of 60 ml. of acetic acid, 60 ml. of water, and 6 ml. of sulfuric acid for 1.5 hr. The reaction mixture solution was then diluted with an equal volume of ethanol, boiled, filtered while hot, and cooled. The separated crystals were recrystallized from ethanol in colorless crystals (3.1 g.), m.p. 235–236°.

Anal. Calcd. for $C_{15}H_{10}O_7$: C, 59.60; H, 3.31. Found: C, 59.35; H, 3.45.

VIIc is soluble in sodium bicarbonate solution with effervescence, and in aqueous sodium hydroxide (2%) with a yellow color. Its ferric chloride reaction is negative.

Demethylation of VIIc. A solution of 0.5 g. of VIIc in 45 ml. of hydrochloric acid was treated, while being heated, with 15 ml. of water, and was then refluxed for 1.5 hr. The reaction mixture was diluted with water to 250 ml., filtered, and kept aside in the ice chest overnight. The yellow crystals, so obtained, were recrystallized from glacial acetic acid (*ca.* 350 mg.) m.p. 280° dec.

Anal. Calcd. for $C_{14}H_8O_7$: C, 58.33; H, 2.78. Found: C, 58.13; H, 2.88.

5-Hydroxy-2-methyl-α-pyrono-5′,6′,6,7-chromone-3′-carboxylic acid (VIId) gives a violet-brown ferric chloride reaction. It is insoluble in water, very sparingly soluble in ethanol, and soluble in sodium bicarbonate solution with yellow color.

Oxidation of VIIc *with alkaline hydrogen peroxide.* To a cooled mixture of 2 g. of VIIc in 50 ml. of aqueous sodium hydroxide (5%) at 0° was added 6 ml. of hydrogen peroxide solution (30%). After half an hour, the reaction mixture was acidified to give yellow crystals of 7-hydroxy-5-methoxycoumarin-3,6-dicarboxylic acid (IX), which upon recrystallization from dilute ethanol, melted at 218–220° dec.; yield, *ca.* 0.8 g.

Anal. Calcd. for $C_{12}H_8O_8$: C, 51.43; H, 2.85. Found: C, 51.63; H, 2.86.

IX is soluble in ethanol with a yellow-blue fluorescence, and in aqueous sodium bicarbonate solution with a strong blue fluorescence. It gives a deep wine-red color with ferric chloride.

5-Methoxy-2-methyl-5′,6′,6,7-α-pyronochromone (VIIa). (a) *From* VIIc. A mixture of 0.8 g. of VIIc, 1 g. of copper bronze, and 4 ml. of quinoline was gently boiled (oil bath) for 10 min. The cooled reaction mixture was acidified with dilute hydrochloric acid, extracted several times with chloroform, and evaporated. The residue was crystallized from ethanol or water to give 40 mg. of VIIa, m.p. 225–228° (dark melt).

Anal. Calcd. for $C_{14}H_{10}O_6$: C, 65.01; H, 3.88. Found: C, 65.09; H, 3.92.

VIIa is insoluble in aqueous sodium hydroxide (4%), but is soluble in alcoholic sodium hydroxide solution with a yellow color. Its ferric chloride reaction is negative. With potassium hydroxide pellets moistened with ethanol, it gives a yellow color.

(b) *From* IIIa. Five grams of IIIa, 10 g. of freshly fused sodium acetate, and 75 ml. of acetic anhydride were refluxed for 5 hr. The reaction mixture was poured into iced water and the brown precipitate was washed with a cold aqueous sodium hydroxide solution (1%). It was then crystallized from dilute ethanol to give VIIa, m.p. 228° (dark melt) (identified by melting point and mixed melting point); yield, *ca.* 2 g.

Demethylation of VIIa. Refluxing a mixture of 200 mg. of VIIa with 40 ml. of dilute hydrochloric acid (50%) for 1 hr., followed by cooling gave 150 mg. of yellow crystals of 5-hydroxy-2-methyl-5′,6′,6,7-α-pyronochromone (VIIe) (from ethanol), m.p. 228–230°. It gives a violet-red color with ferric chloride and a yellow complex soluble in chloroform, with uranyl acetate.[11]

Anal. Calcd. for $C_{13}H_8O_6$: C, 63.93; H, 3.28. Found: C, 63.86; H, 3.35.

Action of alkali on VIIa. *6-Aceto-7-hydroxy-5-methoxycoumarin* (VIII) (70 mg.) was obtained as colorless crystals, m.p. 223–225 dec., upon refluxing 200 mg. of VIIa with 40 ml. of aqueous sodium hydroxide (10%) for 1 hr., followed by acidification. It gives a green color with ferric chloride.

Anal. Calcd. for $C_{12}H_{10}O_5,H_2O$: C, 57.14; H, 4.76. Found: C, 57.16; H, 4.82.

5-Methoxy-α-pyrono-5′,6′,6,7-coumarin (X). A mixture of 1.5 g. of IV,[3b] 20 ml. of acetic anhydride, and 3 g. of fused sodium acetate was refluxed for 5 hr. It was then cooled, poured into iced water, and the separated solid (0.8 g.) was crystallized from ethanol in colorless crystals m.p. 270–272°.

Anal. Calcd. for $C_{13}H_8O_6$: C, 63.93; H, 3.27. Found: C, 63.96; H, 3.20.

X is almost insoluble in water, sparingly soluble in ethanol, and gives a yellow solution with alcoholic sodium hydroxide solution.

CAIRO, EGYPT
U.A.R.

[CONTRIBUTION FROM THE DIVISION OF NUCLEOPROTEIN CHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH; SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Nucleosides. VIII. Synthesis of 5-Nitrocytidine and Related Nucleosides[1]

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The mercuri reaction for pyrimidine nucleoside synthesis was extended to 5-nitrocytosine. Condensation of di(5-nitrocytosine)mercury with poly-*O*-acylglycosyl halides yielded nucleosides in which the sugar moiety was linked to the pyrimidine at position 1. Reduction of the 5-nitro group of these nucleosides (*e.g.*, 5-nitrocytidine) afforded 5-amino analogs which were ring-closed to 1-β-D-glycosyl-2-oxypurines or their corresponding 8-aza analogs. Modifications are given for the synthesis of 1-methyl- and 9-methyl-2-oxypurine and some of the intermediates used in their preparation. 2-Oxy-8-azapurine was synthesized by treatment of 5-aminocytosine with nitrous acid.

