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Preliminary communication

Aminoglycoside antibiotics: chemical transformation of paromamine into 3′-epiparomamine

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Recent studies[1] on the development of bacterial resistance to certain members of the medicinally important aminoglycoside antibiotics[2] have shown that their inactivation is due to the presence in these organisms of adenylating, acetylating, and phosphorylating enzymes. Thus, strains carrying such resistance-transfer factors (RTF) are known to inactivate certain aminoglycoside antibiotics by phosphorylating[3] the 3′-hydroxyl group in the disaccharide portion containing the deoxystreptamine moiety. The appropriate chemical modification of this site could lead to an abberation in the recognition mechanism by such phosphorylating enzymes, thereby decreasing the possibilities of inactivation and broadening the spectrum of antibacterial activity.

We describe in this report the chemical conversion of paromamine[4], the disaccharide portion common to several aminoglycoside antibiotics that are susceptible to enzymic phosphorylation, into its 3′-epimer, namely, 4-*O*-(2-amino-2-deoxy-α-D-allopyranosyl)-2-deoxystreptamine (3′-epiparomamine). Except for the recent work of S. and H. Umezawa and their co-workers[5], who converted neamine and Kanamycin A into their respective 3′,4′-dideoxy derivatives, practically no work, based on a biochemical rationale, has been done in this important area of chemical manipulations of aminoglycoside antibiotics.

Paromamine (1)* was converted into the corresponding tri-*N*-(benzyloxycarbonyl)-di-*O*-isopropylidene derivative 2 by the procedure of S. Umezawa and co-workers[6]. Oxidation of 2 in methyl sulfoxide–acetic anhydride for 3 days at room temperature in the dark, followed by purification by column chromatography on silica gel, gave the glycos-3′-ulose derivative 3 (65%), m.p. 152–154° (*p*-dioxane–cyclohexane); $[\alpha]_D^{25}$ +39° (*c* 0.40, chloroform)**. Reduction of 3 with sodium borohydride in *N*,*N*-dimethylformamide–methanol for 2.5 h at room temperature, followed by addition of water, gave

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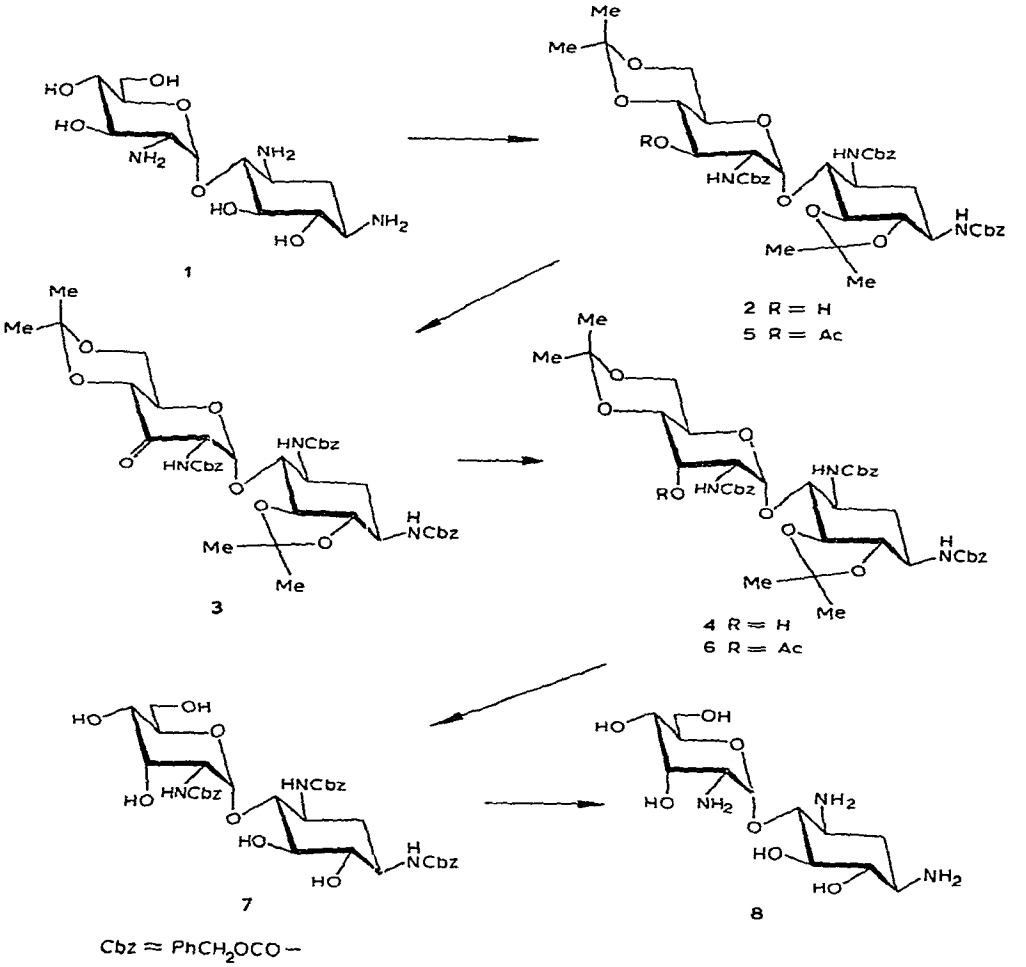
*Obtained by methanolysis of paromomycin I; see ref. 4.
**Crystalline compounds reported in this work were adequately characterized by microanalytical, chromatographic, and n.m.r. spectral data (at 60 and 100 MHz). Melting points are uncorrected.



