sorption spectrum showed maxima at 226 mμ (log ε 4.15) and 288 mμ (log ε 3.58) when run in ethanol containing one drop of sodium hydroxide.

Anal. Calcd. for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.91. Found: C, 71.46; H, 6.87; N, 4.98.

The picrate was prepared by the addition of ethanolic picric acid to an ethanolic solution of 64 mg. of the methine. The picrate was recrystallized from acetone to give 51 mg. of yellow prisms, m.p. 209–211°, $[\alpha]^{22}_{589}$ −19.4°, $[\alpha]^{22}_{436}$ −28.0° (*c* 1.25, acetone–water, 90:10). The infrared spectrum (Nujol) was identical with that of dihydroöxocrinine methopicrate. A mixture melting point was not depressed.

BETHESDA 14, MARYLAND

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, PEARL RIVER LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

The Synthesis of 1-(Aminodeoxy-β-D-ribofuranosyl)-2-pyrimidinones. New 3′- and 5′-Aminonucleosides

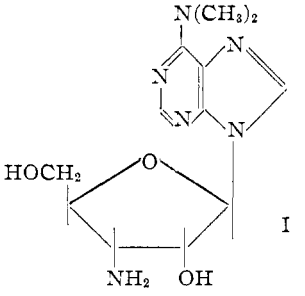
BY HENRY M. KISSMAN AND MARTIN J. WEISS

RECEIVED DECEMBER 7, 1957

1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-thymine (VII) was prepared *via* the condensation of dithyminylmercury (II) with 1-chloro-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranose (V). Condensation of N-acetylcytosinemercury (III) with V gave, after deblocking, 3′-amino-3′-deoxycytidine (IX). The versatile intermediate, 1-(2,3-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)-4-ethoxy-2-pyrimidinone (XVII) was prepared from chloromercuri-4-ethoxy-2-pyrimidinone (IV) and V. Ammonolysis of XVII afforded a second synthesis of IX. Treatment of XVII with butylamine or with dimethylamine gave the corresponding 4-butylamino- (XVIII) and 4-dimethylamino- (X) analogs of IX. Reaction of XVII with methanolic hydrogen chloride produced, after deblocking, 3′-amino-3′-deoxyuridine (XIX). 1-(5-Amino-5-deoxy-β-D-ribofuranosyl)-thymine (XXII) and 5′-amino-5′-deoxycytidine (XXIV) were prepared by similar procedures. Condensation of a mercury derivative of 4-dimethylamino-2(1H)-pyrimidinone (XI) with V gave the *O*-glycosyl derivative XV.

A considerable number of purine nucleosides have been prepared in this Laboratory as analogs of the aminonucleoside, 6-dimethylamino-9-(3-amino-3-deoxy-β-D-ribofuranosyl)-purine (I), derived from the antibiotic puromycin.[1] In the course of this work it was demonstrated that the carcinostatic[2a] and trypanocidal[2b] activities of the aminonucleoside were dependent on the presence of the 3-amino-3-deoxy-D-ribose portion and only to a lesser extent on the nature of the purine moiety. Thus, 3′-amino-3′-deoxyadenosine [6-amino-9-(3-amino-3-deoxy-β-D-ribofuranosyl)-purine[3]] was at least as active as the aminonucleoside, whereas 6-dimethylamino-9-β-D-ribofuranosylpurine[4] had no trypanocidal activity and showed antitumor activity only in tissue culture. Also, a large number of 9-(3-amino-3-deoxy-β-D-ribofuranosyl)-purines with variously substituted amino groups in the 6-position had trypanocidal and *in vivo* antitumor activity.[5] Therefore, it was of interest to determine the effect on the chemotherapeutic activity resulting from the substitution of nucleic acid pyrimidines for the purine portion of the aminonucleoside I. The preparation of several 1-(3-amino-3-deoxy-β-D-ribofuranosyl)-2-pyrimidinones is the subject of this paper.

The most commonly used method for the synthesis of pyrimidine nucleosides has been the one devised by Hilbert and Johnson[6] which involves the condensation of a 2,4-dialkoxypyrimidine with an acylated 1-halo sugar. Good results have been obtained on application of this method to the preparation of pyranosyl nucleosides,[7] but the procedure



I

gave poor yields[8] or compounds of doubtful structure[9] when used with poly-O-acyl-D-ribofuranosyl halides for the synthesis of the naturally occurring pyrimidine nucleosides. No practical synthesis of these latter compounds was available until Fox and his co-workers[10–12] demonstrated the utility of the mercury derivatives of certain 2-pyrimidinones in condensation with suitably blocked glycofuranosyl halides for the preparation of the required 1-β-D-glycofuranosyl-2-pyrimidinone derivatives. Thus, dithyminylmercury (II) upon re-

(1) B. R. Baker, J. P. Joseph and J. H. Williams, THIS JOURNAL, **77**, 1 (1955).

(2) (a) P. L. Bennett, S. L. Halliday, J. J. Oleson and J. H. Williams, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, N. Y., 1954, pp. 766–769; (b) R. I. Hewitt, A. R. Gumble, W. S. Wallace and J. H. Williams, *Antibiotics & Chemotherapy*, **4**, 1222 (1954).

(3) B. R. Baker, R. E. Schaub and H. M. Kissman, THIS JOURNAL, **77**, 5911 (1955).

(4) H. M. Kissman, C. Pidacks and B. R. Baker, *ibid.*, **77**, 18 (1955).

(5) L. Goldman, J. W. Marsico and R. B. Angier, *ibid.*, **78**, 4173 (1956).

(6) G. E. Hilbert and T. B. Johnson, *ibid.*, **52**, 4489 (1930).

(7) J. J. Fox and I. Goodman, *ibid.*, **73**, 3265 (1951).

(8) G. A. Howard, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 1052 (1947).

(9) M. Roberts and D. W. Visser, THIS JOURNAL, **74**, 668 (1952); *cf.* J. O. Lampen, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," Vol. II, The Johns Hopkins Press, Baltimore, Md., 1952, p. 368.

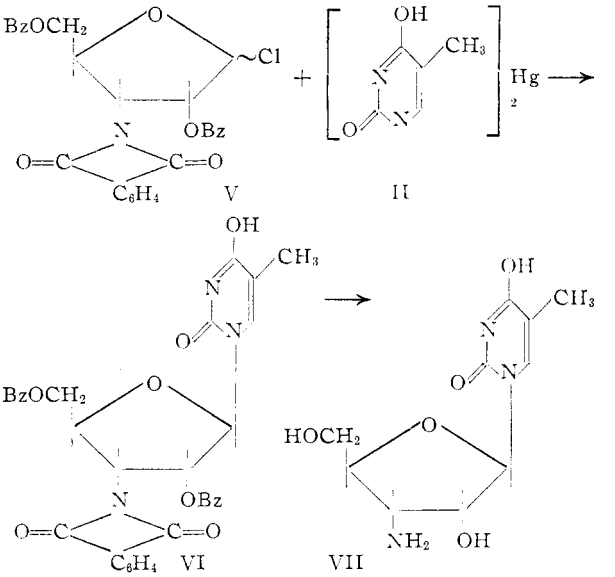
(10) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, THIS JOURNAL, **78**, 2117 (1956). For unequivocal proof of the anomeric configuration of 1-β-D-ribofuranosylthymine, *cf.* J. J. Fox, N. Yung and A. Bendich, *ibid.*, **79**, 2775 (1957).

(11) J. J. Fox, N. Yung, I. Wempen and I. L. Doerr, *ibid.*, **79**, 5060 (1957).

