, amorphous powder, $[\alpha]_D^{25}$ +37° (*c* 1, methanol). Anal. Found: C 49.44, H 6.82, N 8.93%. Calcd. for $C_{26}H_{42}N_4O_{14}$: C 49.20, H 6.67, N 8.83%; NMR (in $CDCl_3$), τ 8.0~8.8 (11 H, broadened signal, cyclohexylidene protons and H-2_{ax}). Mesylation of **III** with mesyl chloride in pyridine gave the corresponding 3′,4′-di-O-mesylated derivative (**V**), m.p. 142~144°C, $[\alpha]_D^{25}$ +50.4° (*c* 1, methanol); NMR (in $CDCl_3$), τ 6.90 and 6.73 (both 3 H singlets, SO_2CH_3), τ 8.4 (10 H, broadened singlet, cyclohexylidene protons). Introduction of 3′,4′-unsaturation into compound **V** was achieved as described in the preceding communication[5] by the use of sodium iodide and zinc dust in DMF at 98°C for 2 hours to give 5,6-O-cyclohexylidene-3′,4′-dideoxy-3′-eno-tetra-N-methoxycarbonylneamine (**VI**) in a yield of 70%, amorphous powder, $[\alpha]_D^{25}$ −39° (*c* 1, methanol). Anal. Found: C 52.08, H 6.78, N 9.10%. Calcd. for $C_{26}H_{40}N_4O_{12}$: C 52.00, H 6.71, N 9.33%; NMR (in $CDCl_3$), τ 4.28 (2 H, slightly broadened singlet, H-3′,4′), τ 8.37 (10 H, broadened singlet, cyclohexylidene protons). Compound **VI** was catalytically hydrogenated to compound **VII**, amorphous powder, $[\alpha]_D^{23}$ +34.3° (*c* 0.6, methanol); NMR (in $CDCl_3$), τ 4.92 (1 H d, J~3.5 Hz, the anomeric proton at C-1′), τ 8.1~8.8 (~15 H broadened signal, cyclohexylidene protons, H-3′,3′,4′,4′ and H-2_{ax}). Deacylation of **VII** with barium hydroxide followed by decyclohexylidenation with 1 N hydrochloric acid gave a ninhydrin-positive product which was purified by column chromatography with Amberlite IRC-50 and 0.1~0.4 N ammonia to give the final product, 3′,4′-dideoxyneamine, $[\alpha]_D^{23}$ +102° (*c* 1, water). The overall yield of 3′,4′-dideoxyneamine starting from neamine was appro-

* Details will be published elsewhere by S. UMEZAWA, H. UMEZAWA, D. IKEDA & T. TSUCHIYA.

Table 1. Antibacterial spectra of 3′,4′-dideoxyneamine and neamine

Test organisms*	Minimal inhibitory concentration (mcg/ml)	
	3′,4′-Dideoxyneamine	Neamine
Staphylococcus aureus FDA 209P	3.12	3.12
Escherichia coli NIHJ	12.5	6.25
〃 K-12 CS-2	6.25	6.25
〃 K-12 ML 1629	12.5	>100
〃 K-12 ML 1630	6.25	>100
〃 K-12 ML 1410	6.25	6.25
Salmonella typhosa T-63	1.56	1.56
Pseudomonas aeruginosa A3	25	>100
〃 No. 11	25	>100
〃 No. 12	25	>100
〃 No. 39	25	>100
〃 No. 45	12.5	>100
〃 No. 67	25	>100
Proteus rettgeri GN 311	50	25
〃 GN 466	12.5	12.5

* Nutrient agar, 37℃ 18 hours.

ximately 30%. Elemental analyses and hydrolysis with 6 N hydrochloric acid confirmed the expected structure. On the hydrolysis, the product gave deoxystreptamine, which was confirmed by paper-chromatography with *n*-butanol-pyridine-water-acetic acid (6 : 4 : 3 : 1).

Synthetic 3′,4′-dideoxyneamine showed antibacterial activity as strong as that of the parent substance, neamine, against most of bacteria tested and, moreover, showed activity against *E. coli* 1629 and 1630 carrying R factor, and *P. aeruginosa* against which neamine showed no activity, as shown in Table 1.

These results and those reported in the preceding communications[4,5] confirm that the removal of the hydroxyl group which is phosphorylated by drug-resistant bacteria gives compounds with activity against the resistant organisms.

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