of sample of insoluble sulfur prepared according to the method in ref. 9. This diffraction pattern[7] was remarkably similar to that of orthorhombic sulfur. It is clear that this insoluble sulfur must have a crystalline structure. Since the line widths were comparable in the two cases, we conclude that the crystal size must be similar (since the half-width of the peak is proportional to particle size). This insoluble sulfur is commonly referred to as amorphous sulfur[10] or μ-sulfur and is thought to consist of long-chain polymers having molecular weights ranging from 18,000 to 73,000.[11] The only differences we have been able to observe between the diffraction patterns of the insoluble sulfur and orthorhombic sulfur are (1) differences in the relative intensities of several of the lines[7] and (2) the insoluble sulfur and the orthorhombic sulfur show a few lines not present in each others pattern; for example, lines at 2.99 Å. (insoluble) and 2.62 Å. (orthorhombic). The significance of these small differences is not clear at present. It is of course possible for polymeric materials to be in a crystalline form; however, one might expect a greater difference in the diffraction patterns of polymeric μ-sulfur and orthorhombic sulfur.

It is interesting that Das in a recent summary of his work[12] (some of it unpublished) has found an orthorhombic X-ray powder diagram for milk of sulfur (which has commonly been described as an amorphous modification) and also the same for colloidal sulfur prepared by the reaction of sulfur dioxide and hydrogen sulfide. In view of our findings and those of Das, it would seem advisable to discontinue the use of the term "amorphous" sulfur for these crystalline forms. The insoluble sulfur in the present paper could more aptly be referred to simply as "insoluble sulfur" or μ-sulfur.

The X-ray diffraction patterns were obtained on a North American Philips X-ray diffractometer using a rotating sample holder of the type previously described.[13] This sample holder minimizes the possibility of orientation effects in powdered samples and thus makes it possible to obtain a more complete and accurate diffraction pattern.

The authors express their appreciation to the Texas Gulf Sulphur Co., Newgulf, Texas, for a fellowship to J. S. K. under which this work was done; to Dr. R. Fanelli of the Texas Gulf Sulphur Co., New York, for helpful information; and to Baroid Division, National Lead Co., Houston, Texas, for the use of the X-ray diffractometer.

(10) Ref. 1, pp. 522, 574, 576; ref. 2, pp. 81, 83; ref. 3, pp. 4, 5, 7, 8; ref. 6, pp. 30, 32, 46; "Facts About Sulfur," Texas Gulf Sulphur Co., New York, N. Y., 1953, p. 19.

(11) H. Specker, *Z. anorg. allgem. Chem.*, **261**, 116 (1950).

(12) S. R. Das in ref. 3, p. 103.

(13) J. L. McAtee, Jr., *Am. Mineralogist*, **41**, 942 (1956).

(14) Baroid Division.

DEPARTMENT OF CHEMISTRY
BAYLOR UNIVERSITY — A. G. PINKUS
WACO, TEXAS — J. S. KIM
BAROID DIVISION — J. L. MCATEE, JR.[14]
NATIONAL LEAD COMPANY — C. B. CONCILIO[14]
HOUSTON, TEXAS

RECEIVED JULY 16, 1957

CHEMISTRY OF THE NEOMYCINS. I. A PARTIAL STRUCTURE FOR NEOBIOSAMINES B AND C[1]

Sir:

Though the isomeric antibiotics neomycins B and C have been investigated extensively and widely employed clinically (as a mixture of the two isomers),[2] the structures of these compounds thus far have resisted complete elucidation. We wish to report here evidence that the neobiosamine[3] portion of both neomycins B and C consists of a diaminohexosido-pentose. In an accompanying Communication it is shown that the same pentose, D-ribose, is found in both isomers.[4]

Methanolysis of neomycin C gave neamine[2b] and methyl neobiosaminide C,[3] the latter, crude product, was carefully chromatographed and isolated as a mixture of its anomeric α- and β-glycosides [*Anal.* Calcd. for $C_{11}H_{21}N_2O_7(OCH_3)$: C, 44.44; H, 7.46; N, 8.64. Found: C, 44.60; H, 7.72; N, 8.55] and characterized as its derivative, methyl N,N'-dibenzoylneobiosaminide C, m.p. 250–252° [*Anal.* Calcd. for $C_{11}H_{19}N_2O_7(OCH_3)(COC_6H_5)_2$: C, 58.63; H, 6.00; N, 5.27. Found: C, 58.52; H, 5.77; N, 5.01]. These analytical values are in excellent agreement with the formulation of neobiosamine C as $C_{11}H_{22}N_2O_8$, a disaccharide composed of diaminohexose and pentose moieties, but do not support the formula $C_{11}H_{22}N_2O_7$, which would be a disaccharide containing desoxydiaminohexose and pentose moieties, as previously proposed.[3]

The α- and β-anomers were separated ($[\alpha]^{25}D$ 113° and $[\alpha]^{25}D$ 61°, respectively) and each was hydrolyzed in dilute hydrochloric acid to neobiosamine C (identical mutarotation value, $[\alpha]^{25}D$ 104°), which gave a single strong papergram spot [R_f 0.227 (TBAW)],[5] both reducing and ninhydrin-positive. The formation of a single compound from hydrolysis of methyl neobiosaminide C argues strongly for the formulation of neobiosamine C as a diaminohexosido-pentose, cleaved with difficulty, rather than as a pentosido-diaminohexose, which would be readily cleaved during hydrolysis to a pentose and a diaminohexose (or methyl diaminohexoside) and thus would give two papergram spots.