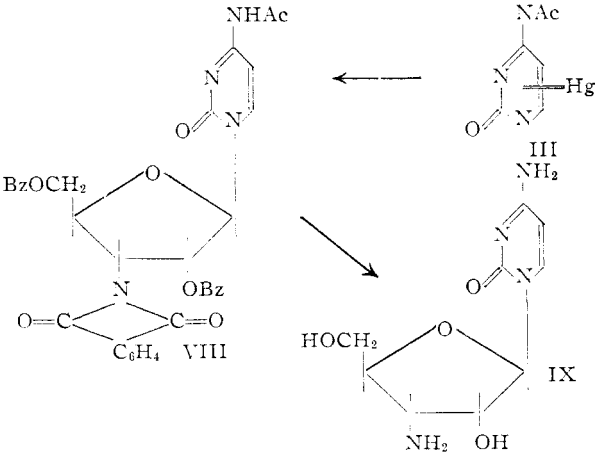
(12) J. J. Fox, N. Yung and D. Van Praag, *Federation Proc.*, **16**, 182, (1957).

action with tri-*O*-benzoyl-D-ribofuranosyl chloride followed by deblocking afforded 1-β-D-ribofuranosylthymine. In a similar manner, cytidine was prepared by the condensation of N-acetylcytosinemercury (III) with two equivalents of the same chloro sugar.[11] Treatment of chloromercuri-4-ethoxy-2-pyrimidinone (IV) with this chloro sugar afforded 1-(tri-*O*-benzoyl-β-D-ribofuranosyl)-4-ethoxy-2-pyrimidinone which could be converted to cytidine on ammonolysis and to uridine by acid hydrolysis and debenzoylation.[11]

We have been able to adapt these methods to the synthesis of a variety of 3′-amino-3′-deoxypyrimidine nucleosides. The most satisfactory derivative of 3-amino-D-ribose for nucleoside synthesis has been found to be 1-chloro-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranose (V)[13] and, therefore, this sugar halide was used for condensation with the mercury pyrimidines mentioned above. Reaction of V with dithyminylmercury (II), under the conditions described by Fox and co-workers,[10] afforded crystalline 1-(2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)-thymine (VI) in 39% yield. Removal of the phthaloyl and benzoyl blocking groups was accomplished by refluxing with butylamine in methanol[5] and there was isolated a 62% yield of crystalline 1-(3-amino-3-deoxy-β-D-ribofuranosyl)-thymine (VII). The anomeric configuration of this nucleoside VII and of its precursor VI was not established experimentally. However, the β-configuration is assumed by analogy to the results obtained by Fox[10] in the synthesis of 1-β-D-ribofuranosylthymine, the β-configuration of which was established conclusively by periodate oxidation to a known dialdehyde.[14] Furthermore, it is reasonable to expect the formation of a β-nucleoside from the reaction of a 1-halo-2-acyl sugar, such as V, with a pyrimidylmercury derivative in view of the results reported previously for the condensation of 1-halo-2-acyl sugars with various purine[3,15] and benzimidazole[16] mercuric chloride derivatives. In all cases, the major product of such a condensation had the C_1–C_2 *trans* configuration.[17] Although nucleosides having the C_1–C_2 *cis* configuration have been obtained in certain instances,[3,5,16] the yields of these compounds have been low and they have always been accompanied by substantially larger amounts of the C_1–C_2 *trans* products. Therefore, the fact that the nucleoside VI was obtained in relatively good yield (39%) supports the assignment of the β-configuration. For similar reasons, the β-configuration is assigned to the other nucleosides (IX, X, XVIII, XIX) reported in this paper.



The synthesis of **3′-amino-3′-deoxycytidine (IX)** was accomplished by the method outlined by Fox and co-workers[11] for the preparation of cytidine. Acetylcytosinemercury (III)[11] was condensed with two equivalents[18] of the 1-chloro sugar V in refluxing xylene and the blocked nucleoside VIII was isolated after chromatography on silica gel as a crystalline solid in 59% yield. Treatment of compound VIII with butylamine in refluxing methanol[5] removed all the blocking groups and afforded crystalline 3′-amino-3′-deoxycytidine (IX), 1-(3-amino-3-deoxy-β-D-ribofuranosyl)-cytosine, in 74% yield.



From the condensation of III with chloro sugar V there also was obtained a highly dextrorotatory,

(13) B. R. Baker, J. P. Joseph and R. E. Schaub, THIS JOURNAL, **77**, 5905 (1955).

(14) Unfortunately, this procedure is not applicable to the aminonucleosides of this investigation. In general, it has been found in this Laboratory that 3-amino-D-ribofuranosides react with periodate to consume two rather than the expected one equivalent of oxidant. It also has been shown that the oxidation products obtained from various 3′-aminonucleosides, such as 3′-aminocytidine (IX), 3′-aminoadenosine[3] and the puromycin aminonucleoside (I) [unequivocally a β-nucleoside; *cf.* B. R. Baker and J. P. Joseph, THIS JOURNAL, **77**, 15 (1955)], were not the dialdehydes which are obtained from the corresponding non-amino ribonucleosides; M. J. Weiss, J. P. Joseph, H. M. Kissman, F. J. McEvoy, R. E. Schaub and A. M. Small, to be published.

(15) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).

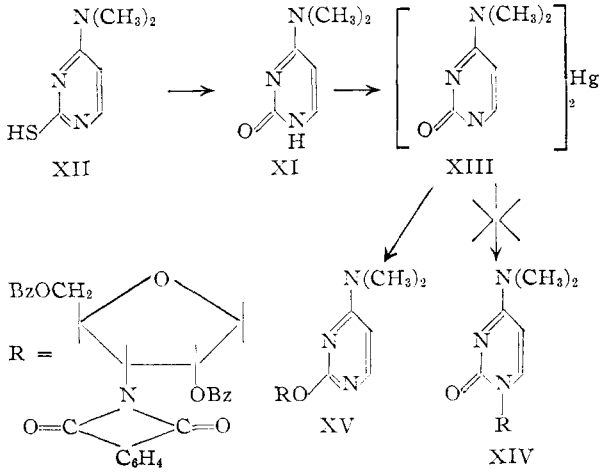
(16) H. M. Kissman, R. G. Child and M. J. Weiss, THIS JOURNAL, **79**, 1185 (1957).

(17) C_1–C_2 refers to the sugar carbons. With a D-ribose derivative, a C_1–C_2 *trans* configuration is therefore the β-configuration.

(18) It should be noted that, contrary to the usual pyrimidine, purine and benzimidazole mercury derivatives, acetylcytosinemercury (III) appears to be a structure in which both valences of mercury are bonded to the pyrimidine moiety. Apparently, an additional equivalent of the chloro sugar is required[11] for cleavage of one of these bonds to give an intermediate, which is then capable of undergoing nucleoside condensation with a second equivalent of the chloro sugar. These phenomena have been discussed in detail by Fox and co-workers.[11]

less polar substance with m.p. 140–141°, which gave combustion values in fair agreement with the formula $C_{27}H_{19}NO_7$. Ultraviolet and infrared spectroscopy indicated the presence of the benzoate and phthalimido groups and the absence of the pyrimidine moiety. No further structural work has been done on this compound, but it is thought to have originated from that first equivalent of the chloro sugar V[18] which reacts but does not condense with the pyrimidine mercury compound.

It was also of interest to prepare **1-(3-amino-3-deoxy-β-D-ribofuranosyl)-4-dimethylamino-2-pyrimidinone** (X) because of a certain similarity between the dimethylamino-2-pyrimidinone portion of X and the 6-dimethylaminopurine portion of the aminonucleoside I. At the same time, it was relevant to determine whether mercury derivatives of N,N-dialkylated cytosines (*i.e.*, 4-dialkylamino-2(1H)-pyrimidinones) could be used in condensation reactions with chloro sugars such as V. Therefore, the synthesis of X was attempted starting with a mercury derivative of 4-dimethylamino-2-(1H)-pyrimidinone (XI). The 4-dimethylamino-2(1H)-pyrimidinone (XI) was prepared from 4-dimethylamino-2-mercaptopyrimidine (XII)[19] by an adaption of the methods reported for the synthesis of cytosine.[20,21] Compound XII was condensed with chloroacetic acid and the resulting 2-carboxymethylthio-4-dimethylaminopyrimidine, without isolation, was hydrolyzed to afford XI in 53% yield. Depending on the proportion of the reagents used, the reaction of 4-dimethylamino-2-(1H)-pyrimidinone (XI) with mercuric chloride could be made to yield either the chloromercuri-pyrimidinyl or the dipyrimidinylmercury (XIII) derivative. The latter compound (XIII) was condensed with chloro sugar V, to give a crystalline reaction product (85% yield) which had the correct combustion values calculated for the anticipated, blocked nucleoside XIV. However, further investigation of this substance showed that it could not have structure XIV. In acid solution, its ultraviolet absorption maximum shifted from 274 to 282 mμ in a period of less than 20 minutes and the final spectrum was very similar to that of 4-dimethylamino-2(1H)-pyrimidinone (XI). This observation indicated a facile hydrolysis to the free pyrimidinone. Additionally, this product reduced Benedict reagent[22] and gave a 67% yield of pyrimidinone XI when refluxed with methanolic butylamine.[23] Such behavior is in contrast to the well known stability of pyrimidine nucleosides toward acidic and basic conditions, but it is in accordance with the lability of *O*-glycosylpyrimidines. These facts strongly suggest the *O*-glycosyl structure XV for the product obtained from this condensation. Formation of *O*-glycosyl derivatives of pyrimidinones previously have been reported to result from the reaction of sugar halides with the silver salts of pyrimidinones.[22,24,25]



The synthesis of X finally was achieved from 1-(2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)-4-ethoxy-2-pyrimidinone (XVII). This versatile intermediate was also used for a second synthesis of 3′-amino-3′-deoxycytidine (IX) and for the synthesis of 3′-amino-3′-deoxyuridine (XIX). The possibility of preparing an intermediate such as XVII by the condensation of a chloro sugar with chloromercuri-4-ethoxy-2-pyrimidinone (IV) was indicated by the work of Fox.[11] Condensation of the chloromercuri derivative IV with the blocked chloro sugar V in refluxing xylene gave, after purification by chromatography on acid-washed alumina, an amorphous material (59% yield) with the characteristics expected for the N-glycosyl derivative XVII.

