Synthesis of Aminosugar Nucleosides[1]

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Several blocked glucosamine-pyrimidine nucleosides were synthesized from 1-chloro-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-, 2-carbobenzyloxyamino-, and 2-carbomethoxyamino-D-glucopyranose (I, II and III, respectively) by the Hilbert–Johnson and the acetylcytosine–mercury procedures. Interconversions of these nucleosides showed that they all had the same configuration at the nucleosidic center. This was unequivocally established to be β by periodate fission of 1-(2-deoxy-2-amino-β-D-glucopyranosyl)-cytosine (XIII) to the known dialdehyde XXIII, and in addition by cleavage studies on the uracil XV. Hydrogenolysis of the carbobenzyloxy-nucleoside VIII gave XXVI, reductive methylation of which afforded the dimethylaminonucleoside XXVII. XXVII could also be made by hydrogenolysis of V to XXVIII, reductive methylation of XXVIII to the dimethylamino compound XXIX and ammonolysis of XXIX to XIV followed by acetylation of XIV.

The antibiotic amicetin, isolated from *Streptomyces plicatus*[3] has been shown to have a structure[4] incorporating an aldosyl-cytosine nucleoside with an aminosugar (in a disaccharide link with the aldosyl moiety) and a peptide (on the cytosine moiety). Amicetin and two other similar antibiotics, baniicetin and plicacetin, isolated from the same fermentation,[3] exhibit interesting antibacterial and antitumor activities. A comparative study of the activities of these antibiotics and their derivatives[5] led to the speculation that aminosugar–pyrimidine nucleosides with an unblocked amino group in the sugar residue and an acylated amino group on the cytosine moiety may possess similar properties. This paper reports the results of a study directed toward the synthesis of simple aminosugar–pyrimidine nucleosides that would incorporate these features and serve as models for the synthesis of amicetin. Although the synthesis of a variety of aminosugar-purine nucleosides[6] has been stimulated by structural

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(3) T. H. Haskell, A. Ryder, R. P. Frohardt, S. A. Fusari, Z. L. Jakubowski, and Q. R. Bartz, *J. Am. Chem. Soc.*, **80**, 743 (1958).

(4) C. L. Stevens, K. Nagarajan, and T. H. Haskell, *J. Org. Chem.*, **27**, 2991 (1962).

(5) T. H. Haskell, *J. Am. Chem. Soc.*, **80**, 747 (1958).

(6) B. R. Baker, R. E. Schaub, J. P. Joseph, and J. H. Williams, *ibid.*, **77**, 12 (1955) and several other publications of that group.

work on puromycin,[7] no pyrimidine analogs had been prepared when this work was undertaken.[8] Glucosamine was chosen because of its ready availability.

The earliest nucleoside synthesis in this work was achieved using 3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-D-glucopyranosyl chloride (I) which already was known in the literature and had been used successfully in the synthesis of a purine nucleoside.[9] Application of the Hilbert–Johnson procedure[10] involving refluxing the chlorosugar (I) with excess 2,4-dimethoxypyrimidine in benzene solution afforded a 49% yield of 1-(3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (IV).[11] The ultraviolet absorption spectrum of IV with a maximum at 276 mμ (ε 5,900),[12] and the stability of the sugar–pyrimidine linkage to acidic and basic conditions (see below) clearly showed that compound IV was a nucleoside and not a glycoside.[13a,b] Ammonolysis of IV proceeded in 59% yield to 1-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-cytosine (XI), which was further characterized as the fully acetylated derivative VII (52% yield). VII could also be formed from the chlorosugar I by application of the acetylcytosine–mercury procedure of Fox and co-workers,[14] which involved addition of 2 moles of the chlorosugar to a suspension of the 1:1 acetylcytosine–mercury salt in refluxing toluene. VII was formed only in very poor yield in this reaction, although yields were considerably better with the two other chlorosugars studied in the course of this work. The nucleoside XI exhibited an intense absorption maximum at 276 mμ in acid solution[8,15a] and showed the presence of a single basic function with pK'a at 3.9.[15b] These features are

(7) P. H. Fryth, C. W. Waller, B. L. Hutchings, and J. H. Williams, *J. Am. Chem. Soc.*, **80**, 2736 (1958).

(8) H. M. Kissman and M. J. Weiss, *ibid.*, **80**, 2575 (1958), have subsequently reported the synthesis of some 3'-deoxy-3'-amino and 5'-deoxy-5'-aminoribose-pyrimidine nucleosides. It may be pointed out, however, that their method of using the phthaloyl group for blocking the aminosugar would not lend itself readily to the synthesis of the model compounds (XXVI and XXVII) prepared in this work.

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

(10) G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, **52**, 4489 (1930); conducting the condensation in refluxing benzene is in fact a slight modification of the procedure adopted by these authors, since they used no solvent for the reaction. In our experience, the use of benzene was found to result in less decomposition of the reactants.

(11) Proof of β-configuration for this and other nucleosides is presented later. For the sake of convenience it is assumed at this stage.

(12) J. E. Austin, *J. Am. Chem. Soc.*, **56**, 2141 (1934), has studied the ultraviolet spectra of N- and O-alkylated pyrimidine derivatives and reports λ_{max} values of 256, 276 and 276 mμ (ε 5,400) for 2,4-dimethoxypyrimidine, 1-methyl-4-methoxy-2-pyrimidinone and 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (XIX), respectively.

(13) (a) P. Newmark and I. Goodman, *ibid.*, **79**, 6446 (1957), reported the formation of glycosides rather than nucleosides in similar reactions of glycosyl halides with substituted alkoxypyrimidines; (b) G. E. Hilbert, *ibid.*, **59**, 330 (1937).

(14) J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *ibid.*, **79**, 5060 (1957); also see reference (8).

characteristic of 1-substituted cytosine derivatives and hence confirm the structural assignment of this compound as XI. The acetyl derivative VII had maxima at 246 and 302 mμ, characteristic of the 4-acetamido-2-pyrimidinone chromophore[15,16] and p*K'*a of 11.1, due to the weakly acidic character of the vinylogous imide structure in the pyrimidine moiety.[5,17]

In initial attempts to remove the protecting group from nucleoside XI, hot aqueous or alcoholic alkali was used. Theoretically, the resulting compound (XIII) could be used for the synthesis of XXVI and XXVII, and for elucidation of the configuration at the nucleosidic center. However, hydrolysis of the acetamido group could not be achieved. Under a variety of mild conditions, XI could be recovered unchanged. Baker and co-workers[9] have encountered similar resistance to hydrolysis of the N-acetyl function in 6-dimethylamino-9-(2'-acetamido-2'-deoxy-β-D-glucopyranosyl)-purine.

Attention then was directed toward the use of other protecting groups for the aminosugar. The carbobenzyloxy group was selected for protecting the amine, since hydrogenolysis could be expected to proceed under mild conditions to liberate the free amine. 1,3,4,6-Tetra - *O* - acetyl - 2 - deoxy - 2 - carbobenzyloxyamino - β - D-glucose was therefore synthesized by a known procedure[9] and converted to the chlorosugar II in 72% yield by saturating an ether–acetic anhydride solution of it with hydrogen chloride. Refluxing the chlorosugar II with excess 2,4-dimethoxypyrimidine in benzene solution afforded the 4-methoxy-2-pyrimidinone nucleoside (V) in 84% yield. Application of the acetylcytosine–mercury procedure[14] to II led to varying yields of nucleoside. Using pure starting materials, a maximum of 25% yield of VIII could be obtained. The conversion of VIII and V to models XXVI and XXVII is taken up in a later portion of the discussion. Now alkaline hydrolysis of VIII to XIII could be expected to proceed more readily than hydrolysis of XI to XIII. Alternatively, the possibility of making the carbomethoxynucleoside (IX) in good yield was studied.

Hydrolysis and decarboxylation of an N-carbalkoxy group under alkaline conditions has been utilized[18] for the synthesis of methyl

(15)(a) Z. A. Shabarova, N. I. Sokolova, and M. R. Prokofyev, *J. Gen. Chem., U. S. S. R.*, **27**, 2891 (1957) [Russian Edition]; **27**, 2928 (1957) [English Edition]; (b) J. J. Fox and D. Shugar, *Biochim. et Biophys. Acta*, **9**, 369 (1952).

(16) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2384 (1956).

(17) Physical data for only a few compounds typical of most of the nucleosides reported in this paper are presented in the introduction, and these are briefly discussed in terms of their structures. Data for all other similar nucleosides are merely recorded in the experimental section.

(18) S. Akiya and T. Osawa, *Chem. Abstracts*, **51**, 17763g (1957); *Yakugaku Zasshi*, **77**, 726 (1957).

glucosaminide. In the present work, 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine[19] was carbomethoxylated using methyl chloroformate to give the N-carbomethoxy derivative[20] and this was converted into the chlorosugar III in 88% yield. III could be obtained only as a waxy or amorphous solid and did not have a sharp melting point, but had the correct analysis. Further, it gave the best results in nucleoside formation, yielding 84% of the 4-methoxy-2-pyrimidinone (VI) in the Hilbert–Johnson reaction[10] and 65% of the acetylcytosine nucleoside (IX) by the procedure of Fox and co-workers.[14]

Several interconversions of these nucleosides were made with a view of relating them to one another. Ammonolysis of V afforded in 75% yield a crystalline compound, m.p. 280–281° (dec.), forming a crystalline picrate and a polyacetate X. Analysis indicated an empirical formula $C_{11}H_{17}N_5O_6$ for this compound and potentiometric titration showed the presence of two weakly basic groups with p*K'*a 2.2 and 4, respectively, in water. On the basis of these data, it was clearly the urea derivative XII. XII was also obtained in fair yields by ammonolyzing VI or IX.

Hydrolysis of nucleoside IV with refluxing 6 *N* hydrochloric acid removed all the blocking groups on the sugar portion and cleaved the methyl ether at the 4-position of the pyrimidine moiety, affording a crystalline hydrochloride (91% yield), also obtained in 53% yield from VI. The free nucleoside, 1-(2-deoxy-2-amino-β-D-glucopyranosyl)-uracil (XV), could be recovered from the hydrochloride in 91% yield by neutralization with diethylamine in alcohol and was further characterized as a picrate, m.p. 254–255° (dec.). XV exhibited a maximum at 259 mμ (H_2O) in the ultraviolet region (ε 9,870) and had an acidic hydroxyl with p*K'*a 9.9 in 50% methanol. These are in good agreement with the values λ_{max} 258.5 mμ (ε 9,190) and p*K'*a 10 in 50% methanol, observed by us for the very similar compound 1-β-D-glucopyranosyl uracil (XX).[10] The amino group in XV had a p*K'*a of 6.1 in 50% methanol, indicating a drop of 1.7 units from glucosamine itself (p*K'*a 7.8 in 50% methanol). This was obviously due to the electron-withdrawing effect of the spatially proximate 2-carbonyl group of the pyrimidinone moiety, and had a good analogy in 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine (XXX) with a p*K'*a of 5.2 in 50% methanol, representing a drop of 1.3 units for each adjacent acetate.