Similar arguments obtain for neobiosamine B. Both α- and β-anomers ($[\alpha]^{25}D$ 13° and $[\alpha]^{25}D$ −17°, respectively) of methyl neobiosaminide B [*Anal.* Calcd. for $C_{11}H_{21}N_2O_7(OCH_3)\cdot H_2O$: C, 42.10; H, 7.66; N, 8.19. Found: C, 41.82; H, 7.42; N, 7.95] are hydrolyzed to constant rotation ($[\alpha]^{25}D$ + 33°) and a single strong papergram spot [R_f 0.251 (TBAW)]; thus neobiosamine B also may be formulated as $C_{11}H_{22}N_2O_8$, a diaminohexosido-pentose.

(1) Presented in part at the XVIth International Congress of Pure and Applied Chemistry, Paris, July 18 to 24, 1957.

(2) For recent reviews, *cf.* (a) J. H. Ford, M. E. Bergy, A. A. Brooks, E. R. Garrett, J. Alberti, J. R. Dyer and H. E. Carter, THIS JOURNAL, **77**, 5311 (1955); (b) J. R. Dyer, Ph.D. Dissertation, University of Illinois, June, 1954; (c) S. A. Waksman, "Neomycin," Rutgers University Press, New Brunswick, N. J., 1953.

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

(4) K. L. Rinehart, Jr., P. W. K. Woo and A. D. Argoudelis, *ibid.*, **79**, 4568 (1957).

(5) TBAW is *tert*-butyl alcohol:acetic acid:water, 2:2:1.

In the accompanying Communication[4] the pentose moiety is shown to be the same for both neobiosamines B and C. The disaccharides presumably differ then in their diaminohexose 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.

$$\underset{\text{Pentose}}{R—O—C_5H_6O(OH)_2—O—}\underset{\text{Diaminohexose (B or C)}}{C_6H_7O(OH)_2(NH_2)_2}$$

Ia, b, c

Ia, R = H
Ib, R = CH_3
Ic, R = $C_{12}H_{25}N_4O_5$ (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.

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS
KENNETH L. RINEHART, JR.
PETER W. K. WOO
ALEXANDER D. ARGOUDELIS
ASTREA M. GIESBRECHT

RECEIVED JUNE 24, 1957

CHEMISTRY OF THE NEOMYCINS. II. THE PENTOSE MOIETY

Sir:

In the preceding Communication[1] it was shown that the neobiosamine portion of both neomycin B and neomycin C consists of a diaminohexosidopentose. We present here evidence that the pentose moiety in both neobiosamines B and C is D-ribose.

Methyl N,N′-dibenzoylneobiosaminide-C[1] 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 aldopentose[2] since its papergram gives an orange-brown color when developed with *p*-dimethylaminoaniline and trichloroacetic acid[5] 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°, very different from the needles of xylosazone, m.p. 162–164°.[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 diaminohexose moieties. The structures of the diaminohexoses, 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.

$$\underset{\text{D-Ribose}}{R—O—C_5H_6(OH)_2—O—}\underset{\text{Diaminohexose (B or C)}}{C_6H_7O(OH)_2(NH_2)_2}$$

Ia, b, c

Ia, R = H; Ib, R = CH_3; Ic, R = $C_{12}H_{25}N_4O_5$ (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.

(1) K. L. Rinehart, Jr., P. W. K. Woo, A. D. Argoudelis and A. M. Giesbrecht, THIS JOURNAL, **79**, 4567 (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 Chemotheraphy*, **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. Bevenue and K. T. Williams, *Arch. Biochem. and Biophys.*, **34**, 225 (1951).

(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).

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS
KENNETH L. RINEHART, JR.
PETER W. K. WOO
ALEXANDER D. ARGOUDELIS

RECEIVED JUNE 24, 1957

EVIDENCE FOR THE HYDROGEN DICHLORIDE ANION: THE COMPOUND $CsHCl_2$

Sir:

The hydrogen bonded HF_2^- 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. Helmholz and M. T. Rogers, THIS JOURNAL, **61**, 2590 (1939); M. T. Rogers and L. Helmholz, *ibid.*, **62**, 1533 (1940); R. V. Wilson and G. H. Cady, *ibid.*, **70**, 1500 (1948); R. Kruh, K. Fowa, and T. E. McEver, *ibid.*, **78**, 1256 (1956).