Conversion of blocked nucleoside XVII to the **dimethylamino derivative X** was effected with methanolic dimethylamine in a sealed tube at 100° followed by complete deblocking with methanolic butylamine.[5] The desired nucleoside was obtained in crystalline form in 31% over-all yield after partition chromatography on Celite. Similarly, the 4-butylamino compound, **1-(3-amino-3-deoxy-β-D-ribofuranosyl)-4-butylamino-2-pyrimidinone (XVIII)**, was prepared by reaction of the blocked nucleoside XVII with butylamine in refluxing methanol. This product (XVIII) could not be obtained in crystalline form, even after partition chromatography, but the amorphous solid which was isolated gave good combustion values and was indicated to be homogeneous by paper chromatography (see Table I).

Heating the blocked 4-ethoxynucleoside XVII in a sealed tube with methanolic ammonia followed by methanolic butylamine treatment produced **3′-amino-3′-deoxycytidine (IX)**. The product was isolated[26] as a crystalline solid in 43% yield and it was identical in all respects with the material prepared from N-acetylcytosine (see above).

(19) P. B. Russell, G. B. Elion, E. A. Falco and G. H. Hitchings, THIS JOURNAL, **71**, 2279 (1949).

(20) G. H. Hitchings, G. B. Elion, E. A. Falco and P. B. Russell, *J. Biol. Chem.*, **177**, 357 (1949).

(21) D. J. Brown, *J. Soc. Chem. Ind. (London)*, **69**, 353 (1950).

(22) E. Fischer and B. Helferich, *Ber.*, **47**, 210 (1914), reported that *O*-glycosylpyrimidines reduced Fehling reagent.

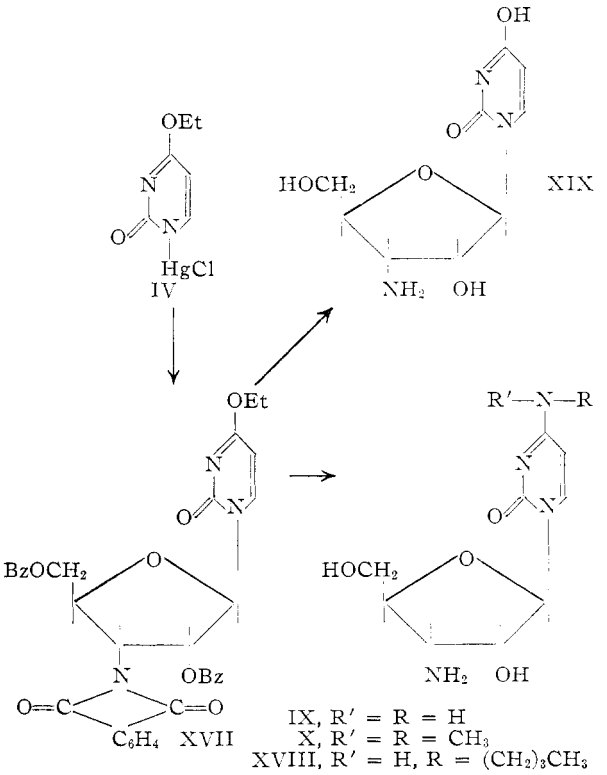
(23) It should be noted that the N-glycosylpyrimidinones discussed in this paper are stable under these conditions.

(24) P. A. Levene and H. Sobotka, *J. Biol. Chem.*, **65**, 469 (1925).

(25) The concurrent development of the "4-ethoxy-2-pyrimidinone" procedure made feasible a synthesis of X from the blocked 4-ethoxynucleoside XVII. Therefore, an investigation of the use of the chloromercuri-4-dimethylamino-2-pyrimidinone was not undertaken. It is conceivable that the use of this derivative might have resulted in the formation of N-glycosides.

(26) In the pilot experiment, crystalline IX could be isolated only after partition chromatography on Celite.

The uridine analog, **3′-amino-3′-deoxyuridine** (**XIX**), was obtained as a crystalline solid in 41% yield by treatment of the blocked 4-ethoxynucleoside XVII with methanolic hydrogen chloride and subsequently with butylamine in methanol.[5]



The availability in this Laboratory of the 1-chloro-2,3-di-*O*-benzoyl-5-deoxy-5-phthalimido-D-ribofuranose (XX)[27] led us to investigate the syntheses of certain 5′-amino-5′-deoxypyrimidine nucleosides. Condensation of XX with dithyminylmercury (II) afforded a product from which the blocked nucleoside XXI could be isolated, after chromatography on alumina, as an amorphous solid (21% yield) with satisfactory analytical values. There was also obtained a crystalline $C_{20}H_{15}NO_5$ product,[28] m.p. 144–145°, which did not contain the pyrimidine moiety. This compound gave a positive Benedict test and decolorized bromine in carbon tetrachloride solution. The substance was not investigated further. Removal of the blocking groups from XXI with methanolic butylamine[5] led to the isolation of amorphous **1-(5-amino-5-deoxy-β-D-ribofuranosyl)-thymine** (**XXII**) (49% yield), which was approximately 70% pure as estimated by comparison of the ultraviolet spectrum with that of 1-β-D-ribofuranosylthymine.[10] It has not been possible to purify this compound as such, but an amorphous hydrochloride salt with satisfactory analytical values was obtained.

The 5-phthalimido halogenose XX also was condensed with acetylcytosinemercury (III) to afford a blocked nucleoside XXIII, which was obtained in crystalline form after silica gel chromatography (54% yield). The $C_{20}H_{15}NO_5$ by-product was also isolated from this reaction. Deblocking of XXIII under the usual conditions yielded amorphous **5′-amino-5′-deoxycytidine** (**XXIV**) (84% pure by ultraviolet analysis) which could not be obtained analytically pure but which was characterized as a crystalline picrate.

The β-configuration was assigned without experimental proof to the 5′-amino-5′-deoxynucleosides XXII and XXIV for reasons analogous to those advanced above for the assignment of the β-configuration to the 3′-aminopyrimidine nucleosides.

Biological Testing.—None of the deblocked nucleosides prepared in the course of this investigation showed any significant antitumor or trypanocidal activities.[29] It must be concluded that substitution of nucleic acid pyrimidines for the purine portion of the aminonucleoside I from puromycin leads to loss of these biological activities.

TABLE I

PAPER CHROMATOGRAPHY OF 3′-AMINO-3′-DEOXYPYRIMIDINE NUCLEOSIDES[a]

Compound	*Rf* values in solvent systems A	B	C
1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-thymine (VII)	0.47	0.62	0.60
3′-Amino-3′-deoxycytidine (IX)	.26	.52	.50
1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-4-dimethylamino-2-pyrimidinone (X)	.52	.69	.69
1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-4-butylamino-2-pyrimidinone (XVIII)	.76	.90	.78
3′-Amino-3′-deoxyuridine (XIX)	.37	.50	.51

[a] The circular paper chromatography apparatus (26 cm. diameter) described by E. Kawerau, *Chromatographic Methods*, 1, No. 2, 7 (1956), was purchased from Shandon Scientific Co., Ltd., London, England. The chromatograms were run on Whatman #1 filter paper (KCT-26) in the following solvent systems: (A) butanol:water; (B) isopropyl alcohol:1 *N* ammonium hydroxide (7:2); (C) ethanol:concd. ammonium hydroxide:water (80:4:16). Spots were located by inspection in ultraviolet light.

Acknowledgment.—We are indebted to Dr. Jack J. Fox and his co-workers of the Sloan-Kettering Institute for making available to us the results of their investigations in this field prior to publication. We wish to thank Miss Arlene M. Small for technical assistance and Dr. J. H. Clark, Messrs. J. F. Poletto and L. J. Binovi for the large scale preparation of certain intermediates. We are grateful to Mr. C. Pidacks and staff for assistance in the use of partition chromatography, Mr. W. Fulmor and staff for spectral and polarimetric work and Mr. L. Brancone and staff for microanalyses.

