the mixture at mass numbers greater than *m/e* 76. The heptaborane contribution to *m/e* 78 was only 10% and to *m/e* 77, only 8% of the observed peak heights.

From the monoisotopic mass spectrum, the $B_6H_{10}^+$ and $B_6H_8^+$ species of hexaborane-12 are relatively more abundant than those of hexaborane 10. Hexaborane-12 resembles hexaborane-10 in that it has no $B_5H_{11}^+$ or $B_5H_{10}^+$ species.

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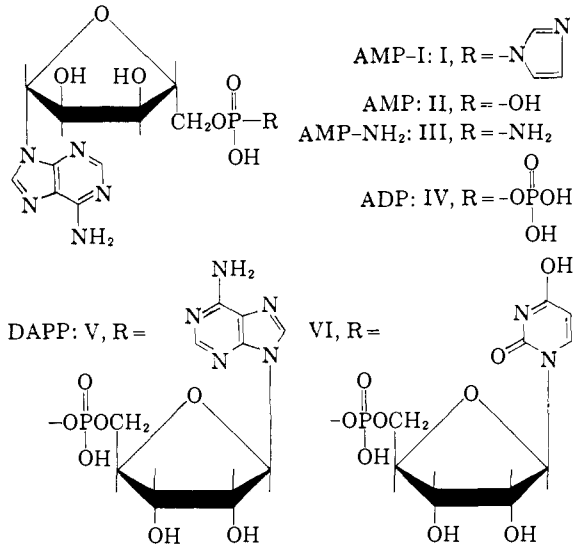
THE PREPARATION OF ADENOSINE-5' IMIDAZOL-1-YLPHOSPHONATE AND ITS REACTIONS WITH NUCLEOPHILES. A NOVEL SYNTHESIS OF NUCLEOTIDE COENZYMES

Sir:

The imidazole ring of a histidine moiety is implicated in the binding or catalytic activity of esterases, proteases, carbohydrases, etc.[1] Enzyme-ATP complexes have been suggested as intermediates in transphosphorylation,[2] and an imidazol-1-ylphosphonate[3] has been suggested[4] as an example of this type of complex. Imidazolylphosphonates, such as imidazol-1-ylphosphonic acid, imidazol-1,3-diyldiphosphonic acid and phenyl imidazol-1-ylphosphonate have been shown to be phosphorylating agents.[4,5,6]

Adenosine-5' imidazol-1-ylphosphonate (AMP-I, I) was chosen as a model for reaction with nucleophiles to provide information bearing on the nature of transphosphorylation. In addition, these reactions exemplify a novel, facile synthesis of nucleotide coenzymes.

Imidazolium AMP-I[7] is prepared readily in anhydrous dimethylformamide by reaction of the imidazolium salt of adenosine-5' phosphate (AMP, II) with 1,1'-carbonyldiimidazole (CDI).[8,9] With equimolar quantities of AMP monohydrate and CDI, the products found by paper chromatography are AMP-I in major amount, unchanged AMP, and P^1,P^2-di-(adenosine-5') pyrophosphate (DAPP, V). With 2–4 moles of CDI to one mole of AMP monohydrate, conversion to AMP-I is nearly quantitative. On Whatman No. 1 paper AMP-I has R_f = 0.43[10] in isopropyl alcohol–ammonia–water (7:1:2), in which solvent it is partly solvolyzed to adenosine-5' phosphoramidate (AMP-NH_2, III), R_f = 0.20.



When imidazolium AMP-I (from one mole of AMP monohydrate and 2 moles of CDI) is allowed to react with AMP monohydrate and the reaction mixture is chromatographed on Dowex-1 (formate), 57% of colorless crystalline DAPP (V) sesquihydrate,[11] m.p. 184–189°, is obtained (Calcd. for $C_{20}H_{26}N_{10}O_{13}P_2 \cdot 1.5H_2O$: C, 34.2; H, 4.16; N, 19.9; P, 8.81. Found: C, 34.3; H, 4.20; N, 20.2; P, 8.49, 8.37), homogeneous by the criteria of paper chromatography in two solvent systems and by paper electrophoresis. Uridine-5' phosphate and AMP-I give P^1-(adenosine-5') P^2-(uridine-5') pyrophosphate (VI), 0.84 as electrophoretically mobile as P^1,P^2-di-(uridine-5') pyrophosphate on Whatman 3MM paper in *p*H 4.8 acetate buffer.[12]

Imidazolium AMP-I (from one mole of AMP monohydrate and 3 moles of CDI), aqueous ammonia, dimethylformamide and *tert*-butyl alcohol, kept at 92° for 11 hours, give AMP-NH_2 (III), isolated in 86% yield as the colorless crystalline 1,3-dicyclohexylguanidinium salt solvated with water and dimethylformamide, m.p. 207–210° dec. (Calcd. for $C_{10}H_{15}N_6O_6P \cdot C_{13}H_{25}N_3 \cdot H_2O \cdot C_3H_7NO$: C, 47.3; H, 7.47; N, 21.2; P, 4.69. Found: C, 47.4; H, 7.25; N, 21.6, 21.3; P, 4.93), which, when recrystallized from aqueous acetone, gives the unsolvated salt, m.p. 236–238° dec.[10]

