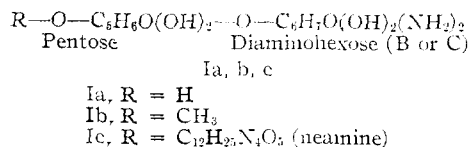


In the accompanying Communication⁴ the pentose moiety is shown to be the same for both neobiosamines B and C. The disaccharides presumably differ then in their diamino-hexose moieties and may be assigned the partial formula Ia, and methyl neobiosaminides B and C, Ib. Since neomycins B and C both contain neamine they may be formulated as Ic.



We wish to express our appreciation to the Public Health Service for a research grant (No. E-1278) in support of this work, and to the Upjohn Company for the generous gift of neomycin samples.

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RECEIVED JUNE 24, 1957

CHEMISTRY OF THE NEOMYCINS. II. THE PENTOSE MOIETY

Sir:

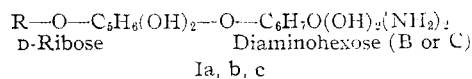
In the preceding Communication¹ it was shown that the neobiosamine portion of both neomycin B and neomycin C consists of a diamino-hexosidopentose. We present here evidence that the pentose moiety in both neobiosamines B and C is D-ribose.

Methyl N,N'-dibenzoylneobiosaminide-C¹ was hydrolyzed with dilute aqueous hydrochloric acid at reflux to give a mixture of products. Purification by partial precipitation, ion exchange resins and chromatography gave a neutral, salt-free carbohydrate fraction, which was shown by color tests, papergrams and rotation to be D-ribose. The compound is an aldopentose² since its papergram gives an orange-brown color when developed with *p*-dimethylaminoaniline and trichloroacetic acid³ and a red color when developed with aniline acid phthalate,⁶ but no gray-green color (indicative of a ketopentose and found in model papergrams of ribulose and xylulose) when developed with orcinol and trichloroacetic acid.⁷ In simultaneous papergrams (at least two chromatograms in each solvent system) with the four aldopentoses and two ketopentoses, the material was shown to give R_f values quite close to those of ribose, but very different from those of the other aldopentoses and the ketopentoses [ribose, R_f 0.553 (TBAW), 0.630 (PhNC), 0.368 (BAW); hydrolysate pentose, R_f

0.547 (TBAW), 0.630 (PhNC), 0.364 (BAW)].⁸ The observed rotation of solutions of the pentose is negative, establishing the compound as D-ribose, rather than the L-isomer, which would give positive rotations.

The pentose obtained from hydrolysis of methyl N,N'-dibenzoylneobiosaminide B also has been shown to be D-ribose by a precisely analogous procedure, involving papergram color tests, R_f values, mutarotation data and osazone form. The R_f values are: ribose, 0.596 (TBAW), 0.308 (BAW); hydrolysate pentose, 0.598 (TBAW), 0.309 (BAW).⁹ The mutarotation value of the isolated pentose was -17.1° , that of D-ribose, determined simultaneously, -17.6° . The moss-like crystal form of the osazone of the hydrolysate pentose, m.p. 158° , was the same as that of ribosazone, m.p. $159-162^\circ$, very different from the needles of xylosazone, m.p. $162-164^\circ$.¹⁰

Since the pentose from both neobiosamines is D-ribose and neomycins B and C both contain neamine,³ the formulas of neobiosamines B and C, of methyl neobiosaminides B and C, and of neomycins B and C may be represented by Ia, Ib and Ic, respectively. The difference between the isomeric antibiotics presumably lies in the diamino-hexose moieties. The structures of the diamino-hexoses, their position of linkage to ribose, the position of ribose attachment to neamine and questions of pyranose *vs.* furanose ring structure will, it is hoped, be the subjects of future publications.



Ia, R = H; Ib, R = CH₃; Ic, R = C₁₂H₂₅N₄O₅ (neamine)

We wish to express our appreciation to the Public Health Service for a research grant (No. E-1278) in support of this work, and to the Upjohn Company for the generous gift of neomycin samples. We also wish to thank Professor W. A. Wood for helpful advice and suggestions.

(8) TBAW is *tert*-butyl alcohol:acetic acid:water, 2:2:1; PhNC is phenol + 1% ammonia + hydrogen cyanide (trace); BAW is *n*-butyl alcohol:acetic acid:water:4:1:5.

(9) Slight variations in absolute values between these R_f 's and those above are due to operator and room temperature differences. Each series is, however, self-consistent and values within a series were obtained in a single chromatogram.

(10) W. Z. Hassid and R. M. McCready, *Ind. Eng. Chem., Anal. Ed.*, **14**, 683 (1942).

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RECEIVED JUNE 24, 1957

EVIDENCE FOR THE HYDROGEN DICHLORIDE ANION: THE COMPOUND C₈HCl₂

Sir:

The hydrogen bonded HF₂⁻ anion,¹ and its crystalline salts with heavy alkali metal cations,²

(1) E. F. Westrum, Jr., and K. S. Pitzer, *THIS JOURNAL*, **71**, 1940 (1949); S. W. Peterson and H. A. Levy, *J. Chem. Phys.*, **20**, 204 (1952).

(2) L. Helmholtz and M. T. Rogers, *THIS JOURNAL*, **61**, 2590 (1939); M. T. Rogers and L. Helmholtz, *ibid.*, **62**, 1533 (1940); R. V. Winsor and G. H. Cady, *ibid.*, **70**, 1500 (1948); R. Kruh, K. Fuwa, and T. E. McEever, *ibid.*, **78**, 4256 (1956).

(1) K. L. Rinehart, Jr., P. W. K. Woo, A. D. Argoudelis and A. M. Giesbrecht, *THIS JOURNAL*, **79**, 4507 (1957).

(2) The presence of a pentose was demonstrated earlier since more vigorous acid hydrolysis of methyl neobiosaminide has been shown to give furfural,³ and is the basis of a chemical assay for neomycin.⁴

(3) J. D. Dutcher, N. Hosansky, M. N. Donin and O. Wintersteiner, *THIS JOURNAL*, **73**, 1384 (1951).

(4) J. D. Dutcher, N. Hosansky and J. H. Sherman, *Antibiotics and Chemotherapy*, **3**, 534 (1953).

(5) R. B. Koch, W. F. Geddes and F. Smith, *Cereal Chem.*, **28**, 424 (1951).

(6) S. M. Partridge, *Nature*, **164**, 443 (1949).

(7) A. Revenne and K. T. Williams, *Arch. Biochem. and Biophys.*, **34**, 225 (1951).