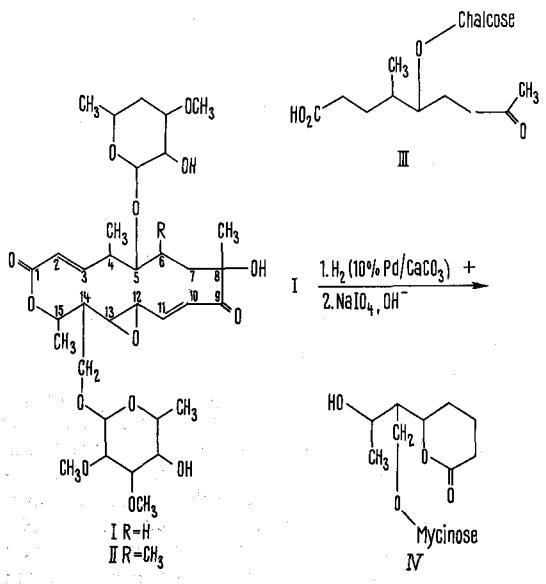
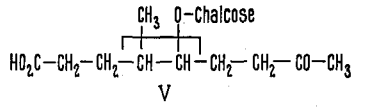
The Structure of Neutramycin

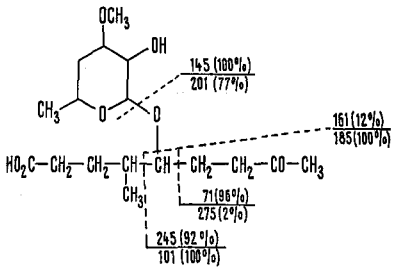
We previously reported the establishment of the molecular formula, the nature of the chromophoric groupings and the presence of chalcose and mycinose in the neutral macrolide antibiotic neutramycin[1]. We would now like to describe experiments which lead to formula I as the most probable one for neutramycin.



Previous experiments accounted for all but 1 of the oxygen atoms. As this remaining oxygen was not easily acetylated, an ether or tertiary hydroxyl seemed likely. Chalcomycin, whose structure was determined to be II[2], contains a tertiary hydroxyl adjacent to the ketonic carbonyl. Considering that neutramycin might be similarly endowed, hexahydroneutramycin was treated with alkaline periodate and indeed found to cleave into 2 C_{17} fragments (III and IV). 1 of the fragments had a molecular formula $C_{17}H_{30}O_8$ and contained a δ-lactone (5.77 μ in the IR). It also contained the sugar mycinose (IR- and NMR-spectra) which was indicated to exist in the β-pyranoside form by the NMR coupling constant for the anomeric hydrogen ($H_1 = 5.42\,\tau$, $J_{1,2} = 8$ Hz). Without going into detail, the NMR-spectra of the mycinosyloxy lactone fragment and its diacetate are in complete agreement with structure IV. This is also the structure proposed (and in which we concur) for the same fragment obtained from the neutral macrolide antibiotic chalcomycin (II) by similar treatment[3]. Comparison of the spectral, physical and chromatographic properties for the mycinosyloxy lactone from both neutramycin and chalcomycin (in our case Aldgamycin D)[4] indicated them in fact to be identical.

The second fragment (III), m.p. 73–74°, crystallized from ethyl acetate-hexane. Anal. ($C_{17}H_{30}O_7$): C, 58.90; H, 8.95; $M^+ = 346$ (mass spectrum); $[\alpha]_D^{25} = -55° \pm 3.1°$ ($c = 0.967$, methanol). Chalcose was discernible from its spectra and the coupling constant for the anomeric hydrogen in its NMR-spectrum ($H_1 = 5.83\,\tau$, $J_{1,2} = 7.5$ Hz) indicated the sugar to exist in the β-pyranoside form. The NMR-spectrum further revealed the gross features of its structure. In addition to the O- and C-methyls of chalcose (3H singlet, 6.56 τ and 3H doublet, 8.77 τ), it revealed the presence of a methyl ketone (3H singlet, 7.87 τ; confirmed by an IR-band at 1709 cm^{-1} and a positive iodoform test), *one* other C-methyl (3H doublet, 9.07 τ) and the anticipated hydroxyl and carboxyl protons (2H broad, 3.75 τ). Integration of the NMR-spectrum revealed the presence of 7 hydrogens in the region (6.3–6.9 τ) for those on carbons attached to an oxygen, 7.5 hydrogens in the region (7.2–7.9 τ) for those on carbons adjacent to carbonyl functions and 12.5 hydrogens in the region (8.0–9.2 τ) for those on carbons not attached to heteroatoms (H_{4e} of chalcose occurs between 7.7 and 8.1 τ and overlaps both regions). Chalcose is attached to a carbon with only 1 hydrogen since chalcose itself accounts for 6 of the 7 hydrogens on carbons attached to an oxygen. 7 hydrogens on carbons adjacent to carbonyls means both the methyl ketone and carboxyl functions are flanked by methylenes. 1 of these occurs as a 1:2:1 triplet (7.35 τ, $J = 6.5$ Hz) while the other consists of a pair of overlapping doublets (7.6 τ, $J = 6$, 9 Hz), thus indicating each of these methylenes to be attached to another methylene. These considerations greatly restrict the possible locations of chalcose and the remaining C-methyl, and indicate partial structure V for the chalcosyloxy ketoacid fragment.