With excess 85% phosphoric acid at −10 to −20°, imidazolium AMP-I (from equal moles of AMP monohydrate and CDI) is converted to adenosine-5' pyrophosphate (ADP, IV), isolated in 25% yield as the yellow crystalline acridinium salt,[13] m.p. 216–217° dec. (Calcd. for $C_{10}H_{15}N_5O_{10}P_2 \cdot C_{13}H_9N$: C, 45.6; H, 3.99; N, 13.9; P,

(1) For a review and leading references see E. A. Barnard and W. D. Stein, *Advances in Enzymol.*, **20**, 51 (1958).
(2) M. B. Hoagland, *Biochim. et Biophys. Acta*, **16**, 288 (1955).
(3) "Imidazolylphosphonate," rather than "phosphoimidazole"[5] or "phosphoroimidazole"[10]; *cf.* report of the A.C.S. Nomenclature, Spelling and Pronunciation Committee, *Chem. Eng. News*, **30**, 4515 (1952).
(4) J. Baddiley, J. G. Buchanan and R. Letters, *J. Chem. Soc.*, 2812 (1956).
(5) T. Rathlev and T. Rosenberg, *Arch. Biochem. and Biophys.*, **65**, 319 (1956).
(6) H. A. Staab, H. Schaller and F. Cramer, *Angew. Chem.*, **71**, 736 (1959).
(7) AMP, imidazole and dicyclohexylcarbodiimide were reported[10] to give an unstable solid tentatively identified as a mixture of AMP-I and AMP.
(8) H. A. Staab, *Ann.*, **609**, 75 (1957).
(9) After completion of the work described here, H. A. Staab, *et al.*,[6] reported the preparation of imidazol-1-ylphosphonic acid and phenyl imidazol-1-ylphosphonate by the reaction of the appropriate phosphate with 1,1'-carbonyldiimidazole.
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(11) An amorphous tetrahydrate is described by S. M. H. Christie, D. T. Elmore, G. W. Kenner, A. R. Todd and F. J. Weymouth, *J. Chem. Soc.*, 2947 (1953).
(12) G. W. Kenner, C. B. Reese and A. R. Todd, *ibid.*, 546 (1958).
(13) T. Wagner-Jauregg, *Hoppe-Seyler's Z. physiol. Chem.*, **239**, 188 (1936).

10.2; adenine:pentose:total P:labile P = 1:1:2:1. Found: C, 46.0, 45.7; H, 4.32, 4.20; N, 13.7; P, 9.30, 9.56; adenine:pentose:total P:labile P = 1:0.97:1.98:0.93), which is indistinguishable from an authentic sample by mixture m.p., comparison of infrared and ultraviolet spectra, and mobility in paper chromatographic and electrophoretic systems, and which is enzymatically active (pyruvate kinase coupled with lactic dehydrogenase).[14]

Solutions of AMP-I, prepared from AMP monohydrate and excess CDI, react with phosphoric acid to produce a mixture of compounds, the nature of which will be described in a future communication.

(14) T. Bücher and G. Pfleiderer, "Pyruvate Kinase from Muscle," in S. P. Colowick and N. O. Kaplan, eds., "Methods in Enzymology," Vol. 1, Academic Press, Inc., New York, N. Y., 1955, pp. 435–440.

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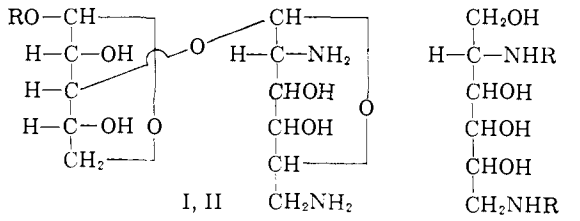
CHEMISTRY OF THE NEOMYCINS. VI. STRUCTURE OF NEOBIOSAMINE B[1]

Sir:

Neobiosamine B[1,2] has been shown to be a disaccharide composed of a diaminohexose, neosamine B,[1,3] linked glycosidically[2] to D-ribose.[1,4] In this report neobiosamine B is shown to have the structure and partial stereochemistry of I.