Ultraviolet absorption spectra and spectrally-determined pK_a values for key compounds in the above syntheses are given.

In a previous report[2] the synthesis of 5-nitrouridine was accomplished by direct nitration of suitably-acylated uridine followed by removal of the protecting groups. Attempts to apply these procedures to the synthesis of 5-nitrocytidine have thus far been unsuccessful.[3] It was of interest to prepare the 5-nitro analog of cytidine for testing as a potential chemotherapeutic agent, for examination as

a substrate in enzymic studies with nucleosidases and nucleoside deaminases, and as a chemical precursor for the preparation of glycosyl derivatives of 2-oxypurine. The total synthesis of 5-nitrocytidine by the mercuri process for pyrimidine nucleoside synthesis[4-6] was therefore undertaken.

As pointed out previously,[7] prior blocking of the amino function of aminooxypyrimidines is not always necessary for the preparation of mercuri derivatives suitable for nucleoside condensations. Whereas mercuri derivatives of cytosine failed to yield nucleosides when condensed with poly-*O*-acylglycosyl halides, a monochloromercuri derivative of 5-carbethoxycytosine afforded good yields of 2′,3′,5′-tri-*O*-benzoyl-5-carbethoxycytidine[7,8] when treated with tri-*O*-benzoyl-*D*-ribofuranosyl chloride.

5-Nitrocytosine[9] formed two types of mercuri derivatives (depending upon the proportion of reactants used) when treated with mercuric chloride in alkali. These were the monochloromercuri-5-nitrocytosine and the di-5-(nitrocytosine)mercury derivatives. Though either of these metal pyrimidines yielded nucleosides in subsequent reactions, more uniform results were obtained with the latter derivative.

Initial experiments were performed with tetra-*O*-acetyl-α-D-glucopyranosyl bromide which condensed smoothly with di-5-(nitrocytosine)mercury (I) in hot toluene to afford a compound whose elemental analysis was consonant with IIa (see Fig. 1).

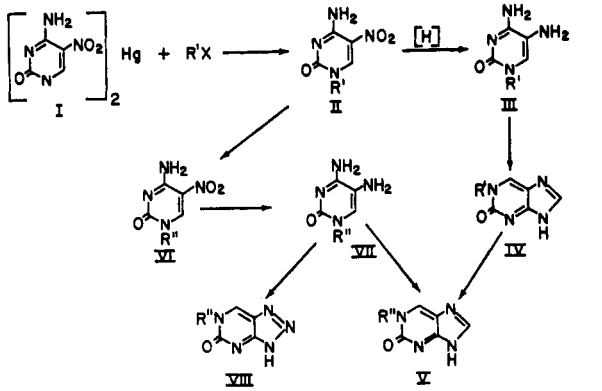

Figure 1

Evidence that the glucosyl moiety in IIa was affixed to position 1 of the pyrimidine residue was obtained from further reactions of IIa as well as from reactions in the *b* series (using ribose rather than glucose) as shown in Fig. 1. Compound IIa was reduced with palladium on charcoal in methanolic acetic acid (3:1) to IIIa, presumably the acetylated glucosyl derivative of 5-aminocytosine, which gave a positive phosphomolybdate test.[10] Treatment of IIIa (R′ = tetra-*O*-acetyl-D-glucopyranosyl) with diethoxymethyl acetate[11] yielded the 2-oxypurine derivative (IVa) which, after saponification with alcoholic ammonia, afforded the free "nucleoside" (Va).

In order to rule out the possibility of position 9 as the site of attachment of the glucosyl moiety to the purine in IVa or Va (which would be the case if glycosylation of I had occurred on the 4-amino group), the spectra of 1- and of 9-methyl-2-oxypurine were examined. Both of these methylated 2-oxypurines had been reported by Johns,[12,13] and they were prepared for this study by modifications of his procedures. Reduction of 1-methyl-5-nitrocytosine with palladium on charcoal (rather than with ferrous sulfate in aqueous ammonia)[12] produced a nearly quantitative yield of 1-methyl-5-aminocytosine. Treatment of this diamine with diethoxymethyl acetate at 120° for one hour yielded 1-methyl-2-oxypurine. 9-Methyl-2-oxypurine was prepared from 4-ethoxy-2(1*H*)pyrimidinone,[14] which was converted to 4-methylamino-2(1*H*)pyrimidinone by reaction with alcoholic methylamine in a sealed tube at 120°. The methylamino derivative was nitrated and then reduced catalytically to 2-hydroxy-4-methylamino-5-aminopyrimidine. 2-Oxy-9-methylpurine was obtained by ring-closure of the diamine with diethoxymethyl acetate.

The ultraviolet absorption spectrum of Va (see experimental section) was essentially similar to that for 1-methyl-2-oxypurine in both the acid and alkaline regions but differed markedly with that for 9-methyl-2-oxypurine (see Figs. 2 and 3). Va is almost certainly, therefore, 1-β-D-glucopyranosyl-

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. CY-3190) and from the Ann Dickler League.

(2) I. Wempen, I. Doerr, L. Kaplan, and J. J. Fox, *J. Am. Chem. Soc.*, 82, 1624 (1960).

(3) I. Wempen, I. Doerr, and J. J. Fox, unpublished data.

(4) J. J. Fox, N. Yung, J. Davoll, and G. B. Brown, *J. Am. Chem. Soc.*, 78, 2117 (1956).

(5) J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *J. Am. Chem. Soc.*, 79, 5060 (1957).

(6) J. J. Fox and I. Wempen, *Advances in Carbohydrate Chem.*, 14, 283 (1959).

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

(8) It would appear that when the basicity of the 4-amino function of cytosine is lowered by the presence of electronegative substituents (*i.e.* nitro or carbethoxy) at position 5, prior blocking of the amino group is unnecessary in order to prepare mercury derivatives adaptable in nucleoside condensations.