Treatment of IV and V with alcoholic hydrogen chloride under transesterification conditions[10] gave the uracils XVI (81%) and XVII

(19) M. Bergmann and L. Zervas, *Ber.*, **64**, 975 (1931).
(20) W. H. Bromund and R. M. Herbst, *J. Org. Chem.*, **10**, 267 (1945).

(58%), respectively. Structure assignment was confirmed by analysis, ultraviolet and infrared spectra and pK'a measurements. Acetylation of XVII afforded a fair yield of the crystalline tri-*O*-acetate XXI, which could also be obtained in one step from V in 97% yield, by the action of hydrogen chloride in a mixture of chloroform and acetic anhydride. Hydrogenolysis of XXI proceeded smoothly in dioxane using a 10% palladium-on-charcoal catalyst and the product (XXV) was isolated in 88% yield as the crystalline hydrochloride, m.p. 230° (dec.). Dioxane was found to be the solvent of choice for this and other reductions reported in this paper, because considerable reduction of the pyrimidine ring occurred in acetic acid or alcohol or other similar polar solvents. The free amino group in XXV had a pK'a at 4.9 in 50% methanol, in good agreement with the value (5.2) found for the similar compound XXX. Treatment of XXV with alcoholic hydrogen chloride removed the ester groups and gave the hydrochloride of XV in 85% yield.

Hydrolysis of the carbomethoxyaminonucleoside IX occurred with hot aqueous barium hydroxide.[18] The precipitated barium carbonate was filtered off and the filtrate acidified with a slight excess of sulfuric acid. From the filtrate the crystalline sulfate of 1-(2-deoxy-2-amino-β-D-glucopyranosyl)-cytosine (XIII) was isolated in 57% yield. In agreement with the proposed structure, XIII had an absorption maximum in the ultraviolet region at 274 mμ (ϵ 12,700) in acid solution and had two basic groups, one with pK'a 3.7 in 50% methanol, representing the 4-amino group of cytosine and the other with pK'a 6.3 in 50% methanol, corresponding to the aminosugar. Further, XIII reduced a little over 2 moles of sodium metaperiodate[21a,b,c] at room temperature in 48 hours. Acetylation of the free base from the crystalline sulfate with acetic anhydride in pyridine gave VII in 66% yield.

The reactions described so far indicate that the various nucleosides formed by the Hilbert–Johnson and the acetylcytosine–mercury procedures all have the same configuration at the nucleosidic center, thus confirming previous indications.[14,21c] Proof that this configuration is β is now discussed. The chlorosugars I,[9] II and III all have high specific rotations, varying from +115 to +130° and hence may be assumed to have the α-configuration, although no rigorous chemical proof is adduced. It has been proposed[9,22] that, irrespective

(21) (a) E. L. Jackson, "Organic Reactions," Vol. II, 1944, p. 341; (b) J. M. Bobbitt, *Advances in Carbohydrate Chemistry*, **11**, 1 (1956); (c) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956); J. J. Fox and L. Wempen, *Advances in Carbohydrate Chemistry*, **14**, 338 (1959).

(22) B. R. Baker, "The Chemistry and Biology of Purines," Ciba Foundation Symposium, Little, Brown & Co., Boston, Mass., 1957, p. 120.

of the configuration of the glycosyl halide used, the nucleoside formed would be *trans* to the 2-acyloxy or acylamino function, giving the more stable β-configuration for glucose or glucosamino derivatives. This result would arise from participation by the 2-substituent on the sugar portion, when possible. This has been proved to be the case in the Hilbert–Johnson reaction on the anomeric pair of acetochloroglucose.[23] The nucleosides obtained from the acetylcytosine–mercury procedure on such halides similarly have been found to have the β-configuration.[19] In fact, the formation of appreciable amounts of α-nucleosides is possible only when there is no participation from the 2-position, as in the case of the 2,3-cyclic carbonate of D-ribosyl bromide[24] or when a deoxyglycosyl halide is used.[25] The assignment of β-configuration to 3′-amino-3′-deoxy- and 5′-amino-5′-deoxycytidine[8] was based on these arguments alone, since periodate fission of these compounds, a simple method useful for assigning configuration,[26] was not helpful due to anomalous overoxidation.[8,27]

The nucleosides prepared in the course of this study may be expected to have the β-configuration, since there is an acylamino function at the 2-position which is capable of participation, when required.[9,22] The close similarity in specific rotations of related nucleoside pairs IV (+33°) and 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-ethoxy-2-pyrimidinone[13b] (+36°) and of XV (+16°) and XX (+21°),[10] lends additional support for the β-configuration. Numerous instances[28–31] can be cited from the literature to show that the molecular rotations of α- and β-anomers of sugar derivatives do not change significantly when a hydroxyl group therein is replaced by an amino group. Nevertheless it was the goal of this investigation to prove the configuration rigorously. Since the diamine XIII was obtained only at a later stage in our work, initial experiments were carried out on XV and its derivatives.

Periodate oxidation of XV led to consumption of 2.35 moles of the reagent in 48 hours. This rate was comparable to that for the cleavage of XX alone or in the presence of 1 mole of ammonium hydroxide

(23) J. J. Fox and I. Goodman, *J. Am. Chem. Soc.*, **73**, 3256 (1951).
(24) R. S. Wright, G. M. Tener, and H. G. Khorana, *ibid.*, **80**, 2004 (1958).
(25) M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *ibid.*, **81**, 4112 (1959).
(26) J. Davoll, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 833 (1946).
(27) M. J. Weiss, J. P. Joseph, H. M. Kissman, A. M. Small, R. E. Schaub, and F. J. McEvoy, *J. Am. Chem. Soc.*, **81**, 4050 (1959).
(28) R. E. Schaub and M. J. Weiss, *ibid.*, **80**, 4683 (1958).
(29) R. Kuhn and W. Bister, *Ann.*, **617**, 92 (1958).
(30) E. E. van Tamelen, J. R. Dyer, H. E. Carter, J. O. Pierce, and E. E. Daniels, *J. Am. Chem. Soc.*, **78**, 4817 (1956).
(31) A. Neuberger and R. P. Rivers, *J. Chem. Soc.*, 122 (1939), and other references cited therein.

(see Table I). However, the solution from XV had a final specific rotation of +30°, compared to +16°, reported[26] for XX. The picrate of XV reduced the theoretical amount of 2 moles of sodium metaperiodate in 4 hours, with no further uptake in 48 hours. Although the solution had $[\alpha]_D$ + 24° at the end of 4 hours, it dropped to +15.6° in 24 hours and did not change in 48 hours (see Table II). In the presence of 1 mole of ammonium picrate, XX consumed 2 moles of periodate during the same period and the solution had $[\alpha]_D$ + 15.5°. Similar results were obtained by using a different salt of XV or by the addition of picric acid, after 2 moles of periodate had been consumed by XV. The cleavage of XX with periodate leads to the dialdehyde XXII,[26] although it was not isolated. The same dialdehyde probably is formed from XV in the presence of a mole of strong acid. The anomalous rotation observed when XV is cleaved as the free base with sodium metaperiodate perhaps arises because the amino group is bound in an aldimine or equivalent structure.[27] The presence of a strong acid may help to hydrolyse the imine to the dialdehyde XXII expected from this reaction. The periodate cleavage of 3-amino-3-deoxy-[27,30,32] and 2-amino-2-deoxy-[27,29,33] pyranosyl hexose derivatives has been found to be normal, although isolation of products seems to have been achieved only in some cases.[29,30,32] The identity of rotations of the periodate cleaved solutions of XV and XX would lend support to the assignment of β-configuration to XV, since the anomeric center in XX has that configuration.[26] However, since the dialdehyde XXII could not be isolated in either study, and since fortuitous coincidence of rotations is not entirely unknown in such reactions,[34] it seemed desirable to reinforce this evidence by other experiments.

The nitrous acid deamination of XV and XXV then was studied. Over-all retention of configuration in this reaction could be utilized to get XX, while a net inversion[35] would still be useful to obtain a mannose nucleoside which could not be made via the Hilbert-Johnson reaction.[13b] However, although both XV and XXV evolved nitrogen on treatment with nitrous acid, uracil and a gummy reducing sugar were the only products from XV, while the product from XXV on deacetylation yielded only a gum and not crystalline XX.

Conclusive evidence for the β-configuration of these nucleosides was obtained by fission of 1-(2-deoxy-2-amino-β-D-glucopyranosyl)-cytosine (XIII). In spite of numerous attempts, the picrate salt

(32) D. R. Walters, J. D. Dutcher, and O. Wintersteiner, *J. Am. Chem. Soc.*, **79**, 5076 (1957).
(33) R. W. Jeanloz and E. Fonchielli, *J. Biol. Chem.*, **188**, 361 (1951).
(34) Footnote (20) in reference (27).
(35) A. B. Foster, E. F. Martlew and M. Stacey, *Chem. and Ind.*, 825 (1953).

required for this reaction could not be obtained crystalline. Hence the periodate fission was run on the free base in the presence of picric acid. At the end of 60 hours, a little over 2 moles of the oxidant had been reduced and a crystalline picrate, m.p. 218–220° (dec.), was obtained in good yield. This was shown to be identical with the picrate of 1-[formyl-(1-formyl-2-hydroxyethoxy)-methyl]-cytosine (XXIII),[36] isolated by Todd and co-workers from the fission of cytidine picrate. The β-configuration of cytidine (XXIV) has been established by X-ray studies and by chemical evidence resting on the formation of a cyclonucleoside from a derivative. This is possible only if the substituents at the 1 and 5 positions of ribose are in the same direction.[37] The anomeric configuration is thus unequivocally established to be β for XIII and for all other nucleosides prepared in this study, since they have been related to XIII.

The configuration of the nucleosides having been thus settled, the synthesis of the model compounds XXVI and XXVII was undertaken. These compounds had the required features of an unblocked aminosugar and an acylated cytosine moiety. Hydrogenolysis of VIII in acetic acid, alcohol, or ethyl acetate using a variety of catalysts led to intractable products, perhaps due to partial reduction of the nucleus. Only by the use of dioxane as a solvent and 10% palladium-on-charcoal as catalyst could the isolation of XXVI be achieved. Even here, the course of the reaction could not be followed because of the variable uptake of hydrogen observed. This may be because the carbon dioxide resulting from the hydrogenolysis of the carbobenzyloxy group was being desorbed to different degrees in different experiments. That the carbobenzyloxy group was indeed split readily was demonstrated by running the hydrogenation for 30 minutes and codistilling the toluene formed with dioxane. A minimum of 0.7 mole of toluene per mole of VIII could be detected by ultraviolet spectrophotometry. Although the product could be obtained in quantitative yield as a crude gum, only 40% of a crystalline material could be recovered. Further crystallizations resulted in further depletion of yields but gave XXVI as a nicely crystalline solid. Ultraviolet and infrared absorption spectra and potentiometric titrations confirmed the structure, although combustion values were erratic.