A decision in favor of III for the chalcosyloxy ketoacid fragment, and therefore I for neutramycin, was made from its mass spectrum. The spectrum is rather complex, but fragmentations occur primarily about the point of attachment of chalcose. These are illustrated in VI. The most significant fragment in the spectrum is m/e = 245. This strong peak contains chalcose since it loses methanol to give m/e = 213 (38%), the process being supported by a metastable ion (185.2). The alternate formulation in V would require fragments at m/e = 273, 247, 99 and 73, and these are either absent or minor in the spectrum.

Cleavage fragments III and IV contain all of the carbons of neutramycin and rational reassembly leads to formula I as most likely for neutramycin. Thus neutramycin differs

1 M. P. KUNSTMANN and L. A. MITSCHER, Experientia *21*, 372 (1965).
2 P. W. K. Woo, H. W. DION and Q. R. BARTZ, J. Am. chem. Soc. *86*, 2726 (1964).
3 P. W. K. Woo, H. W. DION and Q. R. BARTZ, J. Am. chem. Soc. *86*, 2724 (1964).
4 Aldgamycin D, which proved to be identical with chalcomycin, was a co-occurring antibiotic with Aldgamycins E and C: cf. M. P. KUNSTMANN, L. A. MITSCHER and E. L. PATTERSON, in *Antimicrobial Agents and Chemotherapy* (Braun-Bromfield, Ann Arbor, Michigan 1964), p. 87; M. P. KUNSTMANN, L. A. MITSCHER and N. BOHONOS, Tetrahedron Lett. *8*, 839 (1966); G. A. ELLESTAD, M. P. KUNSTMANN, J. E. LANCASTER, L. A. MITSCHER and G. MORTON, Tetrahedron *23*, 3893 (1967).

from chalcomycin in that it lacks the C-6 methyl group which the latter contains. Both neutramycin and chalcomycin fit in with the generally accepted biogenesis of the lactone ring from a polyketide precursor, itself formed from acetate and propionate units. Thus an acetate unit is apparently incorporated into the C-5, C-6 portion of neutramycin whereas a propionate unit is incorporated in chalcomycin[5].

Zusammenfassung. Für das Antibiotikum Neutramycin wird die Strukturformel I abgeleitet.

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Lederle Laboratories, A Division of American Cyanamid Company, Pearl River (New York= 10965, USA), 29 August 1968.

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Polarographic Behaviour of Polycytidylic Acid and its Double-Stranded Complex with Polyinosinic Acid

Our previous results have shown that denatured deoxyribonucleic acid (DNA) is polarographically reducible in a neutral medium, while native double-helical DNA is non-reducible under the same conditions[1,2]. We have explained the polarographic reducibility of denatured DNA by the availability of the potentially reducible bases contained in DNA for the electrode process. It has been presumed that the residues of cytosine, which yields in a monomeric form a direct current (d-c) polarographic reduction step at pH 7, take part in the process[3-5]. Provided the above-mentioned presumptions are correct, it can be expected that polycytidylic acid (poly C), which is at neutral pH a random coil with fluctuating regions of helicity[6], will behave similarly to denatured DNA, while its double-helical complex with polyinosinic acid poly (I) · poly (C) will be polarographically non-reducible. Our experiments confirmed this expectation in principle.

Polarographic measurements were carried out in ammonium formate with sodium phosphate background electrolyte, which had been used earlier for DNA analysis. The instruments used were described in our previous papers[1,2]. Poly C was prepared by using a polynucleotide phosphorylase (2.7.7.8) obtained from *Micrococcus lysodeikticus*[7]. Commercial preparation of poly I (California Corporation for Biochemical Research) was kindly donated by Dr. V. KLEINWÄCHTER. All chemicals used for background electrolyte solutions were of analytical grade.

In 0.5 M ammonium formate with 0.1 M sodium phosphate (pH 7) $2 \times 10^{-4} M$ poly C (related to phosphorus content) produced a d-c polarographic reduction step whose height corresponded to c. 0.6 μA and the half-step potential ($E_{1/2}$) was about -1.34 V, i.e. slightly more positive than $E_{1/2}$ of denatured DNA in the same medium[2]. Poly I was, in agreement with the polarographic non-reducibility of monomeric hypoxanthine at neutral pH[8], inactive. The d-c polarographic step of poly C almost disappeared after the addition of the equivalent amount of poly I (formation of the 1:1 complex was controlled spectrophotometrically).

The oscillopolarographic behaviour of poly C, poly I and poly (I) · poly (C) agreed in principle with their d-c polarographic behaviour, the sensitivity of the estimation of poly C by means of the oscillopolarographic 'first curve technique'[1] was, however, much higher, as compared with d-c polarographic analysis (Figure 1). For the oscillopolarographic analysis only a few tenths of μg of poly C were necessary. The depth of the indentation of poly C depended on the ammonium formate concentration (Figure 2) like the depth of the indentation of denatured DNA. Mixing of poly C with poly I caused a decrease in the depth of the indentation of poly C; however, this indentation did not disappear completely even in the presence of an excess of poly I (Figure 3). It is possible that the presence of a small indentation or a step on the curves of poly (I) · poly (C) may be con-

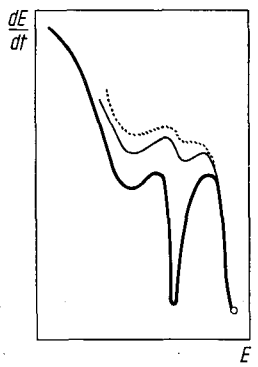

Fig. 1. Right section of the cathodic part of the oscillopolarogram dE/dt against E. $1.7 \times 10^{-5} M$ poly C in 1.0 M ammonium formate with 0.1 M sodium phosphate (pH 7). ——— first curve, ——— second curve, ····· third curve.

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