AMINOGLYCOSIDE ANTIBIOTICS: OXIDATIVE DEGRADATIONS LEADING TO NOVEL BIOCHEMICAL PROBES AND SYNTHETIC INTERMEDIATES[1]

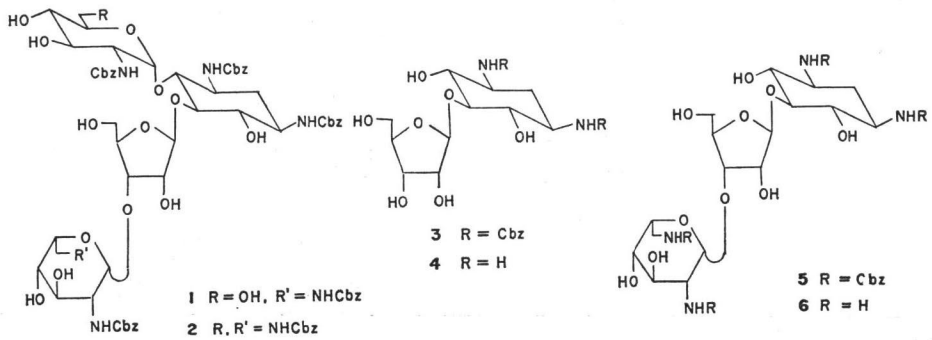
Sir:

Subtle chemical (functional) modifications in the area of aminoglycoside antibiotics[2] are known to have a profound effect on biological activity.[3] Such modifications are based on biological rationales at the molecular level,[3~5] and may involve the chemical alteration of a specific, peripheral functional group in the molecule, with the objective of rendering it inert to the action of inactivating enzymes, such as the phosphotransferases.[3~5] Experience has shown that the maintenance of significant biological activity, invariably imposes the existence of a delicate balance between functional, structural, configurational, and conformational aspects in the aminoglycoside molecule. One of the obstacles in deciphering the relative importance of these aspects has been the limited number of appropriate model compounds.

We report herein, on the preparation of two novel pseudosaccharides by a sequential oxidative, and β-eliminative degradation reaction of the readily available aminoglycoside antibiotics, neomycin and paromomycin.

Treatment of dry penta-*N*-carbobenzoxyparomomycin[1] (**1**) with periodic acid (8 equiv.) in tetrahydrofuran containing 4 Å molecular sieve at 1°C for 20 hours, followed by neutralization with aqueous sodium bicarbonate, gave the corresponding tetraaldehyde resulting from the cleavage of the two diaminosugar subunits, as a chromatographically homogeneous amorphous solid in 89 % yield. The tetraaldehyde underwent smooth β-elimination in methanol containing triethylamine (25°C, 15 hours), to give the pseudodisaccharide derivative **3** in 42~45 % yield. Recrystallization from ethanol gave a pure product, mp 235~237°C (sintering); $[\alpha]_D^{25}$ −44° (*c* 1, MeOH);[6] the corresponding *O*-TMSi derivative showed a fragment at *m/e* 675 corresponding to the deoxystreptamine portion, attached to a $-CH=\overset{+}{O}TMSi$ unit resulting from the anomeric carbon of D-ribosyl portion and migration of an OTMSi group.[7] Compound **3** could also be obtained from neomycin, and from 4′,6′-*O*-benzylidene-penta-*N*-carbobenzoxy-paromomycin.[1] In the latter case the sequence involved periodate oxidation, acid hydrolysis of the acetal function, oxidation, and finally, β-elimination.[8] Hydrogenation of **3** in the presence of palladium hydroxide-on-charcoal catalyst[9] in methanol containing N hydrochloric acid, followed by treatment with an anion-exchange resin, gave the free pseudodisaccharide **4**, which was obtained as a chromatographically homogeneous colorless solid from a mixture of methanol and ethanol (~quant), mp 100~105°C (softening), ~135°C (foaming); $[\alpha]_D^{25}$ −24.6° (*c* 0.22, H_2O);[10] Rf 0.56, R paromamine 1.12; R deoxystreptamine 1.36 ($CHCl_3$ - MeOH - NH_4OH, 1 : 3 : 2).