Reductive methylation of XXVI in dioxane, using aqueous form-

(36) *Chemical Abstracts* nomenclature; the English authors use the name α-(cytosine-1)-α'-hydroxymethyldiglycolic dialdehyde; dialdehydes XXII and XXIII are perhaps better represented as the cyclic hemiacetals; *cf.* J. E. Cadotte and co-workers, *J. Am. Chem. Soc.*, **79**, 691 (1957).

(37) V. M. Clark, A. R. Todd and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

aldehyde and palladized charcoal catalyst, afforded XXVII in 31% overall yield from VIII. However, since VIII itself was formed only in 25% yield from the chlorosugar II under the best conditions, an alternate route to XXVII was explored.

Hydrogenolysis of V proceeded with a reproducible uptake of nearly 1 mole of hydrogen[38] and gave the free aminonucleoside XXVIII in 76% yield. In analogy with XXV and XXX, the amino group in XXVIII had a pK'a at 5.2 in 50% methanol. Ammonolysis of XXVIII afforded a mixture of products from which the acetamidonucleoside XI was isolated in 40% yield. Such O→N migration of acetyl groups in glucosamine derivatives under these conditions is known[39a,b] and in compounds such as XXVIII where there is a stable substituent at position 1, the acetyl group at carbon atom 3 is considered[39a] the one most likely to undergo migration to the nitrogen at position 2. Reductive methylation of XXVIII as before afforded the dimethylaminonucleoside XXIX in 51% yield. Its structure was supported by elemental and group analysis and by ultraviolet and infrared spectra. The pK'a value of XXIX (4.3 in 50% methanol), was lower than that of the corresponding primary amine XXVIII (pK'a 5.2) but this phenomenon had analogies in the literature.[40] A closer model (XXXI) was synthesized by reductive methylation of 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine (XXX) in 62% yield and was found to have a pK'a of 4.2. The presence of a dimethylamino group in XXIX was confirmed further by conversion to XVIII (90% yield) and isolation of dimethylamine as its crystalline *p*-hydroxyazobenzene-*p'*-sulfonic acid salt[5] from the periodate fission of XVIII.

Ammonolysis of XXIX afforded the diamine XIV as a crystalline sulfate (86% yield) which exhibited pK'a's of 3.1 and 5.5 in 50% methanol, corresponding respectively, to the amine in the cytosine portion and the dimethylamino group in the sugar residue. The latter value again showed a drop of about 1 unit from 6.3 for the related primary amine XIII. Structure assignment to XIV was supported by periodate fission and isolation of the dialdehyde (XXIII) picrate (51% yield) and of dimethylamine in 50% yield. Acetylation of XIV afforded in 89% yield a tetraacetyl derivative identical in all respects with XXVII from the reductive methylation of VIII. Although the pK'a value of 2.8 for XXVII was lower than expected,

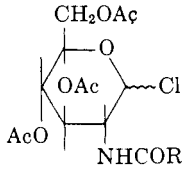
(38) The carbon dioxide formed in this reaction was evidently completely retained by the basic product and was lost later in the work up, on evaporation of the solvent.

(39) (a) F. Maley, G. F. Maley and H. A. Lardy, *J. Am. Chem. Soc.*, **78**, 5303 (1956). (b) T. White, *J. Chem. Soc.*, 1498 (1938).

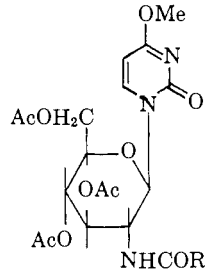
(40) J. D. Roberts and V. C. Chambers, *J. Am. Chem. Soc.*, **73**, 5030 (1951).

there was no doubt about its structure because of the mode of its formation by two independent routes. Further it could be ammonolyzed back in 80% yield to XIV of established structure.

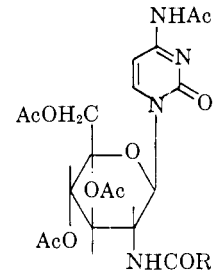
The extension of the Hilbert–Johnson reaction to the synthesis of the 2-deoxy-N-methyl-2-acetamido nucleoside XXXII was briefly studied. Conversion of pentaacetyl-N-methyl-α-D-glucosamine[41] to the chlorosugar even under the vigorous conditions recommended for glucosamine-α-pentaacetate,[42] led to a substantial recovery of starting material. The chlorosugar from the reaction could not be crystallized and was subjected as a syrup to reaction with dimethoxypyrimidine to yield the nucleoside XXXII. The ultraviolet and infrared spectra of XXXII were in agreement with the structure. However, the yield in this reaction was minute and hence further conversions of XXXII were not attempted.



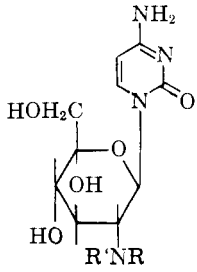
I, R = CH_3
II, R = $C_6H_5CH_2O$
III, R = CH_3O



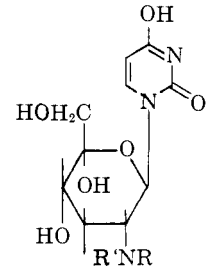
IV, R = CH_3
V, R = $C_6H_5CH_2O$
VI, R = CH_3O



VII, R = CH_3
VIII, R = $C_6H_5CH_2O$
IX, R = CH_3O
X, R = $NHCOCH_3$

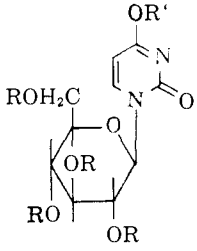


XI, R = H, R' = CH_3CO
XII, R = H, R' = $CONH_2$
XIII, R = R' = H
XIV, R = R' = CH_3

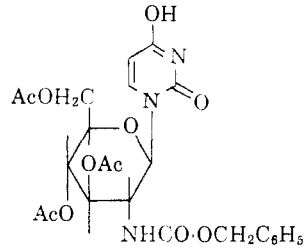


XV, R = R' = H
XVI, R = H, R' = CH_3CO
XVII, R = H, R' = $C_6H_5CH_2OCO$
XVIII, R = R' = CH_3

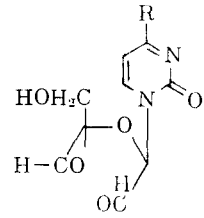
(41) F. A. Kuehl and co-workers, *J. Am. Chem. Soc.*, **69**, 3032 (1947).
(42) D. H. Leaback and P. G. Walker, *J. Chem. Soc.*, 4754 (1957).



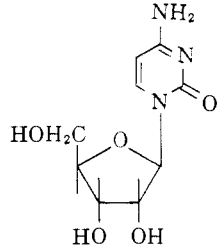
XIX, R = Ac, R' = Me
XX, R = R' = H



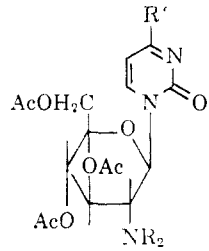
XXI



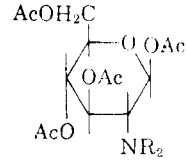
XXII, R = OH
XXIII, R = NH_2



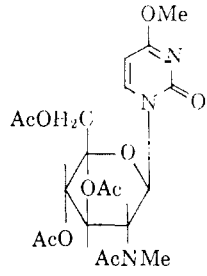
XXIV



XXV, R = H, R' = OH
XXVI, R = H, R' = $NHCOCH_3$
XXVII, R = CH_3, R' = $NHCOCH_3$
XXVIII, R = H, R' = OCH_3
XXIX, R = CH_3, R' = OCH_3



XXX, R = H
XXXI, R = CH_3



XXXII

Biological Testing.—The compounds were tested for antitumor activity in the tissue culture and the mouse tests of the Cancer Chemotherapy National Service Center, the protocols of which tests are described in *Cancer Chemotherapy Reports*, No. 1, Jan., 1959, pp. 42–64. The following compounds were tested for cytotoxicity in the tissue culture test: II, V, VI, VIII, X, XII, XIII, XIV, XVII, XVIII, XX, XXI, XXVIII and XXXII. All were inactive. Compound IX was tested against adenocarcinoma 755 at a dose range of 250–375 mg./kg. and against sarcoma 180 at a dose level of 400 mg./

kg. and found to be nontoxic but inactive in both tumors. Compound XV, in the sarcoma 180 tumor, was toxic at a dose level of 500 mg./kg. and inactive at 125 mg./kg. In both the adenocarcinoma 755 tumor and the lymphoid leukemia L1210, XV was not toxic but inactive at 100 mg./kg.

Experimental

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (IV).—A solution of 5 g. of 1-chloro-3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-D-glucopyranose (I)[9] and 6 ml. of 2,4-dimethoxypyrimidine in 25 ml. of dry benzene was refluxed on the steam bath for 48 hr. The solution was diluted with 20 ml. of ether and filtered from 60 mg. of uracil, m.p. above 300° dec. The filtrate was diluted with petroleum ether to turbidity, and allowed to stand at room temperature for a few hr. The crystals were collected and washed with ether to give 3.40 g. of a solid, m.p. 160–180°. Recrystallization from ethanol–ether–petroleum ether mixture gave 2.45 g. of needles, m.p. 189–191°. Dilution of the mother liquors with more petroleum ether and cooling at 0° gave an additional 0.40 g. of solid, which was dissolved in benzene and filtered from a little insoluble material. Concentration of the filtrate and addition of ether gave 0.20 g., m.p. 185–188°. The mother liquors from the various crystallizations were combined and the solvents evaporated. The residue was taken up in benzene and chromatographed over 100 g. of alumina. Washing with 400 ml. of benzene gave unreacted dimethoxypyrimidine, while elution with 200 ml. portions of benzene containing 0.5% and 1% alcohol, respectively, brought down negligible material. Further elution with 200 ml. of benzene containing 2% alcohol, evaporation of the eluate and crystallization from benzene–ether gave 0.4 g., m.p. 188–190°. A total of 3.05 g. (49%) of the nucleoside (IV) was thus obtained. An analytical sample, crystallized from methanol–ether–petroleum ether gave white needles, m.p. 192°; $[\alpha]^{25}D + 32.9$ (*c* 0.95 in $CHCl_3$); λ_{max}^{EtOH} 276 mμ (ε 5,920).