Experimental[30]

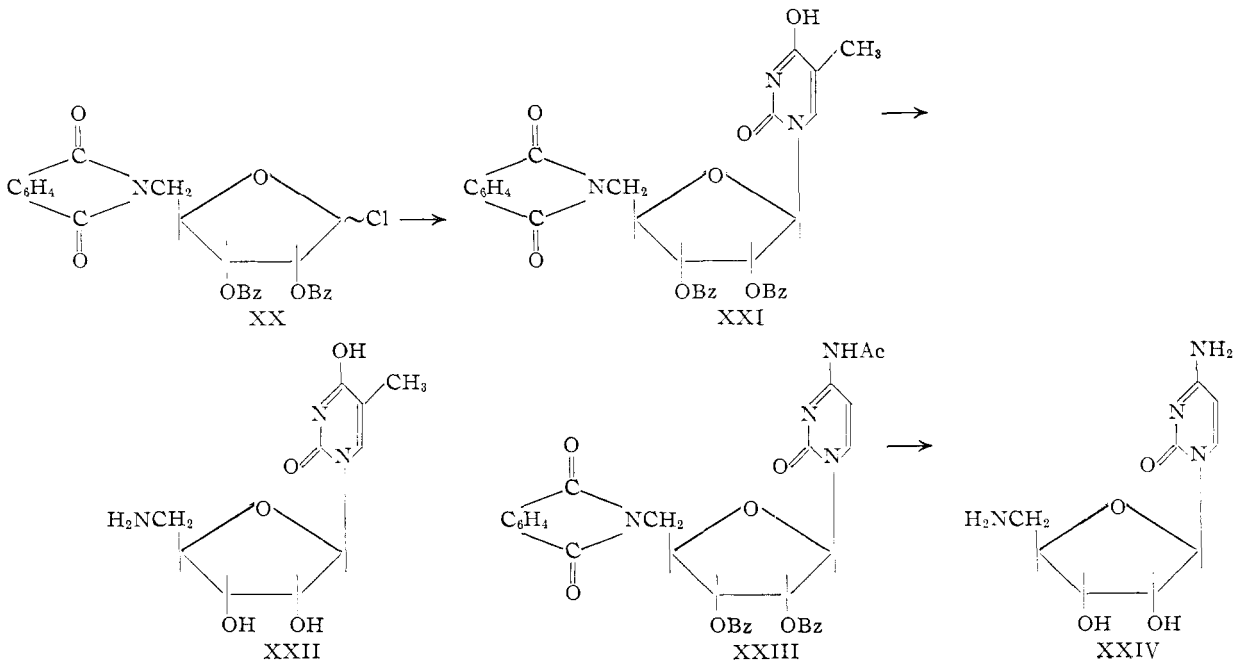
1-(2,5-Di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)-thymine (**VI**).—To an azeotropically dried suspension of 4.51 g. (10 mmoles) of dithyminylmercury[10] (II) in 200 cc. of xylene was added a suspension of 1-chloro-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranose (V),

(27) H. M. Kissman and B. R. Baker, Abstracts of Papers, 130th Meeting of the A.C.S., Atlantic City, N. J., September, 1956, p. 19D.

(28) This formula would fit a structure derived from the chloro sugar XX by subtraction of the elements of benzoyl chloride.

(29) Private communications from Dr. R. I. Hewitt, Miss S. L. Halliday and Dr. J. J. Oleson of these laboratories.

(30) Melting points were taken on a Kofler micro hot-stage and are corrected. Ultraviolet absorption spectra were determined on a Cary recording spectrophotometer and infrared spectra on a Perkin-Elmer double beam spectrophotometer, model 21.



obtained from 11.0 g. (20.8 mmoles) of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-D-ribofuranose,[13] in 75 cc. of xylene. The stirred mixture was refluxed for three hours and was then filtered while hot from 0.923 g. (7.3 mmoles) of thymine.[10] The filtrate was evaporated under reduced pressure and the residue was dissolved partially in 120 cc. of chloroform. The filtered solution was washed with two 20-cc. portions of 30% aqueous potassium iodide solution and then with 20 cc. of water. The chloroform solution was dried over magnesium sulfate, filtered and evaporated *in vacuo* to leave a yellow gum as residue. This was crystallized and recrystallized from ethyl acetate–ethanol to afford 4.87 g., m.p. 218–219° (39% yield based on 20.8 mmoles of 1-*O*-acetyl sugar derivative). Two recrystallizations from ether–methylene chloride gave analytically pure material with m.p. 222–223°, $[\alpha]^{25}D$ −37.8° (*c* 1.98 in chloroform). In the ultraviolet, the compound showed λ_{max}^{MeOH} 263 mμ (ε 14,320 in acid), 263 mμ (ε 13,860 in methanol) and 268 mμ (ε 11,460 in base).[31]

Anal. Calcd. for $C_{32}H_{25}N_3O_9$: C, 64.53; H, 4.23; N, 7.06. Found: C, 64.73; H, 4.23; N, 6.95.

1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-thymine (VII).—A suspension of 0.595 g. (1 mmole) of the blocked thymine derivative VI in 12 cc. of absolute methanol containing 0.7 cc. (7 mmoles) of butylamine was heated under reflux for 18 hours. The solution was evaporated under reduced pressure, the residue was triturated with 80 cc. of water and the mixture was filtered. The precipitate was washed well with water and the combined filtrate and washings were extracted with three 15-cc. portions of ether. The aqueous solution was evaporated *in vacuo* and the residue was dried by a triple evaporation with absolute ethanol. Crystallization from methanol–ethanol afforded 0.188 g. (62%) of crystalline solid, m.p. 214–216°. Analytically pure material was obtained after two recrystallizations from the same solvent mixture. The sample was dried at 110° *in vacuo;* m.p. 223–225°, $[\alpha]^{25}D$ +41.0° (*c* 1.93 in water); λ_{max}^{water} 265 mμ (ε 9,570 in acid), 266 mμ (ε 9,650 in water), 267 mμ (ε 7,270 in base).[31]

Anal. Calcd. for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.34. Found: C, 46.75; H, 6.01; N, 16.26.

1-(2,5-Di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)-4-acetamido-2-pyrimidinone (VIII).—A suspension of the chloro sugar V, obtained from 10.85 g. (20 mmoles) of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-D-ribofuranose,[13] in 50 cc. of xylene was added to an azeotropically dried suspension of 3.51 g. (10 mmoles) of N-acetylcytosinemercury[11,32] (III) in 200 cc. of xylene. The stirred mixture was refluxed for two hours and was then evaporated under reduced pressure. The residue was dissolved in 200 cc. of chloroform and the solution was washed twice with 20-cc. portions of 30% aqueous potassium iodide solution and then with 50 cc. of water. The solution was dried and partially decolorized over magnesium sulfate and Darco,[33] and was freed from solvents *in vacuo* to afford 11.8 g. of viscous residue. This was mixed with 100 cc. of benzene and was decanted from 3 g. of a dark gum. The benzene solution and the gum were chromatographed separately on silica gel.

The benzene solution was added to a column (25 × 4.5 cm.) prepared from 200 g. of silica gel[34] and benzene. The column was washed with 200 cc. of benzene, 100 cc. of benzene–ether (9:1) and 100 cc. of benzene–ether (8:2); these washings were discarded. Elution with 200 cc. of benzene–ether (6:4) afforded crystalline material (compound A). Further elution with 100 cc. of benzene–ether (4:6), 150 cc. of ether, 250 cc. of ether–chloroform (2:3), 150 cc. of chloroform, 100 cc. of chloroform–acetone (9:1) and 100 cc. of chloroform–acetone (6:4) yielded only small quantities of colored gums which were discarded. Elution with 200 cc. of chloroform–acetone (2:8) afforded a second crystalline substance (compound B). There was obtained by recrystallization from ethanol–ethyl acetate 2.9 g. of compound A (m.p. 129–138°) and 2.5 g. of compound B (m.p. 243–246°).

The 3 g. of benzene-insoluble gum was dissolved in 30 cc. of methylene chloride and the dark red solution was chromatographed on a column (2.5 cm. i.d.) containing 50 g. of silica gel (prepared with methylene chloride). Washing with 150 cc. of methylene chloride eluted a small amount of gum. Elution with methylene chloride–acetone (9:1) (150 cc.) and with methylene chloride–acetone (8:2) brought down 0.56 g. of colored gum which was discarded. Further elution with 200 cc. of methylene chloride–acetone (6:4) afforded solid material which was crystallized from ethyl acetate–ethanol; 1.13 g., m.p. 245–248°. This was shown to be identical with compound B as obtained from the first column.[35]

(31) The compound was dissolved in the solvent indicated. Aliquots were diluted 1:10 with 0.1 *N* aqueous hydrochloric acid for the acid spectrum and 1:10 with 0.1 *N* sodium hydroxide for the base spectrum.

(32) H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, **29**, 492 (1903).

(33) Darco and Norit are activated charcoal.

(34) Davison Silica Gel, mesh size 200, a product of the Davison Chemical Co., was used as such.

(35) Product recovery was not satisfactory when the total reaction product was chromatographed on one column from benzene–chloroform (3:1).

The two fractions of **compound B (VIII)** were combined to yield 3.65 g. (59% based on 10 mmoles of the mercury derivative, III) of product. Material obtained in a similar experiment was recrystallized from ethanol–ethyl acetate and from methylene chloride–ether; m.p. 245–246°, $[\alpha]^{25}D$ −20.3° (*c* 1.08 in chloroform); λ_{max}^{MeOH} 305 mμ (ε 13,130 in acid), 298 mμ (ε 9,350 in methanol), 272 mμ (ε 13,130 in base).[31]

In the infrared, the compound showed λ_{max}^{KBr} 5.62 μ (phthalimido carbonyl), 5.78 μ (pthalimido and ester carbonyl), 5.96 μ (*N*-acetyl carbonyl), 6.11 μ (C=N).