(9) H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, 31, 591 (1904).

(10) T. B. Johnson and C. O. Johns, *Am. Chem. J.*, 36, 545, 970 (1914).

(11) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, 79, 5238 (1957).

(12) C. O. Johns, *J. Biol. Chem.*, 11, 73 (1912).

(13) C. O. Johns, *J. Biol. Chem.*, 9, 161 (1911).

(14) G. E. Hilbert and E. F. Jansen, *J. Am. Chem. Soc.*, 57, 552 (1935). A commercial sample (Cyclo Chemical Corp.) was also employed.

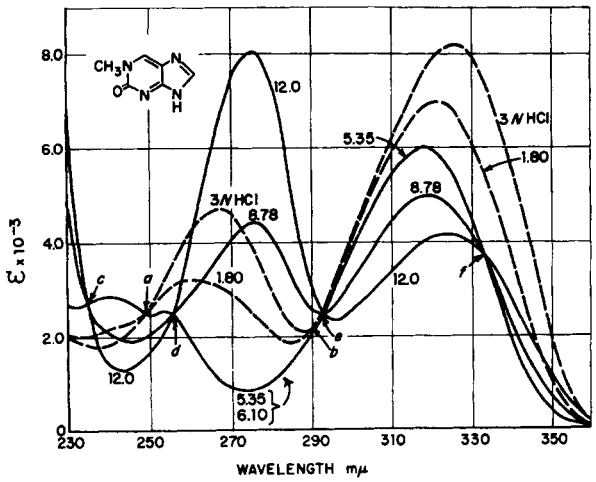

Figure 2

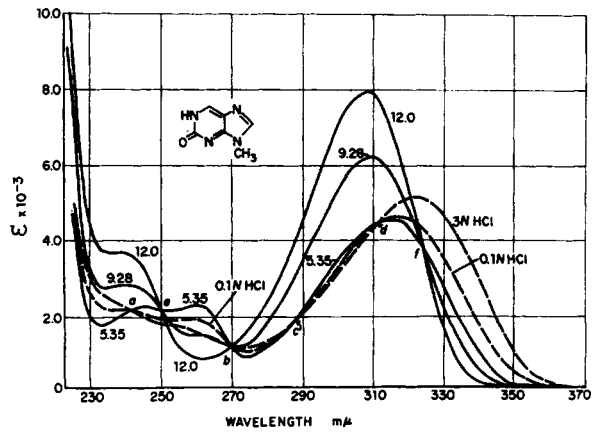

Figure 3

2-oxypurine.[15] On this basis, the assignment of the glucosyl residue to position 1 of the pyrimidine ring in structures IIa and IIIa is warranted.

Attempts to remove the acetyl groups from IIa by methanolic hydrogen chloride at room temperature resulted, unexpectedly, in the cleavage of the glycosyl bond with the formation of 5-nitrocytosine. It was found, however, that treatment of IIa with alcoholic ammonia at room temperature for two days resulted in a 25% yield of 1-β-D-glucopyranosyl-5-nitrocytosine (VIa). The ultraviolet absorption spectrum of this nucleoside was similar to that for 1-methyl-5-nitrocytosine (see Fig. 4 and Experimental). Both substances exhibited spectral shifts in both acid and alkaline regions indicative of two dissociations. This phenomenon has been noted previously in the case of 1-methyl-5-nitrocytosine[16] which, by potentio-

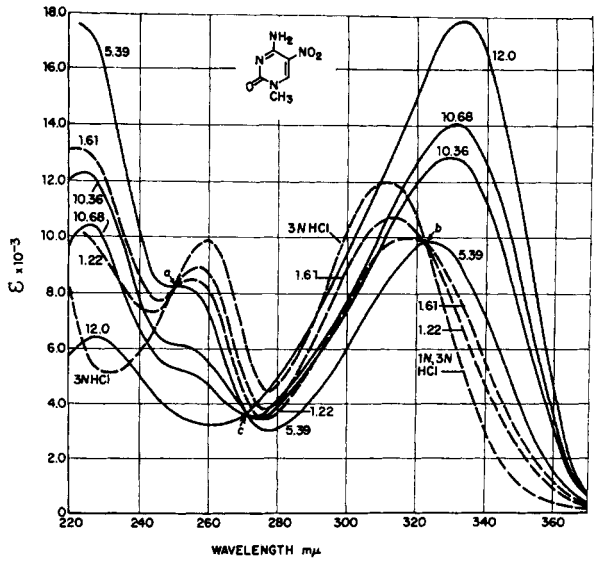

Figure 4

metric titration, was shown to possess both an acidic and basic pK_a.[17]

Condensation of 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride[18] with I yielded the nucleoside IIb in high yield. Deacylation of this blocked nucleoside with three equivalents of sodium hydroxide in 80% ethanol yielded 5-nitrocytidine (VIb, R″ = β-D-ribofuranosyl). The ultraviolet absorption spectrum of VIb was similar to that for VIa as well as for 1-methyl-5-nitrocytosine (see Figs. 4 and 5.). Hy-

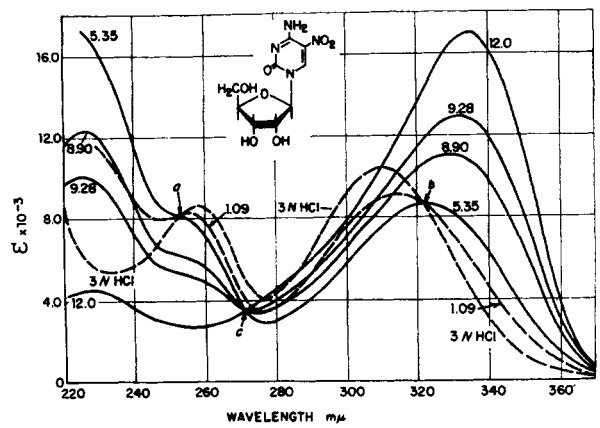

Figure 5

drogenation of VIb with palladium on charcoal catalyst afforded 5-aminocytidine (VIIb) in good yield. The sulfate of this nucleoside was identical with

(15) The *beta* configuration is assigned by analogy with condensations with other mercuripyrimidines[4-7] in which tetra-*O*-acetyl-α-D-glucopyranosyl bromide was employed. For a discussion of the stereochemical specificity of mercuri condensations see B. R. Baker in *The Chemistry and Biology of Purines*, Ciba Foundation Symposium, Little, Brown and Co., Boston, Mass., 1957, p. 120, and ref. 6, p. 336.