Anal. Calcd. for $C_{19}H_{25}N_3O_{10}$: C, 50.11; H, 5.53. Found: C, 49.85; H, 5.69.

1-(2-Deoxy-2-acetamido-β-D-glucopyranosyl)-cytosine (XI).[43]—A suspension of 1 g. of nucleoside (IV) in 25 ml. of ethanol saturated with ammonia at 0° was sealed and heated at 100° for 48 hr. The tube was opened and the contents were filtered to give 0.4 g. (59%) of thick, colorless cubes, m.p. 280–285° dec. Recrystallization from aqueous alcohol gave a sample, m.p. 285–286° dec.; $[\alpha]^{25}D + 105°$ (*c* 1.14 in H_2O); $\lambda_{max}^{0.1\ N\ HCl}$ 276.5 mμ (ε 12,300); p*K'*a 3.9 (50% alcohol).

Anal. Calcd. for $C_{12}H_{18}N_4O_6$: C, 45.85; H, 5.77; N, 17.83. Found: C, 46.00; H, 5.73; N, 17.89.

The picrate was formed in aqueous alcohol and recrystallized from the same solvent pair, m.p. 224–225° dec.

Anal. Calcd. for $C_{18}H_{21}N_7O_{13}$: C, 39.79; H, 3.90; N, 18.05. Found: C, 40.08; H, 3.94; N, 18.04.

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-4-acetamido-2-pyrimidinone (VII). A. By Acetylation of Cytosine Nucleoside (XI).—A suspension of 0.20 g. of the cytosine derivative XI in 20 ml. of pyridine containing

(43) This compound was first prepared and characterized in this laboratory by Dr. E. R. Burrows.

10 ml. of acetic anhydride was boiled for 15 min. to effect complete solution. The solution was then heated over a steam bath for 18 hr. and evaporated *in vacuo* to dryness. The residue was taken up in water and extracted with chloroform. Evaporation of the chloroform layer and crystallization of the residue from ethanol–ether gave 0.16 g. (52%) of the acetyl derivative, m.p. 218–219°. Recrystallization from 1-butanol gave VII, m.p. 219–220°; $[\alpha]^{25}D$ − 9.9° (*c* 0.73 in $CHCl_3$); $\lambda_{max}^{0.1\ NHCl}$ 246 mμ (ε 10,700), 302 mμ (ε 9,060); p*K'*a 11.1 (water).

Anal. Calcd. for $C_{20}H_{26}N_4O_{10}$: C, 49.80; H, 5.43; N, 11.61; O, 33.19. Found: C, 49.87; H, 5.58; N, 11.66; O, 32.81.

B. From Chlorosugar I by the Acetylcytosine–Mercury Procedure.—To an azeotropically dried suspension of 0.53 g. (1.5 mmoles) of acetylcytosine–mercury was added 0.55 g. (1.5 mmoles) of chlorosugar I. The mixture was refluxed for 30 min. with stirring. Another lot of 0.55 g. (1.5 mmoles) of the chlorosugar was added and the heating and stirring continued for an additional hr. During this period the suspension turned into a sticky brown mass and settled at the bottom leaving a yellow solution. The mixture was cooled and excess hexane added. After cooling at 5° for 1 hr., the mixture was filtered. The residue was stirred with 150 ml. of chloroform and filtered from 0.35 g. of insoluble substance. The filtrate was shaken with 20 ml. of 30% aqueous potassium iodide solution and then with water. The dried chloroform extract, on evaporation, left about 0.5 g. of a gum which was dissolved in 3 ml. of benzene and excess ether added to the solution. The precipitate was dissolved in alcohol, and some amorphous material removed by gradual addition of ether. The clear solution on standing deposited 50 mg. of crystals, m.p. 216–218°. Recrystallization from alcohol–ether gave needles, m.p. 219–220°, undepressed by admixture with the acetyl derivative (VII) obtained by procedure A. The infrared spectra of the two preparations were identical.

The decanted benzene–ether solution in the foregoing procedure, on standing overnight, deposited thick cubes which were collected and washed with ether to give 100 mg. of a solid, m.p. 188–189°. Recrystallization from alcohol–ether gave a sample, m.p. 190°, which showed ester and amide carbonyl in the infrared, had no titrable group and no absorption in the ultraviolet region. No structure proof was done on this compound outside of analysis.

Anal. Calcd. for $C_{16}H_{19}NO_8$ (chlorosugar minus the elements of hydrogen chloride): C, 51.06; H, 5.82. Found: C, 51.05, 51.03; H, 5.88, 6.04.

1-Chloro-3,4,6-tri-*O*-acetyl-2-deoxy-2-carbobenzyloxyamino-α-D-glucopyranose (II).—A suspension of 5 g. of 2-carbobenzyloxyamino-β-D-glucopyranose tetraacetate[9] in 125 ml. of dry ether and 15 ml. of acetic anhydride was saturated with dry hydrogen chloride gas at 0 to −10° and the resultant solution held at 0° for 24 hr. The solvents were then removed *in vacuo* below 25°. The residue was taken up in chloroform and washed successively with ice-water, a saturated ice-cold aqueous solution of sodium bicarbonate and then again with ice water. The chloroform layer was quickly dried over sodium sulfate and evaporated *in vacuo*. The residual syrup was rubbed with petroleum ether to induce crystallization. On washing with petroleum ether, 3.4 g. (72%) of chlorosugar II, m.p. 111–112° was obtained. A sample prepared by two crystallizations from benzene–ether–petroleum ether mixture formed colorless needles, m.p. 112–113° dec.; $[\alpha]^{25}D$ + 118.6° (*c* 1.08 in $CHCl_3$). The sample was dried *in vacuo* at room temperature for analysis, since prolonged drying at 60° caused complete decomposition.

Anal. Calcd. for $C_{20}H_{24}ClNO_9$: C, 52.46; H, 5.28. Found: C, 52.53, 52.33; H, 5.23, 5.44.

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-carbobenzyloxyamino-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (V).—A solution of 1 g. of chlorosugar II in 5 ml. of dry benzene and 1.2 ml. of dimethoxypyrimidine was refluxed on the steam bath. In the course of a few hours, a solid began to separate. At the end of 24 hr., 50 ml. of ether was added to the mixture which was then cooled in an ice chest for 1 hr. Filtration and washing with ether gave 1.1 g. of nucleoside V, m.p. 238–241°. Crystallization from alcohol afforded 1 g. (84%) of minute needles, m.p. 242–243°. A second crystallization gave the analytical sample, m.p. 244–245°; $[\alpha]^{25}D$ + 31.1° (*c* 0.79 in $CHCl_3$); λ_{max}^{EtOH} 275 mμ (ε 5,860).

Anal. Calcd. for $C_{28}H_{29}N_3O_{11}$: C, 54.84; H, 5.34; N, 7.67. Found: C, 54.90; H, 5.53; N, 7.69.

1-(2-Deoxy-2-ureido-β-D-glucopyranosyl)-cytosine (XII).—A suspension of 1 g. of nucleoside V in 30 ml. of alcohol saturated with ammonia at 0° was sealed in a tube and heated at 100–110° for 40 hr. The ureide XII had crystallized during this period and was collected by filtration and washed with alcohol to give 0.435 g. (75%), m.p. 275–280° dec. Crystallization from aqueous alcohol afforded white needles, m.p. 280–281° dec.; $[\alpha]^{25}D$ + 71.8° (*c* 0.83 in H_2O); $\lambda_{max}^{0.1\ NHCl}$ 210 mμ (ε 10,400), 276.5 mμ (ε 11,500); p*K'*a 2.2 (estimated) and 4 (in H_2O).

Anal. Calcd. for $C_{11}H_{17}N_5O_6$: C, 41.90; H, 5.44; N, 22.22. Found: C, 41.31; H, 5.67; N, 22.17.

The picrate was formed in hot aqueous methanol and recrystallized from aqueous alcohol; m.p. 208–209° dec.

Anal. Calcd. for $C_{17}H_{20}N_8O_{13}$: C, 37.51; H, 3.70; N, 20.59. Found: C, 37.26, 37.14; H, 3.90, 3.74; N, 20.20, 20.57.

The acetyl derivative was made by refluxing 27 g. of nucleoside XII with 20 ml. of acetic anhydride and 20 ml. of pyridine for 30 min., whereupon complete solution occurred. This was left at room temperature overnight and then evaporated *in vacuo* to dryness. Crystallization from alcohol with the aid of charcoal gave 0.15 g. (33%) of needles, m.p. 263–264° dec.; $[\alpha]^{25}D$ + 42.3° (*c* 0.97 in $CHCl_3$); λ_{max}^{EtOH} 251 mμ (ε 9,560), 280 mμ (ε 8,880); p*K'*a 11.0 (in 50% MeOH).

Anal. Calcd. for $C_{21}H_{27}N_5O_{11}$:[44] C, 48.00; H, 5.18; N, 13.33. Found: C, 48.10; H, 5.40; N, 12.92, 13.16.

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-carbobenzyloxyamino-β-D-glucopyranosyl)-4-acetamido-2-pyrimidinone (VIII).—To an azeotropically dried suspension of 1.94 g. (5.5 mmoles) of acetylcytosine–mercury in 120 ml. of toluene was added 2.52 g. (5.5 mmoles) of chlorosugar II. The mixture was stirred and refluxed for 30 min. A second lot of 2.52 g. (5.5 mmoles) of the chlorosugar then was added and the heating continued for 1.5 hr. The mixture was cooled and after addition of 300 ml. of petroleum ether, held at 5° for 2 hr. It was then filtered, the residue stirred with chloroform and filtered again. The chloroform extract was washed with 30% aqueous potassium iodide solution and then with water, dried and evaporated *in vacuo* to give a gum. This was dissolved in 20 ml. of alcohol and after standing for a day, 1.05 g. of crystals separated, m.p. 230–240°. Crystallized once from alcohol–ether, the nucleoside formed minute needles, 0.78 g., m.p. 256–257°. The combined mother liquors on standing gave an additional

(44) The compound is formulated with the amino group of the urea as being also acetylated because the analysis checks for this structure rather than for the one where it is not acetylated.

crop, 0.10 g., m.p. 255–256°. A total yield of 0.88 g. (27% based on acetylcytosine–mercury used) was thus obtained. An analytical sample, crystallized from alcohol–ether, had m.p. 256–257°; $[\alpha]^{25}D$ − 9.6° (*c* 0.84 in $CHCl_3$); λ_{max}^{EtOH} 250 mμ (ε 15,600), 300 mμ (ε 6,410); p*K'*a 10.4 (in 50% MeOH).

Anal. Calcd. for $C_{26}H_{36}N_4O_{11}$: C, 54.35; H, 5.26; N, 9.75. Found: C, 54.53; H, 5.38; N, 9.70.