Anal. Calcd. for $C_{33}H_{26}N_4O_9$: C, 63.66; H, 4.21; N, 9.00. Found: C, 63.81; H, 4.38; N, 9.17.

Compound A was purified for analysis by several recrystallizations from ethanol–ethyl acetate; m.p. 140–141°, $[\alpha]^{25}D$ +202° (*c* 1.03 in chloroform); λ_{max}^{MeOH} 223 mμ (ε 70,500) and 263 mμ (ε 7,990) in acid, 221 mμ (ε 64,400) and 263 mμ (ε 7,050) in methanol and 214 mμ (ε 73,300) and 262 mμ (ε 3,750) in base; λ_{max}^{KBr} 5.62 μ (phthalimido carbonyl) and 5.80 μ (phthalimido carbonyl and ester carbonyl).[36]

Anal. Calcd. for $C_{27}H_{19}NO_7$ (chloro sugar V minus the elements of benzoyl chloride): C, 69.08; H, 4.08; N, 2.98. Found: C, 68.59; H, 4.16; N, 3.11.

4-Dimethylamino-2-mercaptopyrimidine (XII).[19]—Dithiouracil (30 g., 20.8 mmoles) was shaken with 25% aqueous dimethylamine (180 cc.) in a bomb at 130° for 3.5 hours. The cooled bomb was opened and the contents were collected with 100 cc. of water. The mixture was evaporated *in vacuo* and the residue was mixed with 300 cc. of 2 *N* hydrochloric acid and filtered through Norite.[33] The filtrate was brought to *p*H 8 with concentrated ammonia and the solid which crystallized on standing was collected, washed with water and dried in air. The filtrate and washings were evaporated *in vacuo* and the residue was mixed with 80 cc. of 2 *N* hydrochloric acid. The orange solid which precipitated was removed by filtration and discarded. The filtrate was made alkaline with concentrated ammonium hydroxide and the resulting solid was combined with the first portion of product. Recrystallization from 900 cc. of water with Norit afforded 17.9 g. (55%) of a tan solid with m.p. 266–270° dec. One additional recrystallization from water with Norit gave a white crystalline solid, m.p. 265–270° dec. (lit.[19] m.p. 280–283° dec.).

Anal. Calcd. for $C_6H_9N_3S$: C, 46.43; H, 5.84; N, 27.07; S, 20.66. Found: C, 46.20; H, 6.07; N, 26.69; S, 20.90.

4-Dimethylamino-2(1H)-pyrimidinone (XI).—Water (17 cc.) was added to a mixture of 2.68 g. (17.25 mmoles) of 4-dimethylamino-2-mercaptopyrimidine (XII)[19] and 1.7 g. (18 mmoles) of chloroacetic acid. The mixture was heated on the steam-bath with stirring until all of the solid had dissolved and heating was continued for an additional 40 minutes. Concentrated hydrochloric acid (16 cc.) was added and the solution was allowed to reflux for 2 hours, after which it was kept at room temperature overnight. The solution was evaporated *in vacuo* and the residue was evaporated several times with water in order to remove traces of hydrochloric acid. The residue was then mixed with 2 cc. of water and 4 cc. of concentrated ammonium hydroxide solution. The suspension was chilled and filtered. The dark filtrate was concentrated to a small volume and was again treated with ammonium hydroxide. The mixture was filtered once more and the highly colored filtrate was passed through a column (11.5 × 2.5 cm.) of Amberlite IRA-400 anion exchange resin[37] (OH form). The product was eluted with 300 cc. of water, while colored impurities remained on the column. The effluent was evaporated under reduced pressure and the residue was crystallized from ethanol–isopropyl alcohol to afford 1.28 g. (53%) with m.p. 248–250°, $R_{cytosine}$ 1.03.[38] For analysis, a sample was sublimed *in vacuo;* m.p. 248–249°; λ_{max}^{MeOH} 282 mμ (ε 13,460), 281 mμ (ε 11,130), 284 mμ (ε 9,452).[31]

Anal. Calcd. for $C_6H_9N_3O$: C, 51.78; H, 6.52; N, 30.22. Found: C, 51.90; H, 6.78; N, 30.56.

(36) The 5.96 and 6.11 μ maxima of a 4-acetamido-2-pyrimidinone nucleoside were absent.

(37) This resin is a product of the Rohm and Haas Co., Philadelphia, Pa.

(38) R_f compound/R_f cytosine = $R_{cytosine}$ in butanol:acetic acid:water (50:25:35).

Chloromercuri Derivative.—To a solution of 0.139 g. (1 mmole) of 4-dimethylamino-2(1H)-pyrimidinone (XI) in 1 cc. of 1 *N* sodium hydroxide was added 0.271 g. (1 mmole) of mercuric chloride dissolved in 3 cc. of hot ethanol. The resulting precipitate was collected, washed with water, ethanol and ether and was dried at 74° *in vacuo:* 331 mg. (89%).

Anal. Calcd. for $C_6H_8N_3OHgCl$: C, 19.26; H, 2.15; N, 11.23; Cl, 9.48. Found: C, 20.28; H, 2.56; N, 10.54; Cl, 12.24.

Dipyrimidinylmercury Derivative XIII.—To a solution of 0.139 g. (1 mmole) of XI in 1 cc. of 1 *N* sodium hydroxide solution was added 0.135 g. (0.5 mmole) of mercuric chloride in 2 cc. of hot ethanol. A precipitate formed on scratching. It was collected and washed with water and ethanol by centrifugation. The dried solid (74° *in vacuo*) weighed 0.211 g. (89%).

Anal. Calcd. for $C_{12}H_{16}N_6O_2Hg$: C, 30.22; H, 3.38; N, 17.62; Cl, 0.00. Found: C, 29.03; H, 3.68; N, 16.05; Cl, 1.18.

Condensation of 1-Chloro-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-D-ribofuranose (V) with the Dipyrimidinylmercury Derivative XIII. 4′-Dimethylamino-2′-pyrimidinyl 2,5-Di-*O*-benzoyl-3-deoxy-3-phthalimido-D-ribofuranoside (XV).—To an azeotropically dried suspension of 2.7 g. (5.6 mmoles) of the dipyrimidinylmercury derivative XIII in 200 cc. of xylene was added the chloro sugar V, prepared from 2.7 g. (5.1 mmoles) of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-D-ribofuranose, in 80 cc. of hot xylene. The stirred mixture was allowed to reflux for 3 hours and was then filtered while hot. The precipitate[39] (1.75 g.) was washed with a little xylene and washings and filtrate were combined and evaporated under reduced pressure. The residue was taken up in 100 cc. of chloroform and the resulting suspension was washed with two 15-cc. portions of 30% aqueous potassium iodide solution and then with 15 cc. of water. The organic phase was dried over magnesium sulfate and the filtered solution was freed from solvent *in vacuo*. The residue solidified when triturated with ether. It was collected, washed with ether and dried; 2.6 g. (85% based on V), m.p. 160–168°. For analysis, the substance was recrystallized three times from ethanol; m.p. 165–166°, $[\alpha]^{25}D$ +39.0° (*c* 1.75 in chloroform). The substance reduced Benedict reagent when heated. In the infrared, it showed the carbonyl absorption bands of the benzoyl and phthalimido groups at 5.62 and 5.81 μ. However, there was no absorption in the 5.95–6.05 μ region, indicating the absence of the 2-pyrimidinone carbonyl group. The 5.95 μ (lactam C=O) and 6.10 μ (C=N) bands found in the spectra of normal, blocked pyrimidine nucleosides (*cf.* XVII) was also lacking. In the ultraviolet, the compound showed λ_{max}^{MeOH} 274 mμ (ε 13,400 in acid after 3 minutes), 282 mμ (ε 11,900 in methanol) and 280 mμ (ε 9,450 in base after 3 minutes).[31] Exposure to 0.1 *N* hydrochloric acid caused a shift of the absorption maximum from 274 to 282 mμ in a period of 20 minutes.

Anal. Calcd. for $C_{32}H_{28}N_4O_5$: C, 65.12; H, 4.64; N, 9.21. Found: C, 65.42; H, 4.97; N, 9.39.

Reaction of Compound XV with Methanolic Butylamine.—A solution of 0.2 g. (0.29 mmole) of XV in 10 cc. of methanol containing 1 cc. of butylamine was refluxed overnight and was then evaporated *in vacuo*. The residue was mixed with 40 cc. of water and 20 cc. of ether. The aqueous phase was further extracted with three 10-cc. portions of ether. Evaporation of the water solution left a residue which was purified by sublimation and crystallization from methanol–ethyl acetate to afford 0.027 g. (67%) of 4-dimethylamino-2(1H)-pyrimidinone (XI), m.p. 246–248°. Identity was established by mixed m.p. and by paper chromatography.