(16) D. J. Brown, *J. Applied Chem.*, 9, 203 (1959).

(17) The acidic pK_a was tentatively attributed to tautomerism of the 4-amino to the 4-imino form by the addition of alkali.[16]

(18) R. K. Ness, H. W. Diehl, and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, 76, 763 (1954); H. M. Kissman, C. Pidacks, and B. R. Baker, *J. Am. Chem. Soc.*, 77, 18 (1955). A commercial product of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose (California Corporation for Biochemical Research was utilized for the preparation of the chloro sugar.

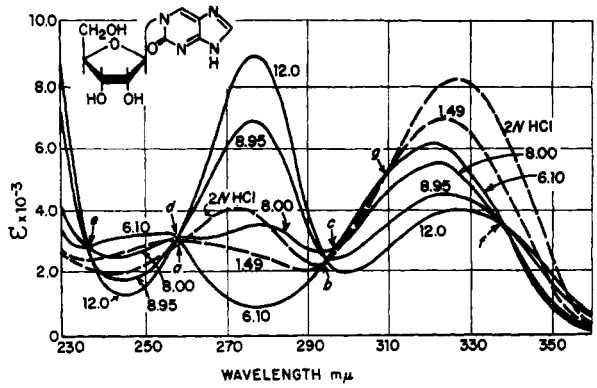

Figure 6

that reported previously by Fukuhara and Visser,[19] who synthesized it from 5-bromocytidine. This identity establishes unequivocally position 1 of IIb as the site of glycosylation in the mercuri condensation reaction and supports a similar assignment in the glucose series (IIa→Va). 1-β-D-Ribofuranosyl-5-aminocytosine (VIIb) was converted to 1-β-D-ribofuranosyl-2-oxypurine (Vb) with diethoxymethyl acetate. Treatment of VIIb with nitrous acid afforded 5-oxy-6-β-D-ribofuranosyl-1-*v*-triazolo(d)pyrimidine (VIIIb).

2-Oxy-8-azapurine, [5-oxy-1-*v*-triazolo(d)pyrimidine], previously prepared[20] by nitrous acid ring closure of 2,4,5-triaminopyrimidine, was synthesized for this study by reaction of 5-aminocytosine with nitrous acid. 5-Aminocytosine was prepared by modifications of the procedure of Johns.[12]

The apparent pK_a values determined spectrally are listed in Table I. It is noteworthy that the acidic pK_a of 1-methyl-5-nitrocytosine (10.55) is reduced appreciably (1.4 pK units) when the methyl group is replaced by a ribofuranosyl moiety (pK 9.12). Though the reason for this effect is not clear, it may, at least conceivably, be related to the effect of the sugar rest (as versus alkyl) upon the ease with which the 4-imino structure in the aglycon is formed. The base-weakening effect of ribosyl versus methyl is much less pronounced when 1-methyl-2-oxypurine and 1-β-D-ribofuranosyl-2-oxypurine are compared (about 0.25 pK units).

The absorption spectrum of 1-methyl- or 1-β-D-ribofuranosyl-2-oxypurine (see Figs. 2 and 6) at pH 6.1 (neutral species) compare favorably with that for the neutral species of 2-oxypurine[21] which would support the contention of Mason[21] that 2-oxypurine (neutral species) exists in solution in the carbonyl form. The fact that the spectrum of the monoanionic species 2-oxypurine differs from that exhibited by the anionic species of the 1-substituted-2-oxypurines (curves for pH 12, Figs. 2, 6) indicates that proton removal in the

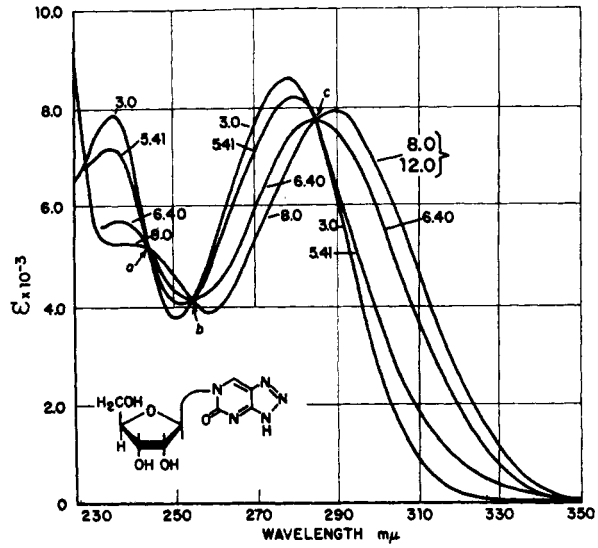

Figure 7

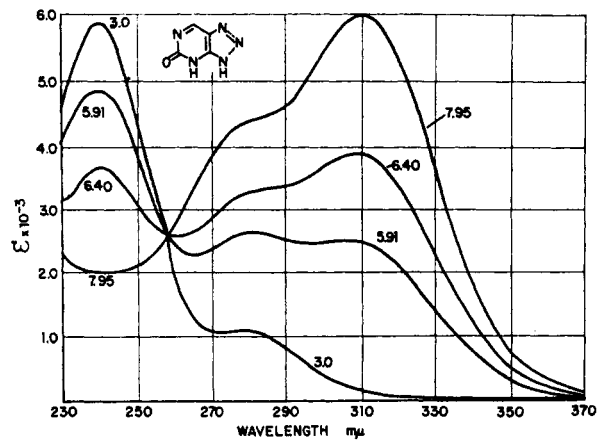

Figure 8

first acidic dissociation of 2-oxypurine occurs from the pyrimidine moiety (pK 8.43[21]). The second acidic pK_a for 2-oxypurine (dianionic species, pK_a 11.90[21]) must therefore apply to proton removal from the imidazole moiety. As expected, 2-oxy-8-azapurine (pK_a 6.18) is a stronger acid than 2-oxypurine.