The mother liquors from the above crystallizations after standing for several days gave a new crop of crystals, 0.12 g., m.p. 168–169°, with some softening above 160°. Recrystallization from alcohol gave long needles, m.p. 170–172°. The compound was not further characterized.[45]

1-Chloro-3,4,6-tri-*O*-acetyl-2-deoxy-2-carbomethoxyamino-D-glucopyranose (III).—A suspension of 3 g. of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-carbomethoxyamino-β-D-glucopyranose[20] in 50 ml. of dry ether and 10 ml. of acetic anhydride was saturated with dry HCl gas at 0°. After standing in the ice box for 30 hr., using the work-up employed in making chlorosugar II, 2.5 g. (88%) of chlorosugar III was obtained as a waxy solid, which on rubbing with petroleum ether formed an amorphous powder, melting indefinitely from 50 to 65°. The substance showed the presence of chlorine and was quite satisfactory for use in further experiments. An analytical sample prepared by crystallization from benzene–ether–petroleum ether mixture had the same melting point behavior, but after drying for 24 hr. *in vacuo* at room temperature, the melting point rose to 82–84°, with some prior shrinking.

Anal. Calcd. for $C_{14}H_{20}ClNO_9$: C, 44.04; H, 5.28; Cl, 9.29. Found: C, 44.20, 44.55; H, 5.47, 5.49; Cl, 9.22, 9.24.

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-carbomethoxyamino-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (VI).—A solution of 0.6 g. of chlorosugar III and 0.6 ml. of 2,4-dimethoxypyrimidine in 2 ml. of dry benzene was refluxed on the steam bath. At the end of 5 hr., a solid began to separate. After 20 hr., 20 ml. of ether was added to the cooled mixture. After filtering and washing with ether, 0.62 g. (84%) of the nucleoside (VI) was obtained, m.p. 206–208°. Recrystallization from methanol–ether gave slender needles, m.p. 207–208°; $[\alpha]^{25}D$ + 39.3° (*c* 1.3 in $CHCl_3$); λ_{max}^{EtOH} 275 mμ (ε 5,940).

Anal. Calcd. for $C_{19}H_{25}N_3O_{11}$: C, 48.41; H, 5.35; N, 8.91. Found: C, 48.51; H, 5.47; N, 8.92.

Ammonolysis of 0.4 g. of the nucleoside by the usual procedure yielded 0.14 g. (52%) of the urea XII, m.p. 275–278° dec., undepressed by admixture with the sample from the previous preparation. The acetyl derivative was also prepared, m.p. and mixture m.p. 263–264° dec.

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-carbomethoxyamino-β-D-glucopyranosyl)-4-acetamido-2-pyrimidinone (IX).—A solution of 3.82 g. (10 mmoles) of chlorosugar III in 40 ml. of toluene was added to an azeotropically dried suspension of 3.51 g. (10 mmoles) of acetylcytosine–mercury in 150 ml. of toluene. After refluxing with stirring for 20 min., a second lot of 3.82 g. (10 mmoles) of the chlorosugar in 40 ml. of toluene was added and the refluxing continued for 80 min. when complete solution occurred. By employing the usual work-up, a crystalline solid was obtained. One recrystallization from alcohol–ether gave 3.26 g. (65%) of

(45) This compound may perhaps be the oxazolone derivative, m.p. 174–175° described by S. Konstas, I. Photaki, and L. Zervas, *Chem. Ber.*, **92**, 1288 (1959). These authors made this compound by the action of titanium tetrachloride or a mixture of phosphorus oxychloride and aluminum chloride on 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-carbobenzyloxyamino-β-D-glucopyranose and postulated its formation through the chlorosugar (II) intermediate.

nucleoside IX, m.p. 260–261°. An analytical sample, recrystallized from alcohol, formed slender needles, m.p. 261–262°; $[\alpha]^{25}D$ + 4.5° (*c* 0.94 in $CHCl_3$); λ_{max}^{EtOH} 249 mμ (ε 17,300), 299 mμ (ε 6,780).

Anal. Calcd. for $C_{20}H_{26}N_4O_{11}$: C, 48.20; H, 5.26; N, 11.24. Found: C, 48.04; H, 5.32; N, 11.34.

Ammonolysis of the nucleoside IX gave the urea XII, m.p. and mixture m.p. 275–278° dec., confirmed further by comparison of the acetyl derivatives.

1-(**2-Deoxy-2-acetamido-β-D-glucopyranosyl**)-**uracil** (**XVI**).—A solution of 0.5 g. of nucleoside IV in 7 ml. of warm methanol was added to 3 ml. of ethanol containing 0.8 g. of hydrogen chloride. After 24 hr., the slightly brown solution was evaporated to dryness *in vacuo* yielding 0.345 g. of a white froth which was dissolved in hot alcohol. On cooling, thick cubes were deposited. These were collected and washed with alcohol to yield 0.28 g. (81%) of uracil XVI, m.p. 215° dec. Recrystallization from methanol–ether gave a pure sample, m.p. 216–217° dec.; $[\alpha]^{25}D$ + 71.3 (*c* 1.05 in H_2O); $\lambda_{max}^{H_2O}$ 258 mμ (ε 9,020); p*K'*a 9.9 (in 50% MeOH).

Anal. Calcd. for $C_{12}H_{17}N_3O_7$: C, 45.71; H, 5.44; N, 13.33. Found: C, 45.45, 45.47; H, 5.66, 5.57; N, 13.11.

1-(**2-Deoxy-2-amino-β-D-glucopyranosyl**)-**uracil** (**XV**).—A solution of 1 g. of nucleoside IV in 10 ml. of water and 10 ml. of concd. hydrochloric acid was refluxed for 10 hr. The solution was then evaporated to dryness *in vacuo*. The residue was dissolved in a small volume of methanol and deposited crystals on standing. These were collected, washed with a little methanol and then with ether to give 0.615 g. (91%) of 1-(2-deoxy-2-amino-β-D-glucopyranosyl)-uracil hydrochloride, m.p. 275–278° dec. Recrystallization from aqueous methanol gave an analytical sample, m.p. 280° dec., with blackening above 240°; $[\alpha]^{25}D$ + 40° (*c* 1.2 in H_2O); $\lambda_{max}^{H_2O}$ 257 mμ (ε 9,790); p*K'*a 6.2, 10.1 (in 50% MeOH).

Anal. Calcd. for $C_{10}H_{16}ClN_3O_6$: C, 38.77; H, 5.21; N, 13.57; Cl, 11.45. Found: C, 38.92, 39.10; H, 5.24, 5.56; N, 12.85; Cl, 11.39.

The same compound could also be prepared by vigorous acid hydrolysis of 1-(3,4,6-tri-*O*-acetyl-2-deoxy-2-carbomethoxyamino-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (VI) in 53% yield, m.p. and mixture m.p. 280° dec. The infrared spectra of the two preparations were superimposable.

A suspension of 0.68 g. of this hydrochloride in 6 ml. of ethanol containing 0.6 ml. of diethylamine was shaken for several hr. The mixture was filtered and the precipitate again shaken as a suspension in 2 ml. of ethanol containing 0.2 ml. of diethylamine. Filtration and washing with ethanol afforded 0.495 g. (91%) of 1-(2-deoxy-2-amino-β-D-glucopyranosyl)-uracil (XV), m.p. 239–240° dec. One crystallization from moist methanol–ether gave needles, m.p. 241–242° dec.; $[\alpha]^{25}D$ + 15.66° (*c* 1.1 in H_2O); $\lambda_{max}^{MeOH-H_2O}$ 259 mμ (ε 9,870); p*K'*a 6.1, 9.9 (in 50% MeOH); p*K'*a 5.87, 9.17 (in water).

Anal. Calcd. for $C_{10}H_{15}N_3O_6$: C, 43.93; H, 5.53; N, 15.36. Found: C, 44.16; H, 5.48; N, 15.34.

The picrate was formed by warming the hydrochloride with alcoholic picric acid. Crystallization from methanol–ether gave yellow cubes, m.p. 254–255° dec., $[\alpha]^{25}D$ + 25.1 (*c* 1.1 in H_2O).

Anal. Calcd. for $C_{16}H_{18}N_6O_{13}$: C, 38.25; H, 3.61; N, 16.73. Found: C, 38.47; H, 3.58; N, 16.72.

1-(**2-Deoxy-2-carbobenzyloxyamino-β-D-glucopyranosyl**)-**uracil** (**XVII**).—A solution of 0.28 g. of nucleoside V in 20 ml. of warm methanol was added to 7 ml.

of alcohol containing 0.5 g. of hydrogen chloride. After leaving for 24 hr. at room temperature, the solution was evaporated to dryness. The residue was dissolved in 5 ml. of alcohol and on standing deposited 0.12 g. (58%) of crystals, m.p. 225–226° dec. Recrystallization from methanol–ether gave a sample of the same melting point; $[\alpha]^{25}D$ − 34° (*c* 0.78 in H_2O); λ_{max}^{EtOH} 258 mμ (ε 9,410).

Anal. Calcd. for $C_{18}H_{21}N_3O_8$: C, 53.07; H, 5.20; N, 10.32. Found: C, 53.25, 52.73; H, 5.53, 5.05; N, 10.58.

1-(**3,4,6-Tri-*O*-acetyl-2-deoxy-2-carbobenzyloxyamino-β-D-glucopyranosyl)-uracil** (**XXI**).—A solution of 1 g. of nucleoside V was converted to 1-(2-deoxy-2-carbobenzyloxyamino-β-D-glucopyranosyl)-uracil as above. The crude product was acetylated without purification by heating with 3 ml. of acetic anhydride at 100° for 4 hr. The solution was evaporated to dryness *in vacuo*. Addition of alcohol to the residue afforded 0.6 g. (62%) of crystalline XXI, m.p. 190–191°. Recrystallization from alcohol–ether yielded shining clusters of needles, m.p. 191–192°; $[\alpha]^{25}D$ − 20.2° (*c* 0.95 in $CHCl_3$); λ_{max}^{EtOH} 256.5 mμ (ε 9,390); p*K'*a 10.7 (in 50% MeOH).

Anal. Calcd. for $C_{24}H_{27}N_3O_{11}$: C, 54.04; H, 5.10; N, 7.88. Found: C, 53.72, 54.24; H, 5.23, 5.28; N, 7.85.

A more direct and satisfactory preparation of this compound (XXI) was as follows: A solution of 1 g. of nucleoside V in 50 ml. of chloroform and 5 ml. of acetic anhydride was saturated with dry hydrogen chloride gas at room temperature. After leaving overnight, the solution was evaporated to dryness *in vacuo* at room temperature. The residual gum was dissolved in alcohol and ether added to it to the point of crystallization. Filtration and washing with ether gave 0.94 g. (97%) of uracil XXI, m.p. and mixture m.p. 189–190°.