Chloromercuri-4-ethoxy-2-pyrimidinone (IV).—To a solution of 4-ethoxy-2(1H)-pyrimidinone[40] (0.14 g., 1 mmole) in 2 cc. of warm water was added 0.271 g. (1 mmole) of mercuric chloride dissolved in 4 cc. of hot ethanol. The mixture was kept at 50–60° to prevent precipitation of the components and there was added dropwise with stirring, 1 cc. of 1 *N* sodium hydroxide solution. The yellow color caused by each drop of sodium hydroxide disappeared on mixing.

(39) Analysis indicated this solid to be at least in part chloromercuri-derivative XI. *Anal.* Found: C, 22.80; H, 2.52; N, 11.00; Cl, 10.77.

(40) G. E. Hilbert and E. F. Jansen, THIS JOURNAL, **57**, 552 (1935).

The last drop produced a permanent, slightly yellow color. The precipitate which had formed during the addition process was collected, washed with water, ethanol, and finally with ether, and was dried at 74° *in vacuo* to afford 0.344 g. (92%) of light yellow powder.

Anal. Calcd. for $C_6H_7N_2O_2$·HgCl: C, 19.21; H, 1.88; N, 7.47; Cl, 9.45. Found: C, 19.54; H, 1.96; N, 7.61; Cl, 9.21.

Very similar results were obtained[11] when mercuric chloride was added to a mixture of the pyrimidinone and sodium hydroxide.

1-(2,5-Di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)-4-ethoxy-2-pyrimidinone (XVII).—To an azeotropically dried suspension of 29.6 g. (78.7 mmoles) of chloromercuri-4-ethoxy-2-pyrimidinone (IV) in 840 cc. of xylene was added chloro sugar V, prepared from 45.5 g. (86 mmoles) of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-D-ribofuranose, as a suspension in 100 cc. of xylene. The mixture was stirred and refluxed for 3 hours and was then filtered while hot through a bed of Celite[41] to remove a small amount of brown solid. The filtrate was evaporated under reduced pressure and the residue was dissolved with warming in 300 cc. of chloroform. The solution was filtered from a small amount of insoluble precipitate (discarded) and was washed twice with 60-cc. portions of 30% aqueous potassium iodide solution and then with 50 cc. of water. The dried (magnesium sulfate) chloroform solution was evaporated *in vacuo* and the residue (34.5 g.) was dissolved in 60 cc. of benzene. A solid product formed as a gel was removed by filtration. After washing with benzene and drying *in vacuo*, there was obtained 2.8 g. of product, m.p. 128–135°. It was not possible to obtain additional quantities of product by crystallization and therefore the benzene mother liquors (200 cc.) were chromatographed on 517 g. of acid-washed alumina.[42,43] The column (31 × 5 cm.) was washed with 400 cc. of benzene and these fractions were discarded. Further elution with 1400 cc. of benzene and 1600 cc. of benzene–ethyl acetate (85:15) yielded, on evaporation of the pooled fraction, a light yellow gum from which there could be obtained by trituration with ethyl acetate 25.5 g. of an amorphous (gel-like) solid, m.p. 130–138°. Combined with the 2.8 g. isolated above this represented a 59% yield (based on XVI) of product with sufficient purity for subsequent steps.

For analysis a sample of the substance was dissolved in methylene chloride–ether, and ether was added to the solution at the b.p. until gel formation started. The solid was collected, washed with ether and dried. This procedure was repeated several times. Material dried at 74° *in vacuo* had m.p. 138–148°, $[\alpha]^{25}D$ +41.5° (*c* 0.92 in chloroform); λ_{max}^{MeOH} 273 mμ (ε 10,830 in acid), 274 mμ (ε 11,020 in methanol) and 273 mμ (ε 10,650 in base)[31]; λ_{max}^{KBr} 5.62 and 5.80 μ (phthalimido and benzoyl carbonyl), 5.95 μ (pyrimidinone carbonyl) and 6.10 μ (C=N).

Anal. Calcd. for $C_{33}H_{27}N_3O_9$: C, 65.02; H, 4.46; N, 6.89. Found: C, 64.73; H, 4.51; N, 7.04.

3′-Amino-3′-deoxycytidine [1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-cytosine (IX)]. (A) From 1-(2,5-Di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)-4-acetamido-2-pyrimidinone (VIII).—A suspension of 4.22 g. (6.8 mmoles) of VIII in 110 cc. of absolute methanol containing 11 cc. of butylamine[5] was allowed to reflux overnight. The light yellow solution was evaporated *in vacuo* and the residue was mixed with 50 cc. of ether and 80 cc. of water. The layers were separated and the water phase was washed with 15 cc. of water, and the aqueous layers were combined and evaporated *in vacuo*. The residue was dried by evaporation with ethanol and was then triturated with hot methanol. The crystalline product which resulted was collected and washed with more methanol. There was obtained 1.43 g. with m.p. 212–215°. Recrystallization from methanol with Norit afforded 1.22 g. (74%), m.p. 220–222°. For analysis, the substance was recrystallized twice more from the same solvent and the sample was dried at 74° *in vacuo:* m.p. 221–223° (slight dec.), $[\alpha]^{25}D$ +91.7° (*c* 0.43 in H_2O); λ_{max}^{water} 277 mμ (ε 13,320 in acid), 270 mμ (ε 8,970 in water) and 272 mμ (ε 9,200 in base).[31]

Anal. Calcd. for $C_9H_{14}N_4O_4$: C, 44.62; H, 5.83; N, 23.13. Found: C, 44.21; H, 5.91; N, 22.71.

(B) From 1-(2,5-Di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)-4-ethoxy-2-pyrimidinone (XVII).—A mixture of 1.83 g. (3 mmoles) of XVII and 50 cc. of methanolic ammonia (saturated at 0°) was heated in a sealed tube at 100° for 16 hours. The tube contents were evaporated *in vacuo* and the residue was dissolved in 35 cc. of methanol containing 3 cc. of butylamine. The solution was refluxed for 16 hours and was then evaporated *in vacuo*. The residue was dissolved in 20 cc. of water and 30 cc. of ether. The layers were separated and the water layer was washed with two 10-cc. portions of ether. The combined ether layers were washed with 5 cc. of water and the combined water solutions were evaporated *in vacuo*. The residue was crystallized from methanol to afford 0.316 g. (43%) with m.p. 204–208° (slight dec.). Material recrystallized twice more from the same solvent (once with Norit) had m.p. 220–222° (slight dec.); a mixed m.p. with material obtained under (A) was undepressed; $[\alpha]^{25}D$ +93.8° (*c* 0.99 in water). Ultraviolet and infrared spectra as well as R_f values (see Table I) in three solvent systems were identical with the product obtained under (A).

Anal. Calcd. for $C_9H_{14}N_4O_4$: C, 44.62; H, 5.83; N, 23.13. Found: C, 44.64; H, 6.02; N, 22.91.

The first few times that this reaction was carried out, the product had to be isolated by partition chromatography on Celite[44] from a butanol–ethyl acetate–water (2:1:1) system. The product was eluted in the third, fourth and fifth hold-back volumes.[45]

1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-4-dimethylamino-2-pyrimidinone (X).—A mixture of 1.83 g. (3 mmoles) of the blocked nucleoside XVII and 35 cc. of methanolic dimethylamine (1 cc. contained 2.52 mmoles of dimethylamine) was heated at 80° in a sealed tube for 16 hours. The tube contents were evaporated *in vacuo* and the residue was treated with butylamine and methanol as described for the synthesis of IX, section B. The crude, deblocked nucleoside (0.84 g. of glass) was partitioned on Celite[44] in the system butanol–ethyl acetate–water (2:1:1). The material was dissolved in 2 cc. of the upper layer of this solvent system and 5 g. of Celite was thoroughly mixed with this solution. This wet Celite was added to the top of a column[4] containing 250 g. of Celite which had been mixed well with 100 cc. of the lower phase of the solvent system. The column (3.8 × 70 cm.) had a hold-back volume[45] of 350 cc. The column was washed with the upper phase of the solvent system and the effluent was allowed to pass through a recording ultraviolet spectrophotometer which had been set at 270 mμ. Some colored impurities were eluted in the first hold-back volume and were discarded. A peak containing material with absorption at 270 mμ was eluted in the last half of the second and all of the third hold-back volume. These last fractions were combined and evaporated *in vacuo* to afford 0.425 g. of residue. This was crystallized from methanol to give 0.301 g., m.p. 203–205°. Recrystallization from methanol–ethanol with Norit afforded 0.247 g. (31%) with m.p. 223–225°, $[\alpha]^{25}D$ +13.2° (*c* 2.20 in ethanol); λ_{max}^{water} 284 mμ (ε 16,170 in acid), 277 mμ (ε 13,580 in water) and 279 mμ (ε 13,460 in base).[31]

Anal. Calcd. for $C_{11}H_{18}N_4O_4$: C, 48.88; H, 6.71; N, 20.73. Found: C, 48.94; H, 6.94; N, 20.45.