A comparison of the spectrum of 1-β-D-ribofuranosyl-2-oxy-8-azapurine (VIII) to that for 2-oxy-8-azapurine (see Figs. 7 and 8) shows that they differ markedly. The neutral species (curves for pH 7.9 for the purine and pH 12 for the nucleoside) are also dissimilar. This overall dissimilarity may be a reflection of tautomeric differences in structure between the free purine and its ribosyl derivative in the undissociated forms.

EXPERIMENTAL[22]

Di-(5-nitrocytosine)mercury (I). 5-Nitrocytosine (46.8 g., 0.03 mole) was suspended in 700 ml. of hot water and

(19) T. K. Fukuhara and D. W. Visser, *J. Am. Chem. Soc.*, **77**, 2393 (1955).

(20) F. Bergmann, G. Levin, and H. Kwietny, *Arch. Biochem. Biophys.*, **80**, 318 (1959).

(21) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

(22) All melting points are uncorrected. Microanalyses by Schwarzkopf Microanalytical Labs., Woodside, N. Y.

TABLE I

SPECTRALLY DETERMINED "APPARENT" DISSOCIATION CONSTANTS[a]

Compound	Basic pK_a	Acidic pK_a
1-Methyl-5-nitrocytosine[b]	1.35	10.55
1-β-D-Ribofuranosyl-5-nitrocytosine (VIb)	1.1 (±0.1)	9.12
1-Methyl-2-oxypurine	1.8 (±0.1)	8.80
1-β-D-Ribofuranosyl-2-oxypurine (Vb)	1.5 (±0.1)	8.55
9-Methyl-2-oxypurine	<1	9.28
2-Oxy-8-azapurine	—[c]	6.18[d]
1-β-D-Ribofuranosyl-2-oxy-8-azupurine (VIIIb)	—[c]	5.90

[a] Values accurate to within 0.05 pH units unless specified otherwise. [b] Mason[21] gives potentiometrically determined pK_a values of <1 and 10.57. [c] The acidic pK_a was not determined. [d] The second acidic pK_a was not determined. Some alteration of the spectrum with time was noted above pH 10.

treated with 300 ml. of 1*N* sodium hydroxide. After the pyrimidine had dissolved, the stirred, hot solution was treated slowly with an alcoholic solution of mercuric chloride (40.5 g., 0.015 mole). A white precipitate formed immediately. The stirred mixture was heated for several more minutes after which it was cooled, filtered, and the precipitate washed repeatedly with distilled water until it was free from chloride ion. The product was washed with alcohol, then ether, and finally dried in a dessicator. The yield was quantitative (76.5 g.). The analysis indicated that it is a monohydrate.

Anal. Calcd. for $C_8H_6N_8O_6Hg.H_2O$: C, 18.18; H, 1.51; N, 21.21. Found: C, 18.05; H, 1.22; N, 21.21.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-5-nitrocytosine (IIa). Di(5-nitrocytosine)mercury (27.0 g., 0.05 mole) was suspended in 1200 ml. of toluene and the stirred mixture dried azeotropically by distillation of approximately 200 ml. of solvent. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.1 mole) was added in two portions to the stirred, refluxing mixture. After 2 hr. at reflux temperature, the stirred solution was concentrated to approximately 500 ml., cooled, and treated with approximately 2 l. of petroleum ether (b.p. 30–60°). The precipitate was separated and treated with chloroform (1 l.). After separation from some insoluble material, the chloroform solution was washed several times with 30% aqueous potassium iodide and finally with water. The chloroform layer was dried over sodium sulfate and filtered. The filtrate was concentrated to dryness and the residue dissolved in hot methanol. After cooling overnight, the product crystallized, 40.0 g., 83%, m.p. 220–222°. Recrystallization from methanol did not alter the melting point.

Anal. Calcd. for $C_{18}H_{22}N_4O_{12}$: C, 44.44; H, 4.53; N, 11.52. Found: C, 45.11; H, 4.68; N, 11.24.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-5-aminocytosine (IIIa). Compound IIa (9.7 g., 0.02 mole) was suspended in a solution of 400 ml. of methanol and 150 ml. of glacial acetic acid and treated with 5 g. of palladium on charcoal (5%). The mixture was shaken under one atmosphere of hydrogen at room temperature for 23 min. during which time an almost theoretical uptake of hydrogen was noted. The mixture was filtered through a Celite pad and concentrated to near dryness. The residual acetic acid was removed by azeotropic distillation with toluene. The residue was dissolved in hot methanol and placed in the refrigerator overnight after which product crystallized (8.3 g.). One recrystallization from a large volume of hot ethanol afforded analytically pure material, m.p. 274–275°.

Anal. Calcd. for $C_{18}H_{24}N_4O_{10}$: C, 47.37; H, 5.27; N, 12.28. Found: C, 47.63; H, 5.22; N, 12.39.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-2-oxypurine (IVa). The 5-amino nucleoside derivative (IIIa) (5.46 g.) was refluxed for 1 hr. in 25 ml. of diethoxymethyl acetate. After removal of the solvent *in vacuo*, the residue was dissolved in methanol, filtered, and cooled. Crystalline product, 3.9 g., was obtained, m.p. 284–285°. An additional 0.6 g. was obtained from the mother liquor. Total yield, 78%.

Anal. Calcd. for $C_{19}H_{24}N_4O_{10}$: C, 48.92; H, 4.72; N, 12.02. Found: C, 49.09; H, 4.56; N, 11.97.