1-(**3,4,6-Tri-*O*-acetyl-2-deoxy-2-amino-β-D-glucopyranosyl)-uracil** (**XXV**).—A solution of 1 g. of carbobenzyloxynucleoside XXI in 40 ml. of purified, acid-free dioxane was subjected to hydrogenation at atmospheric pressure using 0.5 g. of 10% palladium-on-charcoal as a catalyst. At the end of 2 hr., after an uptake of 0.9 mole of hydrogen, absorption slackened considerably. The reaction was stopped at this stage. The mixture was filtered and the filtrate evaporated *in vacuo* at room temperature. Since attempts to crystallize the aminonucleoside failed, it was converted to the hydrochloride salt, using an ether solution of hydrogen chloride. The precipitated salt was collected, washed with ether and dried to yield 0.72 g. (88%) of white solid, m.p. 230° dec. Crystallization from methanol–ether gave an analytical sample, m.p. 232–233° dec.; $[\alpha]^{25}D$ + 29.9° (*c* 0.66 in H_2O); $\lambda_{max}^{0.1\ N\ HCl}$ 256 mμ (ε 9,110); p*K'*a 4.9, 9.6 (in 50% MeOH).

Anal. Calcd. for $C_{16}H_{22}ClN_3O_9$: C, 44.10; H, 5.09; N, 9.64; Cl, 8.02. Found: C, 44.03, 43.92; H, 5.49, 5.40; N, 9.74; Cl, 7.93.

The free base was liberated by dissolving 0.1 g. of the hydrochloride in 2 ml. of water containing 0.1 g. of sodium bicarbonate. The solution was cooled to 0° and shaken with 5 ml. of chloroform containing 0.2 ml. of a 40% solution of carbobenzyloxychloride in toluene. On evaporation of the chloroform layer and crystallization of the residue from alcohol, 30 mg. of crystals, m.p. 160–170°, could be recovered. On two more recrystallizations, the melting point rose to 183–185°, and was not depressed by admixture with the carbobenzyloxynucleoside (XXI).

Deacetylation of Nucleoside XXV.—A solution of 0.2 g. of the hydrochloride of XXV in 3 ml. of methanol was mixed with 5 ml. of ethanol saturated with hydrogen chloride gas. Crystals started to separate from the clear solution at the end of a

few hr. These were collected after 24 hr. and washed with a little methanol and then with ether to give 0.12 g. (85%) of XV·HCl, m.p. 270–274° dec., undepressed by admixture with the hydrochloride of nucleoside XV. The infrared and ultraviolet absorption spectra of the two samples were identical.

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-amino-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (XXVIII).—A solution of 1 g. of nucleoside V in 50 ml. of purified, acid–free dioxane was subjected to hydrogenation at atmospheric pressure, using 0.5 g. of 10% palladium-on-charcoal as catalyst. The uptake of hydrogen was rapid and amounted to 0.95 in mole 1.75 hr., when it stopped almost completely. The mixture was filtered and the filtrate evaporated to dryness *in vacuo* at room temperature. Recrystallization of the residual crystalline solid from alcohol–ether gave 0.57 g. (75%) of XXVIII, m.p. 207–208°. Another crystallization from the same solvent mixture afforded an analytical sample as slender needles, m.p. 208–209°; $[\alpha]^{25}D$ + 42.2° (*c* 1.3 in $CHCl_3$); λ_{max}^{EtOH} 276 mμ (ε 5,290); p*K*'a 5.2 (in 50% MeOH).

Anal. Calcd. for $C_{17}H_{23}N_3O_9$: C, 49.39; H, 5.61; N, 10.17; 3 CH_3CO, 31.23. Found: C, 49.31, 49.72; H, 5.67, 5.91; N, 10.08; CH_3CO, 32.07.

One attempt to make the hydrochloride using an alcoholic solution of the base and ethereal hydrogen chloride, led to demethylation in the pyrimidine ring. The resultant salt has m.p. 227–230° dec., undepressed by admixture with the hydrochloride of XXV.

The nucleoside XXVIII could be carbomethoxylated using methyl chloroformate in chloroform solution to give a rather poor yield of VI.

1-(2-Deoxy-2-amino-β-D-glucopyranosyl)-cytosine (XIII). A. From Carbomethoxy Nucleoside IX.—A solution of 0.6 g. of nucleoside IX and 2 g. of barium hydroxide monohydrate in 60 ml. of water was heated on the steam bath for 45 min. and then left at room temperature overnight. The solution was brought to pH 3–4 by the addition of 22 ml. of 1.22 *N* sulfuric acid. The precipitated barium sulfate was removed with the aid of Celite and the filtrate evaporated to near dryness *in vacuo* at room temperature. The residue crystallized on rubbing with aqueous methanol and gave 0.28 g. (57%) of the sulfate dihydrate, decomposing at about 250°. An analytical sample was obtained as minute needles from aqueous methanol. This blackened and partially decomposed between 240 and 250° in a preheated bath but when heated in a bath from room temperature, the compound blackened at about 250° without melting or decomposing: $[\alpha]^{25}D$ + 49.8° (*c* 1 in H_2O); $\lambda_{max}^{0.1\ N\ HCl}$ 210 mμ (ε 10,300), 274 mμ (ε 12,700); $\lambda_{max}^{pH\ 7}$ 236 mμ (ε 8,370), 267 mμ (ε 8,740); p*K*'a 3.7, 6.3 (in 50% MeOH).

Anal. Calcd. for $C_{10}H_{18}N_4O_9S \cdot 2\ H_2O$: [46] C, 29.54; H, 5.46; N, 13.79; neutralization equivalent, 203.2. Found (for samples from different lots dried under various conditions): C, 29.20, 29.25, 29.49, 29.70; H, 5.63, 5.69, 5.50, 5.50; N, 14.06; neutralization equivalent, 200.4, 203.9.

B. From 1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-amino-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (XXVIII).—A solution of 0.2 g. of nucleoside XXVIII in 8 ml. of alcohol saturated with ammonia at 0° was left in a sealed tube at 100–110° for 45 hr. The tube was then opened and the solution concentrated to 4 ml., whereupon crystals started separating. After cooling at 5° for 2 hr., filtering and washing with alcohol, 60 mg. (40%) of 1-(2-deoxy-2-acetamido-β-D-gluco-

(46) Attempts to estimate the water of hydration by the Fischer method indicated some water to be present, but results were grossly inaccurate because of poor solubility of the compound in the solvents commonly used for this estimation.

pyranosyl)-cytosine (XI), m.p. 285° dec., was obtained. The melting point was undepressed by admixture with a sample from the previous preparation. The identity was further confirmed by making the picrate, m.p. and mixture m.p. 224–225° dec.

The mother liquor from the foregoing crystallization was evaporated to dryness. A few drops of sulfuric acid in 2 ml. alcohol were added to the residue, followed by ether. The gummy precipitate crystallized on rubbing with aqueous methanol. Recrystallization from aqueous methanol gave 25 mg. (12%) of a solid, m.p. 250° dec., undepressed by admixture with the sulfate of nucleoside XIII; the ultraviolet absorption spectrum, $\lambda_{max}^{0.1\ N\ HCl}$ 274.5 mμ (ε 12,900) and the infrared spectrum of this sample were also identical with those of the sample from preparation (A).

Acetylation of 1-(2-Deoxy-2-amino-β-D-glucopyranosyl)-cytosine (XIII).—An aqueous solution of 0.675 g. of the crystalline sulfate dihydrate was neutralized with aqueous barium hydroxide solution. Excess barium was precipitated by bubbling carbon dioxide into the suspension. The mixture was filtered and the filtrate evaporated *in vacuo* at room temperature. The free base thus obtained was boiled with 10 ml. of acetic anhydride and 5 ml. of pyridine for 10 min. and the solution left overnight at room temperature. The residue obtained by evaporation of this solution *in vacuo* was dissolved in 10 ml. of water and the solution extracted with chloroform. The dried chloroform layer was evaporated to dryness and the residue crystallized from methanol–ether to give 0.53 g. (66%) of the acetyl derivative, m.p. 218–219°. After two recrystallizations, the melting point was 219–220°. The melting point was undepressed by admixture with nucleoside VII and their infrared and ultraviolet spectra were superimposable.

Periodate Cleavage Studies.—1-β-D-Glucopyranosyluracil (XX) required in the late experiments was made by the procedure of Hilbert and Johnson.[10] A solution of 0.456 g. (1 mmole) of 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (XIX) in 5 ml. of hot methanol was mixed with 2 ml. of alcohol containing 40% of its weight of hydrogen chloride gas. The glistening hard crystals that had separated at the end of 24 hr. were collected, washed with alcohol and then with ether to afford 0.259 g. (95%) of 1-β-D-glucopyranosyluracil hemihydrate, m.p. 195–197°; $[\alpha]^{25}D$ + 19.8 (*c* 1.1 in H_2O); $\lambda_{max}^{H_2O}$ 258.5 mμ (ε 9,190); p*K'*a 10 (in 50% MeOH); p*K'*a 9.15 (water, *cf.* ref. 15b).

Fission of 1-(2-Deoxy-2-amino-β-D-glucopyranosyl)-uracil (XV).—A solution of 69.9 mg. (0.255 mmole) of uracil XV in 10 ml. (10 mmoles) of 0.2 *N* sodium metaperiodate solution was kept at room temperature in a volumetric flask. Two ml. aliquots were withdrawn at various intervals and the consumption of periodate determined by standard procedure.[21a] The changes in rotation of the solution were measured using an aliquot in a 1 dm. polarimetric tube. For comparison, similar data were gathered for the cleavage reaction of 75.7 mg. (0.28 mmole) of 1-β-D-glucopyranosyluracil using 10 ml. (10 mmoles) of 0.2 *N* sodium metaperiodate solution in the presence of 0.29 ml. (0.28 mmole) of 0.9716 *N* aqueous ammonium hydroxide. Table I presents the comparative data.

Fission of 1-(2-Deoxy-2-amino-β-D-glucopyranosyl)-uracil (XV) Picrate.—The cleavage reaction was studied as before using 93.6 mg. (0.19 mmole) of the picrate and 10 ml. of (10 mmoles) 0.2 *N* metaperiodate solution. Comparison was made with the fission of 70 mg. (0.255 mmole) in the presence of 58 mg. (0.255 mmole) of picric acid and 0.26 ml. (0.255 mmole) of 0.976 *N* ammonium hydroxide solution. The data are presented in Table II.