1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-4-butylamino-2-pyrimidinone (XVIII).—A mixture of 1.83 g. (3 mmoles) of the blocked nucleoside XVII and 40 cc. of absolute methanol containing 7 cc. of butylamine was heated under reflux for 16 hours. The dark yellow solution was evaporated under reduced pressure and the solid was dissolved in 50 cc. of ether and 60 cc. of water. The layers were separated and the aqueous phase was extracted three times with 15-cc.

(41) Celite, a product of the Johns-Manville Corporation, is diatomaceous earth.

(42) Reagent grade alumina (Merck and Co., Inc.) was washed with 1 *N* hydrochloric acid and then with water till neutral. It was dried at 170–178° for 24 hours.

(43) Experience with similar, blocked nucleosides, obtained subsequent to this experiment (see below), showed that silica gel chromatography would probably have given better results in the purification of XVII.

(44) The material used for partition chromatography was Celite[41] 545 which had been washed with 6 *N* hydrochloric acid and then with distilled water until neutral and finally with methanol. The material was dried at 50°.

(45) Hold-back volume is defined as the volume of solvent necessary to fill the packed column.

portions of ether. The combined ether layers were extracted with 15 cc. of water and the combined aqueous solutions were evaporated *in vacuo*. The residual gum (0.96 g.) was dissolved in 5 cc. of the lower and 5 cc. of the upper phase of the system butanol–ethyl acetate–water (1:6:1), and 10 g. of Celite was added and mixed with the liquid. The mixture was packed on top of a column prepared from 250 g. of Celite which had been mixed with 125 cc. of the lower phase of the solvent mixture. The column (3.8 × 72.5 cm.) had a hold-back volume[46] of 450 cc. It was developed with the upper phase of the system and the column effluent was allowed to run through a recording ultraviolet spectrophotometer which had been set at 270 mμ. Yellow material in the first hold-back volume was discarded. A large amount of material with ultraviolet absorption at 270 mμ was eluted in the second and through 250 cc. of the third hold-back volume. Fractions containing this peak were pooled and evaporated *in vacuo*. The residue (0.518 g.) could not be made to crystallize, but it was purified further by precipitation with ethyl acetate from an ethanol solution. After two such precipitations there was obtained 0.249 mg. (28%). The amorphous material liquefied between 95–98° and had $[\alpha]^{25}D$ +65° (*c* 1.07 in water); λ_{max}^{water} 279 mμ (ε 15,220 in acid), 271 mμ (ε 12,530 in water) and 273 mμ (ε 12,530 in base).[31]

Anal. Calcd. for $C_{13}H_{22}N_4O_4$: C, 52.33; H, 7.43; N, 18.78. Found: C, 52.44; H, 7.63; N, 18.27.

3′-Amino-3′-deoxyuridine [1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-uracil (XIX)].—Blocked nucleoside XVII (2.43 g., 4 mmoles) was suspended in 40 cc. of methanol and there was added 18 cc. of a 27% (by weight) methanolic hydrogen chloride solution.[46] The mixture was kept at room temperature with exclusion of atmospheric moisture for 24 hours and was then filtered from a small amount of white precipitate. The filtrate was evaporated *in vacuo* and the residue was evaporated four times with ethanol in order to remove traces of hydrogen chloride. The gummy product was kept in a vacuum desiccator over KOH pellets overnight. It was then dissolved in 40 cc. of methanol containing 7 cc. of butylamine and the solution was refluxed for 16 hours. Evaporation *in vacuo* left a solid residue which was mixed with 50 cc. of ether and 60 cc. of water. The layers were separated and the water layer was further washed with three 15-cc. portions of ether. The combined ether washings were extracted with 15 cc. of water and the combined aqueous solutions were evaporated under reduced pressure. The residual glass (1 g.) was dissolved in 3 cc. of the upper and 3 cc. of the lower phase of a butanol–water mixture and the solution was mixed with 6 g. of Celite. This mixture was packed on top of a column which had been prepared from 250 g. of Celite and 125 cc. of the aqueous phase of the butanol–water system. The column (5 × 72 cm.; hold-back volume, 483 cc.) was developed with the upper phase of the solvent system and the column effluent was allowed to pass through the recording ultraviolet spectrophotometer (set at 262 mμ) as before. The contents of the first hold-back volume were discarded. The second and first half of the third hold-back volumes contained material with absorption at 262 mμ. These fractions were pooled and evaporated *in vacuo* to afford a residue which was crystallized and recrystallized from methanol–ethanol; 0.428 g. (44%), m.p. 180–181°.[47] For analysis the material was recrystallized twice more from the same solvent mixture; m.p. 183–184°, $[\alpha]^{25}D$ +67° (*c* 1.0 in water); λ_{max}^{water} 260 mμ (ε 9,860 in acid), 261 mμ (ε 10,090 in water) and 262 mμ (ε 7,780 in base).

Anal. Calcd. for $C_9H_{13}N_3O_5$: C, 44.44; H, 5.39; N, 17.28. Found: C, 44.72; H, 5.59; N, 17.42.

1-(2,3-Di-*O*-benzoyl-5-deoxy-5-phthalimido-β-D-ribofuranosyl)-thymine (XXI).—A mixture of 5.29 g. (10 mmoles) of 1-*O*-acetyl-2,3-di-*O*-benzoyl-5-deoxy-5-phthalimido-D-ribofuranose,[29] 180 cc. of ethereal hydrogen chloride (saturated at 0°) and 1 cc. of acetyl chloride was allowed to stand at −3° for 72 hours. The light yellow solution was evaporated at room temperature *in vacuo* and the residual gum was freed from hydrogen chloride by a triple evaporation with dry toluene *in vacuo*. The gummy chloro sugar XX was dissolved in 20 cc. of xylene and the solution was added to an azeotropically dried suspension of 2.25 g. (5 mmoles) of dithyminylmercury[10] (II) in 200 cc. of xylene. The stirred mixture was allowed to reflux for three hours and was then filtered while hot. Some 0.7 g. of thymine was recovered in this manner. The filtrate was evaporated *in vacuo* and the residue was dissolved in 120 cc. of chloroform. The solution was washed with three 15-cc. portions of a 30% aqueous potassium iodide solution and then with 20 cc. of water. The chloroform layer was dried over magnesium sulfate and the filtered solution was evaporated under reduced pressure. The gum (5.51 g.) was dissolved in 20 cc. of ethyl acetate and was filtered through Norit; a solid crystallized out on standing. This was collected, washed with 50% ethanol in ethyl acetate and dried *in vacuo;* 0.562 g., m.p. 144–145°. The substance decolorized bromine in chloroform and gave a strong positive Benedict reaction. It had $[\alpha]^{25}D$ 0° (*c* 2.0 in chloroform). Spectral data indicated the absence of the pyrimidine moiety in this substance.[28]

Anal. Calcd. for $C_{20}H_{15}NO_5$[28]: C, 68.76; H, 4.33; N, 4.01. Found: C, 68.94; H, 4.01; N, 4.06.

The ethyl acetate filtrate from which this solid had been separated was evaporated under reduced pressure to afford 3.7 g. of fluffed glass. This was dissolved in 10 cc. of benzene and the solution was added to a column of 100 g. of acid-washed alumina[42] which had been prepared with benzene. Elution of the column (3.5 × 11 cm.) with 150 cc. of benzene yielded a small amount of gum which was discarded. Further elution with this solvent (200 cc.) afforded 0.2 g. of solid with m.p. 144–145° (crystallized from methylene chloride–ether). The compound was shown to be identical with the $C_{20}H_{15}NO_5$ product obtained above. Further washing of the column with 150 cc. of benzene, 300 cc. of benzene–ethyl acetate (9:1), 250 cc. (75:25), 200 cc. (1:1), 150 cc. of ethyl acetate–benzene (8:2) and 150 cc. of ethyl acetate yielded portions of yellow gum which were not further investigated. Elution with 400 cc. of ethyl acetate–ethanol (9:1) afforded 1.8 g. of an amorphous solid. This was purified by solution in methylene chloride and addition of ether at the b.p. until gel formation started. Thus, there was obtained 1.25 g. (21%) of a white powder which melted at 120–124°. For analysis, the material was dissolved in a small amount of acetone, and cyclohexane was added at the b.p. till cloudy. The amorphous solid, so obtained, was dried *in vacuo* at 76° for 3 hours; $[\alpha]^{25}D$ −32.6° (*c* 1.01 in ethanol); λ_{max}^{MeOH} 264 mμ (ε 13,860 in acid), 265 mμ (ε 13,140 in methanol), 269 mμ (ε 9,550 in base).[31]

Anal. Calcd. for $C_{32}H_{25}N_3O_9$: C, 64.53; H, 4.23; N, 7.06. Found: C, 64.44; H, 4.44; N, 7.27.