1-(β-D-Glucopyranosyl)-2-oxypurine (Va). The blocked purine nucleoside (IVa, 4.7 g.) was dissolved in a small amount of methanol and treated with approximately 100 ml. of alcoholic ammonia (previously saturated at 0°). The flask was securely stoppered and the reaction allowed to stand at room temperature for 1 day. The solvent was removed and the residue dissolved in hot ethanol with a few drops of water to effect solubility. After the addition of charcoal, the solution was filtered and the filtrate cooled overnight. Crystalline material (needles) was obtained, 2.6 g., m.p. 285–290°. One recrystallization from methanol afforded pure material, m.p., darkens at 285° and becomes black at 295°. $[\alpha]_D^{25}$, +57° (*c*, 0.7 g., H_2O).

Anal. Calcd. for $C_{11}H_{14}N_4O_6$: C, 44.29; H, 4.70; N, 18.79. Found: C, 44.68; H, 4.96; N, 18.60.

Spectral properties: As with 1-methyl-2-oxypurine, Va exhibited two dissociations in the pH region of 3*N* hydrochloric acid to pH 13. The basic dissociation showed two isosbestic points at 254 and ~300 mμ. In 3*N* HCl: maxima at 272 and 330 mμ, minima at 230 and 293 mμ. At pH 5.0 (neutral species): maxima at 245, 253, and 323 mμ, minima at 250 and 277 mμ. At pH 13.0 (anionic species): maxima at 275 and 330 mμ, minima at 241 and 297 mμ. Isosbestic points for the acidic dissociation (pH 5–13) at 235, 256, 293, and 340 mμ.

1-(β-D-Glucopyranosyl)-5-nitrocytosine (VIa). Three grams of the acetylated nucleoside (IIa) in 250 ml. of alcoholic ammonia (saturated at 0°) was securely stoppered and shaken at room temperature for 1 hr. The solution was allowed to remain at room temperature for 3 days after which it was filtered from some insoluble material and the filtrate concentrated to dryness. The residue was dissolved in hot methanol, filtered, and cooled, 0.8 g., 42%. One recrystallization from 30 ml. of hot ethanol (with the addition of several drops of water to solubilize) afforded pure product, m.p. 243–245°.

Anal. Calcd. for $C_{10}H_{14}N_4O_8$: C, 37.33; H, 4.40; N, 17.62. Found: C, 37.64; H, 4.33; N, 17.24.

Ultraviolet light absorption properties resemble those for 1-methyl-5-nitrocytosine (see Fig. 4): in 3*N* hydrochloric acid: maxima at 254 and 335 mμ, ϵ_{max} 9,780 and 10,940, respectively; minima at 230 and 274 mμ, ϵ_{min} 5940 and 4510 respectively: at pH 5.35: shoulder at 255 mμ, maximum at 318 mμ, ϵ_{max} 8690, minimum at 275 mμ, ϵ_{min} 3080; pH 12: maxima at 228 and 333 mμ, ϵ_{max} 5050 and 19,290, respectively, minimum at 253 mμ, ϵ_{min} 2830.

1-(Tri-O-benzoyl-β-D-ribofuranosyl)-5-nitrocytosine (IIb). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-ribose[18] (0.04 mole) was added to approximately 600 ml. of anhydrous ether and the mixture saturated at 0° with hydrogen chloride. After 3–5 days, the solution was resaturated at 0° with hydrogen chloride. After another day the solvent was removed *in vacuo* (bath temperature not exceeding 35°) to a sirup and the sirup treated with 50 ml. of anhydrous benzene. The benzene was removed *in vacuo* and the syrup again treated with benzene and the process repeated. The syrup was dissolved in anhydrous toluene. The toluene solution of the halogenose (2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride) was added in three portions at 10-min. intervals to a stirred, refluxing suspension of di(5-nitrocytosine)mercury (0.02 mole) in 200 ml. of toluene. (The suspension had previously been azeotroped by removal of 100 ml. of toluene by distillation). Approximately 5 min. after the addition of the last portion of the halogenose the refluxing, stirred mixture was almost clear. Upon removal of approximately half of the solvent by distillation using a take-off adapter, the product

started to precipitate in gelatinous form. The reaction mixture was poured into 1500 ml. of petroleum ether, cooled, and filtered. The precipitate was taken up in chloroform and filtered from approximately 1 g. of insoluble material.

The chloroform solution was washed twice with 300 ml. portions of 30% aqueous potassium iodide solution and twice with water. The chloroform layer was dried with sodium sulfate, filtered, and the filtrate evaporated to dryness. A residue was obtained which was crystallized from glacial acetic acid as fine needles. The precipitate was separated, triturated with ether, and filtered. Total yield 19.6 g., 82%, m.p. 218–219°, $[\alpha]_D^{25}$, −133° (*c*, 0.2 g., chloroform).

Anal. Calcd. for $C_{30}H_{24}N_4O_{10}$: C, 60.01; H, 3.99; N, 9.33. Found: C, 60.17; H, 4.05; N, 9.46.

5-Nitrocytidine (VIb). Six grams of the benzoylated nucleoside (IIb) was suspended in 150 ml. of 80% ethanol. Ten milliliters of 1*N* sodium hydroxide was added and the reaction flask shaken. Two more similar additions of alkali were made in the course of 30 min. After the addition of the alkali (0.03 mole) complete solution was obtained. The reaction was left to stand at room temperature for 2 hr.

The solution (*p*H 8–9) was brought to *p*H 2 by the addition of hydrochloric acid. Most of the alcohol was removed *in vacuo* and the remaining aqueous solution extracted several times with chloroform to remove the benzoic acid. The product started to precipitate out of the water layer. The water layer was concentrated to half the volume and, upon cooling, silky-white needles precipitated. The precipitate was separated and triturated several times with ethanol, then with ether, and dried. Yield 2.8 g., (86%), m.p., shrank at ~120°, began to brown at ~150°, and blackened between 175–300°, $[\alpha]_D^{25}$ −21° (*c*, 0.7 g., water).

Anal. Calcd. for $C_9H_{12}N_4O_7$: C, 37.50; H, 4.17; N, 19.40. Found: C, 38.18; H, 4.92; N, 19.13.