TABLE I

Compound cleaved	Time, hr.	IO_4^- uptake moles	$[\alpha]^{25}D$ degrees
1-(2-Deoxy-2-amino-β-D-glucopyranosyl)-uracil	0		+15.7[a]
	12	2.28	..
	16	2.28	+27.7[b]
	36	2.30	+27.5
	48	2.35	+32.3
	60		+29.5
	108		+30.3
1-β-D-Glucopyranosyl-uracil (in presence of 1 mole of ammonium hydroxide)[47]	0		+19.8[a]
	12	2.28	+16.9[b]
	18	2.28	+16.9
	36	2.25	+16.0
	48	2.19	+16.0

[a] In water. [b] These and subsequent rotations are based upon the theoretical concentration of dialdehyde product that should be in the solution.

TABLE II

Compound cleaved	Time, hr.	IO_4^- uptake moles	$[\alpha]^{25}D$ degrees
1-(2-Deoxy-2-amino-β-D-glucopyranosyl)-uracil picrate	0	..	+25.1[a]
	1	1.66	..
	2	1.79	..
	3	1.92	..
	4	1.99	+24.4[b]
	24	2.06	+15.6[b]
	48	2.05	+15.6[b]
1-(β-D-Glucopyranosyl)-uracil (in presence of 1 mole of ammonium picrate)	0	..	+19.8[a]
	1	1.78	..
	2	1.85	..
	3	1.90	..
	4	2.00	..
	24	2.00	+15.5[b]
	48	2.00	+15.5[b]

[a] In water. [b] Rotation based upon theoretical concentration of dialdehyde product that should be in the solution.

Fission of 1-(2-Deoxy-2-amino-β-D-glucopyranosyl)-cytosine (XIII). A. As the Sulfate Salt.—A solution of 52.5 mg. of the sulfate dihydrate in 7 ml. of 0.2 *N* sodium metaperiodate solution was left at room temperature. At the end of 48 hr. an uptake of 2.13 moles of IO_4^- was observed.

B. In Presence of Picric Acid.—An aqueous solution of 0.202 g. (0.5 mmole) of the sulfate dihydrate was neutralized with 20.2 ml. of 0.049 *N* barium hydroxide solution. A little solid carbon dioxide was added to the mixture to precipitate any excess barium ions. The mixture was filtered and the filtrate evaporated to

(47) J. Davoll, *et al.*,[25] found that this compound when cleaved alone with sodium metaperiodate took up 1.98 moles of periodate and that the resultant solution had $[\alpha]_D^{1.5}$ + 16.0°.

dryness *in vacuo* at room temperature to leave 0.145 g. (106%) of the gummy base. Picric acid (275 mg., 1.2 mmoles) was added to a solution of the base in 5 ml. of water. With a little warming, the picric acid went into solution and a gummy picrate separated on cooling. A solution of 0.61 g. of sodium metaperiodate in 5 ml. of water was added to the mixture which was left at room temperature with occasional shaking. After a few hours the yellow gum began to change over into a crystalline precipitate. At the end of 60 hr. the mixture was filtered and the crystals washed with a little water. The filtrate was made up to 50 ml. and an aliquot on titration by standard procedure showed that 2.59 moles of periodate had been consumed during this period.[48] The crystalline precipitate on drying weighed 0.15 g. (65%), m.p. 210–212° dec. On two recrystallizations from water, a pure specimen of 1-[formyl-(1-formyl-2-hydroxyethoxy)-methyl]-cytosine (XXIII)[36] picrate was obtained, m.p. 218–220° dec. (rapid heating); $[\alpha]^{26}D$ + 56.1° (*c* 0.58 in pyridine).

Anal. Calcd. for $C_{15}H_{14}N_6O_{12}$: C, 38.31; H, 3.00; N, 17.87. Found: C, 38.13; H, 2.93; N, 17.76.

A sample was made for comparison according to the directions of Davoll, *et al.*[26] A solution of 0.24 g. of cytidine picrate and 0.3 g. of sodium metaperiodate in 10 ml. of water after 48 hr. at room temperature gave 0.17 g. of the dialdehyde, m.p. 220° (dec. and charring). The mixture melting points of the two samples were the same. Their infrared spectra were superimposable and X-ray powder patterns were indistinguishable.

1-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-4-acetamido-2-pyrimidinone (XXVI).—A solution of 0.2 g. of the carbobenzyloxyaminonucleoside VIII in 8 ml. of dioxane was subjected to hydrogenation at atmospheric pressure using 0.1 g. of 10% palladium-on-charcoal as catalyst. After 2.5 hr. the mixture was filtered and the filtrate evaporated to dryness. The residual gum was brought to crystallize by addition of ether to its methanol solution and evaporation to the point where a solid began to appear. After standing overnight in the icebox, the mixture was filtered to give 60 mg. (39%) of a solid. The products from two batches were combined and recrystallized twice from methanol–ether to give beautiful rosettes; the melting point behavior of this compound was unsatisfactory. On a Kofler hot stage, it melted sharply to a gum at 145–146°, but flowed, however, only above 160°. In a bath, it shrank and softened at 137°, flowing occurring at 165–167°; $\lambda_{max}^{H_2O}$ 211 mμ (ε 15,800), 249 mμ (ε 15,700), 297 mμ (ε 7,350); $\lambda_{max}^{0.1\ N\ HCl}$ 210 mμ (ε 13,700), 250 mμ (ε 12,900), 300 mμ (ε 7,880); pK'_a 5 ~ 6, 9 ~ 10 (in 50% MeOH).

Anal. Calcd. for $C_{18}H_{21}N_4O_9$: C, 49.09; H, 5.49; N, 12.72. Found: C, 48.35, 49.82, 49.83; H, 5.60, 5.84, 5.69; N, 12.40, 12.65, 12.35.

Attempts to characterize this compound further as a hydrochloride resulted in partial deacetylation of the cytosine moiety in the process of crystallization, as evidenced by the appearance of an intense peak at 273 mμ, characteristic of cytosine and 1-substituted cytosines.[15] The picrate could not be crystallized either.

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-dimethylamino-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (XXIX).—A solution of 0.85 g. of aminonucleoside XXVIII in 20 ml. of acid-free dioxane containing 1.7 ml. of aqueous formaldehyde (36–38%;

(48) The true periodate uptake may be somewhat nearer to 2 moles because of a little retention of the oxidant by the flocculent precipitate, even after washing. This could be inferred from the observation that a trace of iodine was liberated by suspending a little of the washed, moist precipitate in aqueous potassium iodide solution in the presence of sodium bicarbonate.

reagent grade supplied by the Coleman and Bell Co.) was subjected to hydrogenation at atmospheric pressure, using 0.85 g. of 10% palladium-on-charcoal as a catalyst. Hydrogen uptake was rapid initially but after 1 mole had been consumed, it slackened appreciably. The next mole was taken up over a period of several hr. and in some experiments, the hydrogenation stopped at a total of 1.8–1.9 moles. At the end of this period, the mixture was filtered from the catalyst and the filtrate evaporated at room temperature *in vacuo* to remove all the dioxane and part of the formaldehyde. The residual syrup crystallized on rubbing with 5–10 ml. of ice water. The precipitate was collected and washed with a little water and then crystallized once from methanol–ether mixture to give 0.405 g. of a solid, m.p. 262–263°. The aqueous filtrate from the first crystallization was extracted with chloroform. Evaporation of the chloroform extract and crystallization of the residue yielded an additional 60 mg., m.p. 261–262°. A total of 0.465 g. (51%) of dimethylaminonucleoside XXIX was thus obtained. An analytical sample was obtained as colorless needles from methanol–ether and had m.p. 262–263°, with change in crystalline shape at 240–250°; $[\alpha]^{25}D$ + 82.8 (*c* 0.83 in $CHCl_3$); λ_{max}^{EtOH} 275 mμ (ε 5,870); p*K'*a 4.3 (in 50% MeOH).

Anal. Calcd. for $C_{19}H_{27}N_3O_9$: C, 51.70; H, 6.17; N, 9.52; OMe, 7.03; 2 NMe, 6.81. Found: C, 51.36, 52.00; H, 6.18, 6.14; N, 9.74, 9.40; OMe, 6.85; NMe, 6.15.

One attempt to obtain the dimethylaminonucleoside XXIX directly from the carbobenzyloxyaminonucleoside V in the presence of formaldehyde by simultaneous hydrogenolysis and reductive methylation resulted in no uptake of hydrogen and 84% recovery of starting material in a somewhat impure state.

In another experiment, the unstable dimethyl derivative of nucleoside XXVIII was isolated, and then reduced to the dimethylamino compound. A solution of 0.10 g. of nucleoside XXVIII in 10 ml. of dioxane containing 0.2 ml. of 35% aqueous formaldehyde was shaken for 40 min. and then evaporated *in vacuo* below room temperature. On rubbing with water, a solid separated. Filtration and washing with water afforded 0.1 g. (94%) of solid, m.p. 210–213°. One crystallization from methanol raised the melting point to 215–216°. The mixture melting point with starting material was elevated to 229–230° dec., with preliminary blackening. The compound could not be satisfactorily purified for analysis, but catalytic hydrogenation using 10% palladium-on-charcoal in dioxane by the usual procedure gave a 37% yield of dimethylaminonucleoside XXIX, m.p. and mixture m.p. 262–264°.

1-(2-Deoxy-2-dimethylamino-β-D-glucopyranosyl)-uracil (XVIII).—A solution of 0.25 g. of nucleoside XXIX in 3 ml. of warm methanol was mixed with a saturated solution of hydrogen chloride gas in 4 ml. of alcohol. The gelatinous precipitate that separated initially changed into chunky crystals after 24 hr. These were collected and washed with alcohol to yield 0.175 g. (90%), of the hydrochloride, m.p. 258–261° dec. Recrystallization from aqueous alcohol gave 0.125 g. of a pure sample, m.p. 261–263° dec.; $[\alpha]^{25}D$ + 49.4° (*c* 0.72 in H_2O); $\lambda_{max}^{H_2O}$ 255 mμ (ε 9,660); p*K'*a 5.3, 9.8 (in 50% MeOH).

Anal. Calcd. for $C_{12}H_{20}ClN_3O_6$: C, 42.67; H, 5.97; N, 12.44. Found: C, 42.85; H, 6.10; N, 12.40.