The subtle conformational and configurational difference between the two diaminosugar units in neomycin B led us to expect a preferential oxidation of the neosamine B unit in the *N*-protected antibiotic. Indeed, treatment of *N*-carbobenzoxyneomycin B,[11] with periodic acid (1.5 equiv.) in tetrahydrofuran containing 4 Å molecular sieve at 1°C for 20 hours, gave a product that showed a positive aniline hydrogen phthalate test for aldehydes, although there was little difference in chromatographic mobility, compared to the parent neomycin



derivative. Treatment of the dialdehyde with triethylamine in methanol (55~60°C, 2 days), followed by chromatographic purification, gave the pseudotrisaccharide derivative **5**, as a colorless solid (63 %, based on recovered starting material). Recrystallization from 2-propanol gave the pure pseudotrisaccharide derivative, mp 228~230°C; $[\alpha]_D^{25}$+22.9° (*c* 0.9, dioxane); the corresponding *O*-TMSi derivative showed peaks consistent with the structure when analyzed by mass spectrometry.[12] Hydrogenation over 20 % palladium hydroxide-on-charcoal in methanol containing N hydrochloric acid, followed by neutralization with an anion-exchange resin, gave the free pseudotrisaccharide **6** as an amorphous solid, in 83 % yield, mp 210~212°C (dec.); $[\alpha]_D^{25}$+22.5° (*c* 0.24, H_2O); Rf 0.45; R paromamine 0.90; R paromomycin 1.45 ($CHCl_3$-MeOH-NH_4OH, 1:3:2). Compounds **4** and **6** were analyzed by field desorption mass spectrometry,[13] and showed the expected M^+H peaks in each case. No detectable amounts of ribostamycin could be found in the crude mixture containing **6**, which confirms the preferential oxidation of the neosamine C portion.

Compound **6** has been recently found[14] as a minor component in commercial samples of neomycin. This has led to the suggestion[14] that it may be a biosynthetic precursor to the antibiotic.[15] In addition to being potential substrates for such biosynthetic studies, compounds **4** and **6** and their immediate precursors can also be regarded as versatile synthetic intermediates. Structurally, they represent the only remaining natural combinations of deoxystreptamine-containing subunits of the 5-linked aminoglycoside antibiotics, and as such, they are very useful biochemical probes. In this regard, we also report that while compound **4** was devoid of antibacterial activity, the pseudotrisaccharide **6** was found to be active in the standard assay,[16] the activity being 4~10 times less than that of neamine depending on the organism. This finding is particularly significant, as it bridges the last gap in relating structural and functional requirements for antibiotic activity in this series.

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References

1) Reported in part at the "Symposium on Aminoglycoside Antibiotics", 58th Meeting of the Chemical Institute of Canada, Toronto, Ontario. May 25-28, 1975; for previous papers in this series, see TAKAMOTO, T. & S. HANESSIAN, Aminoglycoside antibiotics: Chemical transformation of paromomycin into a bioactive pseudotrisacc haride; Tetrahedron Lett. 1974: 4009~4012, 1974; OGAWA, T.; T. TAKAMOTO & S. HANESSIAN,: Aminoglycoside antibiotics: synthesis of 6-*O*-(β-D-ribofuranosyl) paromamine. Tetrahedron Lett. 1974: 4013~4016, 1974; and references cited therein.