Conversion of 5-nitrocytidine to 5-nitrouridine. 5-Nitrocytidine was dissolved in hot water and treated with an excess of hydrochloric acid and sodium nitrite. After 24 hr. an additional amount of acid and sodium nitrite were added. After several days at room temperature, the reaction mixture was chromatographed (ascending) in water-1-butanol (86/14) (Schleicher and Schuell paper #597) and gave one spot (R_f 0.81) identical with that for 5-nitrouridine. The absorption spectral properties of the solution agreed with that for 5-nitrouridine[2]: *p*H 3; maxima at 238 and 305 mμ, minimum at 262 mμ.

5-Aminocytidine (VIIb). 5-Nitrocytidine (VIb, 3.3 g.) and 3.3 g. of palladium charcoal (5%)[23] were suspended in 300 ml. of methanol containing 5 ml. of glacial acetic acid and shaken at room temperature under one atmosphere of hydrogen. After 5 min. an almost theoretical uptake of hydrogen occurred. The reaction mixture was filtered through diatomaceous earth and the filtrate concentrated *in vacuo* to near dryness. After trituration with methanol, the white precipitate was collected (2.2 g.) and dissolved in hot methanol to which several drops of water were added to effect solubility. Upon cooling, small needles precipitated, m.p. 211–212° dec., $[\alpha]_D^{25}$ +4° (*c*, 2.7 g., water).

Anal. Calcd. for $C_9H_{14}N_4O_5$: N, 21.68. Found: N, 21.49.

Ultraviolet absorption properties: at *p*H 7.2; maxima at 222 and 298 mμ, ϵ_{max} 13,900 and 6,220, respectively; minimum at 264 mμ, ϵ_{min} 2520: in 1.0*N* hydrochloric acid; maximum at 304 mμ, ϵ_{max} 6240; minimum at 258 mμ, ϵ_{min} 1180; at *p*H 4.31; maximum at 298 mμ, ϵ_{max} 6130; minimum at 264 mμ, ϵ_{min} 2230 (ref. 19 reported at *p*H 4.3; maximum at 304 mμ, ϵ_{max} 5663).

The hydrochloride salt was obtained by bubbling hydrogen chloride into a suspension of 5-aminocytidine in methanol. A clear solution was obtained which upon cooling afforded product in almost quantitative yield. The hydrochloride was recrystallized from 95% methanol to yield fine clusters, m.p., turned brown at ~175°, blackened at ~190°, did not melt up to 320°.

Neut. equiv.: calcd. for $C_9H_{15}N_4O_5Cl$: 294. Found: 294.

The sulfate salt was prepared by adding several drops of concentrated sulfuric acid to an alcoholic solution of 5-aminocytidine in methanol. A white precipitate was obtained, m.p., darkened at 205°, decomposed at 212° (Fukuhara and Visser[19] report a similar m.p.).

1-β-D-Ribofuranosyl-2-oxypurine (Vb). Two grams of 5-aminocytidine or its hydrochloride salt (VIIb) was refluxed in 20 ml. of diethoxymethyl acetate at 120° for 3 hr. After this time, the reaction solution gave a negative phosphomolybdate test. The solvent was removed *in vacuo* and the residue was dissolved in a small volume of hot water, treated with charcoal, filtered, and cooled. A white precipitate formed which was separated and recrystallized twice from a small volume of hot water. Product was obtained in the form of fine, white rosettes, 0.95 g. (52%), m.p. 207–208°. $[\alpha]_D^{25}$ +93° (*c*, 0.7, water). For light absorption properties, see Fig. 6.

Anal. Calcd. for $C_{10}H_{12}N_4O_5$: C, 44.79; H, 4.48; N, 20.89. Found: C, 44.31; H, 4.67; N, 20.53.

5-Oxy-6-(β-D-ribofuranosyl)-1-v-triazolo(d)pyrimidine (VIIIb). 5-Aminocytidine (1.29 g., 0.005 mole) or its hydrochloride salt was dissolved in 2.5 ml. of 2*N* hydrochloric acid and treated with 0.340 g. of sodium nitrite. The reaction was maintained at approximately 0 to 5° in an ice bath during which time the reaction was stirred with a glass rod. A white precipitate formed which was triturated with a small volume of water and filtered. One gram of product was obtained (75%), $[\alpha]_D^{25}$ +50° (*c*, 0.23 g., water). For spectral properties, see Fig. 7.

Anal. Calcd. for $C_9H_{11}N_5O_5$: C, 40.01; H, 4.09; N, 26.00. Found: C, 40.21; H, 4.39; N, 25.62.

1-Methyl-5-nitrocytosine. This compound was prepared by nitration of 1-methylcytosine. 1-Methylcytosine[24] (0.26 g.) was dissolved in 1 ml. of concd. sulfuric acid and treated gradually with 0.66 ml. of fuming nitric acid (density 1.50). Some evolution of heat was noted. After 15 min. standing the solution was poured into ice-water and neutralized with concentrated ammonium hydroxide. White needles formed on chilling which were separated and recrystallized from water to afford short needles, m.p., sintered at 268°, melted at 271–273°. A mixed melting point with the product obtained by the alkylation of 5-nitrocytosine according to Johns[12] was not depressed. (See Fig. 4 for spectral properties.)

1-Methyl-5-aminocytosine. Four grams of 1-methyl-5-nitrocytosine and 2.0 g. of palladium-charcoal[23] were suspended 450 ml. of water and shaken at room temperature under 1 atm. of hydrogen. After consumption of an almost quantitative amount of the gas, the reaction was removed and filtered hot from catalyst. The filtrate was concentrated to 200 ml. *in vacuo* and filtered from 0.4 g. insoluble material. The filtrate was cooled and pale-yellow prisms precipitated. One recrystallization from hot ethanol to which water had been added to effect solubility afforded pale yellow needles, m.p., turned brown at ~180° and decomposed above 220° (essentially similar melting point properties were reported by Johns[12] and by Brown[25]). A phosphomolybdate test was positive.

Light absorption properties: in 3*N* hydrochloric acid: maximum at 290 mμ, minimum at 247.5 mμ; in 0.01*N* hydrochloric acid, maxima at 217 and 312 mμ, minimum at 260 mμ; at *p*H 7; maximum at 296 mμ, minimum at 262 mμ.