A solution of 80 mg. of the hydrochloride and 0.16 g. of sodium metaperiodate in 3 ml. of water was allowed to stand for 48 hr. Excess iodate and periodate ions were precipitated by the addition of aqueous barium hydroxide solution. The mixture was filtered, the filtrate was made strongly alkaline and then distilled

into a solution of 70 mg. of *p*-hydroxyazobenzene-*p'*-sulfonic acid in 5 ml. of water. The red solution was evaporated to dryness and after fractional crystallization to remove excess sulfonic acid, 17 mg. (22%) of the dimethylamine salt was obtained as orange needles, m.p. 215–216° dec., m.p. undepressed by admixture with an authentic specimen.[5]

1-(2-Deoxy-2-dimethylamino-β-D-glucopyranosyl)-cytosine (XIV).—A solution of 0.35 g. of nucleoside XXIX in 20 ml. of alcohol saturated with ammonia at 0° was heated in a sealed tube for 40 hr. The tube was opened and the solution evaporated to dryness. The residual gummy base would not crystallize and was converted into the sulfate by addition of 2 drops of concd. sulfuric acid to its alcoholic solution. The salt was completely precipitated by addition of ether. Crystallization from aqueous alcohol afforded 0.285 g. (86%) of the sulfate monohydrate, m.p. 240° dec. with blackening above 230°; $[\alpha]^{25}D$ + 56.7° (*c* 1.05 in H_2O); $\lambda_{max}^{0.1\ N\ HCl}$ 272.5 mμ (ε 12,200); p*K'*a 3.1, 5.5 (in 50% MeOH).

Anal. Calcd. for $C_{12}H_{22}N_4O_9S \cdot H_2O$:[49] C, 34.61; H, 5.81; N, 13.46; neutralization equivalent, 208.2. Found: C, 34.36, 34.98; H, 5.63, 5.87; N, 13.55, 13.36; neutralization equivalent, 202.3.

Periodate Fission of 1-(2-Deoxy-2-dimethylamino-β-D-glucopyranosyl)-cytosine (XIV).—A solution of 0.243 g. of the sulfate monohydrate was neutralized with barium hydroxide and the base isolated as a gum by the usual procedure. This was dissolved in 10 ml. of water containing 0.80 g. of sodium metaperiodate and 0.31 g. of picric acid. After 70 hr. at room temperature, the picrate salt was collected and washed with a little water to afford 0.14 g. (51%), m.p. 205–210° dec.; on two recrystallizations from water, the melting point rose to 215–216° dec.; $[\alpha]^{25}D$ + 55° (*c* 0.67 in pyridine). Identity was established by mixture melting point with the picrate of dialdehyde XXIII from the fission of cytidine[36] and the superimposability of their infrared spectra.

Anal. Calcd. for $C_{15}H_{14}N_6O_{12}$: C, 38.31; H, 3.00. Found: C, 38.18, 37.89; H, 3.19, 3.17.

The mother liquors from this reaction were combined and passed through a 15 × 2 cm. column of Dowex 50 (25–50 mesh). After washing with water until the eluate was neutral, the column was rinsed with 125 ml. of 2 *N* hydrochloric acid and the eluate evaporated *in vacuo* to dryness. The residue was dissolved in a little water and after addition of 20 ml. of 10% potassium hydroxide solution it was distilled into an aqueous solution of 0.175 g. of *p*-hydroxyazobenzene-*p'*-sulfonic acid. Evaporation of this solution and crystallization from alcohol, afforded 90 mg. (50%) of the dimethylamine salt, m.p. and mixture m.p. with authentic specimen,[5] 216–217° dec. Identity was further confirmed by comparison of infrared and ultraviolet absorption spectra and X-ray powder patterns.

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-dimethylamino-β-D-glucopyranosyl)-4-acetamido-2-pyrimidinone (XXVII). A. From 1-(2-Deoxy-2-dimethylamino-β-D-glucopyranosyl)-cytosine (XIV).—An aqueous solution of 0.27 g. of the sulfate monohydrate of nucleoside XIV was neutralized with aqueous barium hydroxide. The free base, isolated by the usual procedure, was then refluxed with 5 ml. of acetic anhydride and 2 ml. of pyridine for 5 min. The solution was allowed to stand overnight and then evaporated to dryness *in vacuo*. The residue was stirred with 5 ml. of water and extracted with chloroform. The chloroform layer was

(49) After drying to constant weight *in vacuo* at 100°, the sample still had a correct analysis except for a hemihydrate: Calcd., C, 35.47; H, 5.71; N, 13.80. Found: C, 35.18, 35.44; H, 5.52, 5.77; N, 13.43, 13.58.

dried over sodium sulfate and evaporated to dryness *in vacuo*. The residue was triturated with ether and the crystalline solid collected by filtration. There was thus obtained 0.27 g. (89%) of the acetyl derivative, m.p. 258–259°. Crystallization from 1-butanol gave needles, m.p. 259–260°; $[\alpha]^{25}D$ + 73.4° (*c* 0.92 in $CHCl_3$); $\lambda_{max}^{0.1\ N\ HCl}$ (initial) 251 mμ (ε 15,700), 297.5 mμ (ε 7,540); $\lambda_{max}^{0.1\ N\ HCl}$ (after 24 hr.) 270.5 mμ (ε 12,410); p*K'*a 2.8 (in 50% MeOH).

Anal. Calcd. for $C_{20}H_{28}N_4O_9$: C, 51.27; H, 6.02; N, 11.96; O, 30.74; 4 CH_3CO, 36.77. Found: C, 50.90, 51.04; H, 6.32, 6.14; N, 11.75, 12.14; O, 30.34, 30.60; CH_3CO, 36.40.

The hydrochloride was prepared in chloroform solution by the addition of ethereal hydrogen chloride. The crude compound was hygroscopic, shrank above 150° and melted at 180° dec. By addition of sodium bicarbonate to an aqueous solution of the salt, the base could be recovered unchanged. An attempt to crystallize the hydrochloride from ethanol–1-butanol afforded, after several days, white crystals, m.p. 228–229° dec. This compound however had an ultraviolet absorption maximum at 276 mμ (E_1^1 222), indicating considerable hydrolysis of the acetylcytosine moiety to free cytosine.[15]

B. From 1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-amino-β-D-glucopyranosyl)-4-acetamido-2-pyrimidinone (XXVI).—A solution of 0.2 g. of carbobenzyloxyaminonucleoside VIII in 10 ml. of dioxane was subjected to hydrogenation using 0.1 g. of 10% palladium-on-charcoal as a catalyst. The mixture was filtered without isolating the aminonucleoside XXVI. Aqueous formaldehyde solution (0.3 ml.) was added to the filtrate and reductive methylation carried out, using 0.15 g. of fresh catalyst. In the course of 20 hr., about 1.6 moles of hydrogen (theoretical, 2 moles) was absorbed. Since no more hydrogen was taken up, the mixture was filtered and the filtrate evaporated *in vacuo* to dryness. The residual syrup was triturated with 5 ml. of ice water, whereupon crystallization occurred. After cooling at 5° for 1 hr., the mixture was filtered and the residue washed with a little water to give 50 mg. (31%) of product, m.p. 255–257°. After one crystallization from methanol–ether, the dimethylaminonucleoside XXVII was obtained as needles, m.p. and mixture m.p. 259–260°. The ultraviolet and infrared absorption spectra of the two samples were identical.

Ammonolysis of 1-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-dimethylamino-β-D-glucopyranosyl)-4-acetamido-2-pyrimidinone (XXVII).—A solution of 35 mg. of nucleoside XXVII in 5 ml. of alcohol saturated at 0° with ammonia was heated in a sealed tube at 100–110° for 48 hr. The solution was evaporated to dryness and the gummy base converted into the sulfate salt. Crystallization from aqueous alcohol gave 25 mg. (80%) of product, m.p. 234–236° dec., undepressed by admixture with the sulfate salt of nucleoside XIV. Identity was confirmed by comparison of infrared and ultraviolet absorption spectra.

1-(3,4,6-Tri-*O*-acetyl-2-deoxy-N-methyl-2-acetamido-β-D-glucopyranosyl)-4-methoxy-2-pyrimidinone (XXXII).—A solution of 4 g. of pentaacetyl-N-methyl-α-glucosamine[41] in 20 ml. of acetic anhydride was cooled to 0° and saturated with dry hydrogen chloride gas. The solution was kept at room temperature for 24 hr., then cooled to 0°, saturated with hydrogen chloride gas and set aside for another 3 days. The solution was then evaporated *in vacuo*, the residue dissolved in chloroform and shaken successively with ice water, cold sodium bicarbonate solution and again ice water. The chloroform layer was dried and evaporated *in vacuo*. The residual syrup was triturated with ether, whereupon a crystalline solid separated. This was collected and washed with a little ether to give 1.6 g.,

m.p. 158–159°, identical with the starting pentaacetate. The ether filtrate, on evaporation, left a halogen-containing syrup which was then refluxed with 3 ml. of 3,4-dimethoxypyrimidine and 15 ml. of benzene on a steam bath for 40 hr. The solution was decanted from the black tar that had formed and subjected to chromatography over a column of 50 g. of alumina. After removal of excess unreacted dimethoxypyrimidine by elution from alumina with benzene, the material eluted by the first 50 ml. of benzene containing 0.5% alcohol was discarded. The nucleoside was obtained from the eluate of the second 50 ml. portion of benzene containing 0.5% alcohol. Evaporation of this fraction and rubbing with ether afforded approximately 0.1 g. of needles which were recrystallized from alcohol–ether, m.p. 227–229°; λ_{max}^{EtOH} 276 mμ (ε 4,800).

Anal. Calcd. for $C_{20}H_{27}N_3O_{10}$: C, 51.16; H, 5.80; N, 8.95. Found: C, 50.74; H, 6.12; N, 9.54.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-dimethylamino-β-D-glucopyranose (XXXI).—To a solution of 1.91 g. (0.5 mmole) of β-1,3,4,6-tetra-*O*-acetylglucosamine hydrochloride (XXX)[19] in 10 ml. of water was added 2 g. of sodium acetate. The base which was liberated was extracted with 3 × 25 ml. of chloroform. The chloroform extract was evaporated *in vacuo*. The residue was dissolved in 20 ml. of dioxane containing 3 ml. of a 35% aqueous solution of formaldehyde. Hydrogenation was carried out at atmospheric pressure using 0.5 g. of 10% palladium-on-charcoal as a catalyst. Uptake of hydrogen ceased at 2 moles at the end of 15 hr. The mixture was filtered and the filtrate evaporated *in vacuo*. The syrupy residue, smelling strongly of formaldehyde, was dissolved in water and extracted with chloroform. The chloroform layer was dried over sodium sulfate and evaporated. Addition of ethereal hydrogen chloride to an ether solution of the residue gave the crystalline hydrochloride salt, 1.32 g. (62%), m.p. 167–168°. Recrystallization from alcohol–ether gave colorless prisms, m.p. 168–170° dec., $[\alpha]^{25}D$ + 28.5° (*c* 0.6 in H_2O); p*K'*a 4.2 (in 50% MeOH).

Anal. Calcd. for $C_{16}H_{26}ClNO_9$: C, 46.67; H, 6.37; Cl, 8.61. Found: C, 46.54, 46.37; H, 6.31, 6.34; Cl, 8.54.

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