2) UMEZAWA, S.: Structures and syntheses of aminoglycoside antibiotics Advan. Carbohyd. Chem. 31: 111~182, 1974;
UMEZAWA, H.: Recent Advances in Chemistry and Biochemistry of Antibiotics. Microbial Chemistry Research Foundation, Tokyo, 1964, p. 67;
HANESSIAN, S & T. H. HASKELL,: in "The Carbohydrates: Chemistry and Biochemistry," W. PIGMAN & D. HORTON eds., Academic Press, Inc., New York, N. Y. 2nd edition, 1970, Vol. IIA, p. 59;
RINEHART, Jr. K. L.: "The Neomycins and Related Antibiotics," E. R. Squibb Lectures on Chemistry of Microbial Products, John Wiley & Sons, Inc., New York, N. Y., 1964

3) UMEZAWA, H.: Biochemical mechanism of resistance to aminoglycoside antibiotics. Advan. Carbohyd. Chem. 31: 183~225, 1974

4) DAVIES, J.: Structure-activity relationships among the aminoglycoside antibiotics. Antimicr. Agents & Chemoth. -1967: 297~303, 1968

5) BENEVISTE, R. & J. DAVIES: Mechanism of antibiotic resistance in bacteria. Ann. Rev. Biochem. 42: 471~506, 1973

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6) Crystalline compounds gave microanalytical data that were in agreement with their structures.
7) The silylation procedure and pertinent mass spectral specifications have been previously described, see DE JONGH, D. C.; J. D. HRIBAR, S. HANESSIAN & P. W. K. WOO: J. Amer. Chem. Soc. 89: 1469~1470, 1967; DE JONGH, D. C.; T. RADFORD, J. D. HRIBAR, S. HANESSIAN, M. BIEBER, G. DAWSON & C. C. SWEELEY; J. Amer. Chem. Soc. 91: 1728~1740, 1969
8) The isomeric mixture, 4(6)-β-D-ribofuranosyl *N*-carbobenzoxydeoxystreptamine, has been obtained by a conventional glycosidation reaction. T. OGAWA & S. HANESSIAN, unpublished results.
9) PEARLMAN, W.: Noble metal hydroxides on carbon: non pyrophoric dry catalysts. Tetrahedron Lett. 1967: 1663~1664, 1967
10) Free pseudosaccharides contained variable amounts of carbonic acid depending on the periods of exposure to air.
11) Prepared in the usual way from neomycin, and purified by chromatography over silica gel; $[\alpha]_D^{23}+32.9°$ (*c* 0.54, $CHCl_3$).
12) The corresponding *N*-acetyl *O*-TMSi derivative gave the following ions: $C_{16}H_{33}N_2O_5Si_2$ (neosaminyl B portion), calcd. 389.1298; found 389.1949; $C_{16}H_{33}N_2O_4Si_2$ (deoxystreptamine portion), calcd. 373.1979; found, 373.1987; $C_{31}H_{73}O_{10}Si_5$ (ribosyldeoxystreptamine with CHOTMS unit), *m/e* 767; $C_{27}H_{57}N_2O_9Si_4$ (neobiosaminyl portion), *m/e* 665, *etc.*
13) We thank Professor K. L. RINEHART, Jr., University of Illinois, for these measurements.
14) CLAES, P. J.; F. COMPERNOLLE & H. VANDERHAEGHE: Chromatographic analysis of neomycin. Isolation and identity of minor components. J. Antibiotics 27: 931~939, 1974
15) For work pertaining to the biosynthesis of the neomycins, see FOGHT, J. L.: Biosynthetic studies on the neomycins. Ph. D. thesis, University of Illinois, 1963, SCHIMBOR, R. F.: The Microbiological incorporation of labeled intermediates into the neomycin antibiotics; Ph. D. thesis, University of Illinois, 1966; RINEHART, Jr., K. L. & SCHIMBOR, R. F., Neomycins, in "Antibiotics biosynthesis." D. GOTTLIEB & P. D. SHAW, Eds., Springer-Verlag, New York, Inc., 1967, pp. 359~372.
16) Courtesy of Ayerst Laboratories, Montreal; we thank Drs. H. BAKER and S. RAKHIT for their collaboration.