4-Methylamino-2(1H)-pyrimidinone. 4-Ethoxy-2(1*H*)pyrimidinone[14] (2.0 g.) and 40 ml. of 30% ethanolic methylamine were heated in a sealed tube for 12 hr. at 120°. After cooling

(23) Purchased from Baker and Co., Newark, N. J.

(24) G. E. Hilbert, *J. Am. Chem. Soc.*, 56, 190 (1934); E. H. Flynn, J. W. Hinman, E. L. Caron, and D. O. Woolf, *J. Am. Chem. Soc.*, 75, 5871 (1953); G. W. Kenner, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 855 (1955).

(25) D. J. Brown, *J. Applied Chem.*, 5, 358 (1955).

and opening the tube, a white precipitate was separated and recrystallized from dilute ethanol, 1.4 g., m.p. ~270° dec. Johns,[13] who prepared this compound from 2-ethylmercapto-4-chloropyrimidine, reported a similar melting point, while Brown[25] reported ~275–278° dec. for this compound prepared from 2-mercapto-4-methylaminopyrimidine.

4-Methylamino-5-amino-2(1H)-pyrimidinone. 4-Methylamino-5-nitro-2(1*H*)pyrimidinone (obtained by nitration of the above compound according to Johns[13]) was suspended in water with half its weight of palladium-charcoal and shaken with hydrogen (1 atm.) until the theoretical uptake of the gas was observed. After filtration from catalyst and concentration of the filtrate to near dryness, precipitation of product occurred (45% yield), m.p. decomposition with prior browning at 220°. Johns[13] reported 225° (dec. eff.) and Brown[25] gave above 220° dec. The compound gave a positive phosphomolybdate test.

2-Oxy-9-methylpurine. The above methylamino pyrimidine (0.45 g.) was refluxed in 5 ml. of diethoxymethyl acetate for 2 hr. after which the mixture was cooled, filtered, and the tan precipitate recrystallized from 3–5 ml. of hot water to which 1 drop of concentrated ammonium hydroxide had been added. Upon cooling, 0.25 g. of needles was obtained. m.p. darkening at ~250°, melting with decomposition to a red liquid at 305–306° (Johns[13] reported browning at ~290°, melting with decomposition and effervescence at ~310°). The product gave a negative test with phosphomolybdate. For spectral properties see Fig. 3.

1-Methyl-2-oxypurine. One gram of 1-methyl-5-aminocytosine was heated with 20 ml. of diethoxymethyl acetate for 1 hr. at 120–130°. The dark amber solution was concentrated to dryness *in vacuo* and the residue dissolved in hot water. After cooling and filtration, the precipitate (0.6 g.) was recrystallized from hot water (with charcoal) to afford a white crystalline product. The melting point was indeterminate, as found previously by Johns,[12] decomposing slowly over 280°. For spectral properties, see Fig. 2. The compound gave a negative phosphomolybdate test.

5-Aminocytosine. Reduction of 5-nitrocytosine (1.5 g.) was carried out in a manner similar to that employed for 4-methylamino-5-nitro-2(1*H*)pyrimidinone (*vide supra*). After separation from catalyst, the filtrate was concentrated to about 30 ml. Thin prisms separated from the cooled solution. After recrystallization from 75% ethanol, (including treatment with charcoal), fine needles were obtained, 0.9 g., m.p., turned brown at ~200° with no definite decomposition point. A similar product was obtained by the procedure of Johns.[12] A phosphomolybdate test[10] was positive.

5-Oxy-1-v-triazolo(d)pyrimidine (2-oxy-8-azapurine). 5-Aminocytosine (1.1 g.) was dissolved in 7 ml. of 2*N* hydrochloric acid and treated with 0.01 mole of sodium nitrite. A white precipitate formed almost immediately. The precipitate was triturated several times with water and filtered. The precipitate was then washed with water, alcohol, ether, and dried; melting point, turned brown at ~240° and exploded at ~256°. Bergmann *et al.*[20] reported darkening at about 250° with no decomposition even at 300°

Anal. Calcd. for $C_4H_3N_5O$: C, 35.03; H, 2.20; N, 51.09. Found: C, 34.89; H, 2.33; N, 50.87.

The product was anhydrous while that reported previously[20] had an analysis corresponding to a monohydrate.

Spectrophotometric studies. Measurements were made with a Cary recording spectrophotometer, model 11, using techniques and buffers previously described.[4, 26] The apparent pk_a values are accurate to within 0.05 *p*H units unless specified otherwise and were determined spectrophotometerically by methods utilized previously.[4, 27]

Key to figures. All the spectra listed were run in aqueous solutions at *p*H values (or normality of hydrochloric acid solutions) indicated on the curves. 0.01*N* sodium hydroxide was taken as *p*H 12.0. The italicized letters refer to isobestic points.[28]

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[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, HARVARD MEDICAL SCHOOL, AND THE MASSACHUSETTS GENERAL HOSPITAL]

2-Amino-2-deoxy-D-idose (D-Idosamine) and 2-Amino-2-deoxy-D-talose (D-Talosamine)[1]

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Ammonolysis of methyl 4,6-*O*-benzylidene-2,3-di-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside in the presence of sodium methoxide and subsequent *N*-acetylation afforded methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-idopyranoside (VI) and methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-α-D-idopyranoside. Hydrolysis of VI followed by acetylation gave 2-acetamido-3,4-di-*O*-acetyl-1,6-anhydro-2-deoxy-β-D-idopyranose identical to the compound synthesized from D-xylose. Mesylation of VI, followed by Walden inversion at C_3 and hydrolysis gave D-talosamine hydrochloride, identical to the compound synthesized from D-lyxose.

The isolation of D-talosamine from hydrolyzates of chondroitin sulfate[3] and its synthesis from D-lyxose[4] have been reported recently. The synthesis of D-talosamine described in this paper had been completed at the time of the above publications, and its report seems of interest because a different stereospecific route has been used. It is also a further example of the method used for the synthesis of D-allosamine[5] and D-gulosamine.[6]

In 1943, W. H. Myers and G. J. Robertson[7] reported briefly the action of ammonia on methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-gulopyrano-