the C-3′ epimeric derivative **4** (75%), m.p. 174–176°; $[\alpha]_D^{25}$ +26° (*c* 0.50, chloroform), which could readily be distinguished from the D-*gluco* analog **2** by t.l.c. (1:1 benzene–ethyl acetate).

Additional spectroscopic and chemical evidence was obtained as follows. Acetylation of **2** and **4** with acetic anhydride–pyridine gave the respective acetates **5**, m.p. 123–125°; $[\alpha]_D^{25}$ +46.1° (*c* 1.0, chloroform), and **6**, m.p. 106–108°; $[\alpha]_D^{25}$ +35° (*c* 0.54, chloroform). The acetyl resonances in the n.m.r. spectra ($CDCl_3$) of these compounds were clearly distinguishable, and corresponded to an equatorially attached acetoxyl group for **5** (*τ* 8.07) and an axially attached acetoxyl group for **6** (*τ* 7.87). In the spectrum of a mixture of the two compounds, these values did not change. Mild acid hydrolysis of **4** with 80% aq. acetic acid for a few min at 50° and 30 h at room temperature, followed by dilution of the mixture with water, gave tri-*N*-(benzyloxycarbonyl)-3′-epiparomamine (**7**), m.p. 232–234° (methanol); $[\alpha]_D^{25}$ +44° (*c* 0.80, *N*,*N*-dimethylformamide) and +32° (*c* 0.41, *p*-dioxane). Removal of the benzyloxycarbonyl groups

from 7, with concomitant glycoside cleavage, by treatment with aq. methylCellosolve–acetic acid–hydrochloric acid[6], afforded 2-deoxystreptamine and a component identified as D-allosamine[7] by chromatography in two solvent systems* and by its transformation into D-ribose after degradation with ninhydrin[8]. Concurrent hydrolysis and degradation of tri-*N*-(benzyloxycarbonyl)paromamine showed the presence of D-arabinose, as expected. Hydrogenolysis of the benzyloxycarbonyl groups of 7, in methanol in the presence of 20% Pd–C, afforded 3′-epiparomamine (8) as an amorphous, white solid, $[\alpha]_D^{25}$ +73° (*c* 0.60, water), which was converted into the hydrochloride, m.p. >~240° (gradual dec.), $[\alpha]_D^{25}$ +72.5° (*c* 0.60, water); R_{Gm} 0.28 (Solvent A)*; paromamine has R_{Gm} 0.30 in the same solvent system†.

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* Thin-layer chromatography was performed on paper and cellulose with 5:5:1:3 pyridine–ethyl acetate–acetic acid–water (Solvent A) [F. G. Fischer and H. J. Nebel, *Z. Physiol. Chem.*, 302 (1955) 10]; and 5:5:1:3 butyl alcohol–pyridine–acetic acid–water (Solvent B), respectively. Compounds were detected with ninhydrin.

† Paromamine and 3′-epiparomamine could also be distinguished by chromatography on plates of silica gel with the upper phase of 2:1:1 chloroform–methanol–17% ammonium hydroxide.