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.

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

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.

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

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. Kolb, K. Fowa, and T. E. McEver, *ibid.*, **78**, 4256 (1956).

are well known. Chlorine is known to form hydrogen bonds under favorable circumstances,[3] but chlorine compounds analogous to the alkali metal bifluorides have not been reported. We now wish to report the compound CsCl·HCl, obtained by simply passing hydrogen chloride into rather concentrated aqueous cesium chloride solutions.

In a typical run 2 g. of cesium chloride was dissolved in 1.5 ml. of water and hydrogen chloride was bubbled through the solution with intermittent cooling. As the solution approached saturation with respect to hydrogen chloride, about 1 g. of the new compound was precipitated as colorless to pale yellow anisotropic needles. The solid lost hydrogen chloride and reverted to isotropic cesium chloride when attempts were made to dry it in air or *in vacuo*; but the compound could be obtained with only slight loss of hydrogen chloride when drying was carried out at 30° in a stream of hydrogen chloride. Several samples dried in this way gave ratios Cs:H:Cl varying from 1:0.7:1.4 to 1:1.0:2.0.

Examined in their mother liquors under the polarizing microscope, the crystals were observed[4] to be perfect orthorhombic prisms, with $2V \sim 70°$, moderately high birefringence, and positive optic sign. As the HCl concentration in the system decreased with time, the needles were transformed first into an anisotropic polycrystalline phase, and eventually into isotropic CsCl.

Cesium appears to be unique among the alkali metals in its tendency to form an "acid chloride." Attempts to prepare similar compounds of rubidium and potassium so far have led only to the normal chlorides. We wish to advance the hypothesis that the new cesium compound contains HCl_2^- anions with a hydrogen-bonded structure (Cl—H· · ·Cl⁻) analogous to that of the HF_2^- ion; and further, that the HCl_2^- ion forms isolable crystalline salts only with very large cations. Other compounds which may contain the HCl_2^- anion are the "dihydrochlorides" by pyridine and other tertiary amines,[5] and the remarkably stable siliconium chloride–hydrogen chloride salts.[6] Structural studies on $CsHCl_2$ are in progress.

(3) K. Nakamoto, M. Margoshes and R. E. Rundle, THIS JOURNAL, **77**, 6480 (1955); L. R. Zumwalt and R. M. Badger, *J. Chem. Phys.*, **7**, 87 (1939); THIS JOURNAL, **62**, 305 (1940).

(4) Preliminary crystallographic examination was carried out with the help of Mr. Terence Patrick of the Department of Geology, University of Wisconsin.

(5) F. Kaufler and E. Kunz, *Ber.*, **42**, 385, 2482 (1909); F. Ephraim, *ibid.*, **47**, 1828 (1914).

(6) W. Dilthey, *Ber.*, **36**, 923 (1903); *Ann.*, **344**, 304 (1906); R. Riley, R. West and W. Erby, unpublished work.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN ROBERT WEST
MADISON 6, WISCONSIN

RECEIVED JULY 8, 1957

THE DEGRADATION OF LEUCINE-DERIVED CAROTENES

Sir:

In previous papers on the incorporation of labeled leucine into carotene by *Phycomyes blakesleeanus*, we have shown that only the 4-C of leucine is incorporated in sufficient quantity to permit its occurrence in each isoprene unit.[1] Incorporation of the 1-C is insignificant, and for the 3-C and 2-C atoms it is too low to account for the increased synthesis of β-carotene in the presence of leucine,[2] so that the selective use of an intact residual C_5 decarboxylated fragment of leucine becomes untenable.

We have therefore located some of the labeled leucine carbons in the carotene molecule in an attempt to explain the unique role of the 4-C atom.

The labeled carotene specimens, each *ca.* 3.5 mg., diluted with carrier to 19 to 32 mg. were insufficient for a complete analysis such as Grob and Bütler[3] performed. They were sufficient for recovery of the acetic acid obtained by chromic acid oxidation,[4] 6 moles of which are yielded per mole of carotene. The carboxyl groups are derived from the 5,5, 9,9, 13, and 13 carbons, and the methyl groups from corresponding side chains.

The acetic acid was decarboxylated in the Schmidt reaction to give CO_2 and methylamine. The CO_2 was converted to $BaCO_3$ and counted. The methylamine, crystallized as the hydrochloride, was burned and the CO_2 handled in the same way.

The *dl*-leucine labeled in positions 2, 3 and 4 and the β-carotene were prepared as described previously.[1,2] At the temperature of boiling water, we oxidized[5] about 20 mg of β-carotene, from which 3.5 to 5.3 mg. is radioactive.

The acetic acid obtained by four successive distillations was titrated by NaOH, 0.1 *N*. The yield of acetic acid varied from 75 to 85%. The calculated quantities of NaN_3 and H_2SO_4 were added to the CH_3COONa, obtained by evaporation of the solution (42 mg. NaN_3 and 0.9 ml. H_2SO_4 for 40 mg. CH_3COONa).[6] The liberated CO_2 was collected as $BaCO_3$. Then the methylamine was distilled into concd. HCl, crystallized as hydrochloride and burned; the CO_2 was collected as $BaCO_3$. The results are tabulated:

Leucine[a]	Total carotene, mg.	Radioactive carotene, mg.	CO_2 c.p.m.[b] per mg. $BaCO_3$	CH_3NH_2·HCl, c.p.m.[b] per mg. $BaCO_3$
4C	20.1	3.5	298 ± 11.0	4.2 ± 1.1
2C	20.2	3.5	53 ± 7.6	1.6 ± 0.8
3C	32.4	3.5	1.8 ± 0.4	10.1 ± 2.7
3C	19.6	5.3	2.0 ± 0.6	7.2 ± 1.3

[a] The counts added to the media differed in each case. [b] The standard deviation was computed to include self-absorption, weighing and counting errors.

The radioactivity of the 4-C is concentrated in the carboxyl group. Thus, the 4-C of leucine is located in positions 5,5, 9,9, 13 and 13 of the carotene molecule.

This strongly suggests that leucine provides an iso C_3 fragment in which the 4-C is centrally located. The distribution for the 2-C of leucine is the same as for the 4-C. Therefore we may assume that a C_2 fragment, also provided by leucine, can

(1) H. Yokoyama, *et al.*, THIS JOURNAL, **79**, 2029 (1957)
(2) C. O. Chichester, *et al.*, *J. Biol. Chem.*, **214**, 515 (1955).
(3) E. C. Grob and R. Bütler, *Helv. Chim. Acta*, **39**, 1975 (1956).
(4) E. C. Grob and R. Bütler, *ibid.*, **37**, 1908 (1954).
(5) P. Karrer and A. Helfenstein, *ibid.*, **13**, 1084 (1930).
(6) C. Schnerch and E. H. Huntress, THIS JOURNAL, **71**, 2283 (1949).