1-(5-Amino-5-deoxy-β-D-ribofuranosyl)-thymine (XXII).—A mixture of 0.595 g. (1 mmole) of the blocked thymine derivative XXI, 15 cc. of anhydrous methanol and 1 cc. of butylamine was heated under reflux for 16 hours. The dark yellow solution was evaporated under reduced pressure and the residue was worked up with ether and water as described for the preparation of VII. From the combined aqueous solutions there was obtained by evaporation 0.23 g. of glass which was dissolved in a small amount of methanol and filtered through Norit. The solution was diluted with ethanol at the b.p. until cloudy. The amorphous precipitate which formed on standing was collected and washed with ethanol and ether. The dried amorphous solid weighed 0.187 g. which was approximately 69% pure by ultraviolet analysis.[48] This would indicate a 49% over-all yield. The substance could not be obtained in crystalline form or in a greater state of purity.

A hydrochloride was prepared with methanolic hydrogen chloride. The solution was evaporated to a small volume and an amorphous precipitate was obtained by the addition of ether. The solid was purified by precipitation with ethyl acetate from ethanol solution. It was dried *in vacuo;* $[\alpha]^{25}D$ −4.0 ± 2° (*c* 0.51 in water); λ_{max}^{water} 265 mμ (ε 9,100 in acid), 265 mμ (ε 9,100 in water) and 267 mμ (ε 7,050 in base).[31]

Anal. Calcd. for $C_{10}H_{15}N_3O_5 \cdot HCl$: C, 40.88; H, 5.50; N, 14.30. Found: C, 40.56; H, 5.85; N, 13.61.

1-(2,3-Di-*O*-benzoyl-5-deoxy-5-phthalimido-β-D-ribofuranosyl)-4-acetamido-2-pyrimidinone (XXIII).—A solution of

(46) G. E. Hilbert, THIS JOURNAL, **59**, 330 (1937).

(47) The compound could be isolated crystalline without partition chromatography, but samples so obtained did not give satisfactory combustion values.

(48) The extinction at 264 mμ was compared to that of the corresponding thymine riboside.[10]

the chloro sugar XX, prepared as described above (*cf.* preparation of XXI) from 5.29 g. (10 mmoles) of 1-*O*-acetyl-2,3-di-*O*-benzoyl-5-deoxy-5-phthalimido-D-ribofuranose, in 50 cc. of xylene was added to an azeotropically dried suspension of 1.75 g. (5 mmoles) of acetylcytosinemercury (III)[11] in 180 cc. of xylene. The stirred mixture was allowed to reflux for three hours and was then evaporated *in vacuo*. The residual brown gum was dissolved in chloroform (200 cc.) and the solution was washed with two 20-cc. portions of 30% aqueous potassium iodide solution and with 40 cc. of water. The organic phase was dried and partially decolorized over magnesium sulfate and Norit. The filtered solution was evaporated *in vacuo* and the residual gum (6.05 g.) was dissolved in 10 cc. of chloroform and 10 cc. of benzene. The solution was chromatographed on a column prepared from 120 g. of silica gel in benzene–chloroform (1:1). The column (3.5 × 29.5 cm.) was eluted with 400 cc. of this solvent mixture and the 0.22 g. of gum obtained from the pooled fractions was discarded. Elution was continued with 200 cc. of chloroform, 200 cc. of chloroform–acetone (98:2) and 150 cc. of chloroform–acetone (97:3). Solid material was isolated from the following fractions which were eluted with 100 cc. of chloroform–acetone (97:3) and 100 cc. of chloroform–acetone (95:5). By crystallization and one recrystallization from ethanol–ethyl acetate there was obtained 1.03 g. (29% based on 10 mmoles of sugar derivative XX), m.p. 145–146°, undepressed by admixture of the $C_{20}H_{15}NO_5$ compound isolated in the preparation of XXI. Continued elution of the column with the last mentioned solvent mixture (150 cc.), with 350 cc. of chloroform–acetone (9:1) and with 100 cc. of chloroform–acetone (85:15) afforded gummy material (1.06 g.) which was discarded. Washing the column with 450 cc. of chloroform–acetone (65:35) yielded a solid which was crystallized and recrystallized from acetone to afford 1.69 g. (27% based on 10 mmoles of XX; 54% based on III), m.p. 147–150°. For analysis the substance was recrystallized twice more from acetone; m.p. 146–148°. Material dried at 74° *in vacuo* still contained acetone of crystallization. The compound showed $[\alpha]^{25}D$ +37.8° (*c* 0.98 in $CHCl_3$); λ_{max}^{MeOH} 283 mμ (ε 10,880 in acid), 298 mμ (ε 7,480 in methanol) and 272 mμ (ε 11,700 in base).[31]

Anal. Calcd. for $C_{33}H_{26}N_4O_9 \cdot C_3H_6O$: C, 63.52; H, 4.74; N, 8.23; C–CH_3, 4.42. Found: C, 63.24; H, 4.83; N, 8.01; C–CH_3, 3.91.

5′-Amino-5′-deoxycytidine [1-(5-Amino-5-deoxy-β-D-ribofuranosyl)-cytosine (XXIV)].—A mixture of 1.86 g. (3 mmoles) of the 4-acetamidopyrimidinone derivative XXIII, 40 cc. of absolute methanol and 7 cc. of butylamine was heated under reflux for 16 hours. The reaction mixture was worked up as described in the preparation of VII and the residue (0.74 g.) obtained by evaporation of the combined water layers was dissolved in 30 cc. of hot methanol and filtered through Norit. The filtrate was evaporated to a small volume and decanted from a small amount of yellow gum. Ethanol was added at the b.p. until the solution became cloudy. The solid which precipitated on standing was collected, washed with ethanol and ether. The vacuum-dried material (0.37 g.) was amorphous and melted around 145°; ultraviolet analysis indicated a purity of 84%[49]; the 0.37 g. of product, therefore, represented a 74% yield. The compound as such could not be purified further and was characterized through the **picrate** prepared in methanol and recrystallized from methanol–ethanol; m.p. 218–220° dec., $[\alpha]^{25}D$ +21.5° (*c* 1.02 in methyl Cellosolve).

Anal. Calcd. for $C_{15}H_{17}N_7O_{11} \cdot H_2O$: C, 36.81; H, 3.91; N, 20.04. Found: C, 36.51; H, 3.23; N, 19.37.

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(49) The extinction of cytidine at 269 mμ was used for comparison.

COMMUNICATIONS TO THE EDITOR

sym-DIPHENYLPYROPHOSPHORODIAMIDIC ACID: A NEW SUBSTRATE FOR THE COLORIMETRIC ESTIMATION OF ENZYMES

Sir:

N-Phosphorylated nitrogen mustards which have been shown to be non-toxic are potentially cytotoxic agents with a high selectivity for cells with high phosphamidase activity.[1a–g] The distribution of phosphamidase enzymes in malignant tissues is, therefore, a matter of interest in chemotherapy. Studies have shown that some tumors do have high phosphamidase activity.[2a–d] However, confirmation, extension and exploitation of these results have been hampered by the fact that the substrates used are unstable below *p*H 5.0 where the enzyme(s) is active.[3a–c] On the basis of the structural features apparently necessary for enzymatic activity[4] and from theoretical considerations with regard to stability, pyrophosphoroamidic acids, although previously unreported, appeared attractive as substrates for enzymes of the phosphamidase type. In addition, chromogenic pyrophosphoroamidic acids would be satisfactory prototypes for toxagenic analogs potentially useful in the treatment of cancer.

We report here the synthesis of *sym*-diphenylpyrophosphorodiamidic acid (II). O-Benzylphenylphosphoroamidic acid,[5] m.p. 118–119°, on treatment with dicyclohexyl carbodiimide in dimethylformamide gave *sym*-O,O-dibenzyldiphenylpyrophosphorodiamidate (I) (m.p. 142–143°; *Anal.* Calcd. for $C_{26}H_{26}N_2P_2O_5$: C, 61.44; H, 5.16; N, 5.52. Found: C, 61.44; H, 5.53; N, 5.49). Debenzylation of I with hydrogen over palladium afforded the dibasic pyrophosphoroamidic acid II which was isolated both as the dicyclohexylammonium salt (m.p. 223–224°; *Anal.* Calcd. for $C_{24}H_{40}N_4O_5P_2$; C, 54.75; H, 7.62; N, 10.64.

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(4) Active substrates have in common structures with at least one ionizable phosphoric acid group, ref. 2a.

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