I, II

D-Ribose — Neosamine B — IIIa,b,c

I, R = H (Neobiosamine B)
II, R = CH_3 (Methyl neobiosaminide B)

IIIa, R = H
IIIb, R = 2,4-$(O_2N)_2$-C_6H_3
IIIc, R = $COCH_3$

N,N′-Bis-(2,4-dinitrophenyl)-neosaminol B (IIIb), obtained by sodium borohydride reduction of neosamine B to neosaminol B (IIIa) and subsequent dinitrophenylation,[1] consumed 1.91 mole of sodium metaperiodate with formation of only 0.03 mole of formaldehyde (chromotropic acid method). Periodate–permanganate oxidation[5,6] of the same compound (IIIb) gave glycine DNP, R_f 0.384 (BEW 415),[7] 0.260 (AA)[7] [authentic sample: R_f 0.381 (BEW 415), 0.267 (AA)] and L-serine DNP, $[\alpha]^{27}D$ +68° (*c* 0.25, 4% aq. $NaHCO_3$), R_f 0.365 (BEW 415), 0.240 (AA) [authentic sample: R_f 0.365 (BEW 415), 0.240 (AA)]. Identical oxidation of N-2,4-dinitrophenyl-D-glucosaminol, m.p. 163–164° [*Anal.* Found: C, 41.46; H, 4.94; N, 11.74.], also gave L-serine DNP, $[\alpha]^{25}D$ +66° (*c* 0.325, 4% $NaHCO_3$), R_f 0.365 (BEW 415). Similar, confirmatory, results were obtained with the N,N′-diacetyl derivative (IIIc)[8] and will be reported in the full paper. The structure and partial stereochemistry of neosaminol B are thus as shown in (IIIa).

Neosamine B consumed 2.56 mole of periodate in 35 min. with formation of only 0.02 mole of formaldehyde; this result establishes the compound as an aldohexose rather than a ketohexose (which would have given formaldehyde). Further, it exists in the pyranose form in methyl neobiosaminide B (II) since the latter compound[2] consumed 1.98 mole of periodate in 40 min. with formation of only 0.05 mole of formaldehyde. Ribose was recovered from hydrolysis of the oxidized methyl neobiosaminide B. Its protection from periodate oxidation establishes (1) that neobiosamine B must be a neosaminidoribose, rather than a ribosido-neosamine (a conclusion reached earlier from the relative ease of hydrolysis of methyl neobiosaminide B and neobiosamine B),[2] and (2) that neosamine B is *not* linked at the C-4 or C-5 position of ribose (which would have necessitated a three-mole periodate uptake with no recovered ribose).

The position of the ribose linkage was determined ultimately by periodate oxidation of neobiosaminol B, obtained by borohydride reduction of neobiosamine B.[1] The reduced disaccharide consumed 4.2 mole of periodate during one hour with formation of 1.6 mole of formaldehyde; this establishes the linkage at ribose C-3 (rather than C-2).

This position was confirmed by methylation studies. Methyl neobiosaminide B was N-acetylated with acetic anhydride and silver acetate,[9] then O-methylated with methyl iodide and barium oxide.[10] The product was hydrolyzed in dilute hydrochloric acid to 2,4-O,O-dimethyl-D-ribose, $[\alpha]^{26}D$ −30.5° (*c* 2.6, H_2O), R_f 0.61, R_{ribose} 2.18 (BAW 415)[7] [lit.[11] values for 2,5 (and 3,5-)-O,O-dimethyl-D-ribose: R_f 0.69, R_{ribose} 2.30 (BAW 415)]. On paper electrophoresis in borate buffer the isolated dimethylribose did not migrate,[11] and it consumed only 0.04 mole of periodate in 52 hr.; these observations establish the C-2 O-methyl group. The C-4 O-methyl group was demonstrated by reducing the dimethylribose with sodium borohydride to 2,4-O,O-dimethylribitol. In the reduced compound the methyl groups were located by its lack of reactivity with sodium metaperiodate (0.00 mol. uptake in 79 hr.) and with periodate–permanganate spray reagent and by its optical inactivity within experi-

(1) Paper V, K. L. Rinehart, Jr., A. D. Argoudelis, W. A. Goss, A. Sohler and C. P. Schaffner, THIS JOURNAL, **82**, in press (1960).

(2) K. L. Rinehart, Jr., P. W. K. Woo, A. D. Argoudelis and A. M. Giesbrecht, *ibid.*, **79**, 4567 (1957).

(3) K. L. Rinehart, Jr., P. W. K. Woo and A. D. Argoudelis, *ibid.*, **80**, 6461 (1958).

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

(5) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, **33**, 1701 (1955).

(6) R. U. Lemieux and H. F. Bauer, *Anal. Chem.*, **26**, 920 (1954).

(7) BEW 415: *n*-butyl alcohol:ethyl alcohol:water, 4:1:5, by volume. AA: isoamyl alcohol containing 1% acetic acid. BAW 415: *n* butyl alcohol:acetic acid:water, 4:1:5, by volume.

(8) A. D. Argoudelis, Ph.D. Thesis, University of Illinois, August, 1959.

(9) T. White, *J. Chem. Soc.*, 428 (1940).

(10) R. Kuhn, H. H. Baer and A. Seeliger, *Ann.*, **611**, 236 (1957).

(11) D. M. Brown, D. I. Magrath and A. R. Todd, *J. Chem. Soc.*, 1442 (1954).