were collected by filtration, washed with ethanol, and air-dried. The compounds were purified as follows: The dry salts were dissolved in water, treated with Darco G-60, filtered and concentrated in a vacuum desiccator. The pentose phosphates, all of which crystallized during concentration of their aqueous solutions, were recrystallized from 80% ethanol. The sirups of the hexosephosphates which did not crystallize from aqueous solution were taken up in a minimum amount of boiling 95% ethanol. Crystallization occurred as the solutions cooled to room temperature in a desiccator.

At this stage of purification the β-D-glucose 1-phosphate prepration still gave a positive qualitative test for α-D-glucose 1-phosphate when tested with potato phosphorylase.[12] Accordingly, all eight of the sugar phosphate preparations were subjected to two additional recrystallizations by the procedure previously described.

The thrice-recrystallized β-D-glucose 1-phosphate preparation gave an inconclusive test for α-anomer contamination when it was treated with potato phosphorylase. The limit of contamination was therefore estimated by an enzymatic assay in which the α-D-glucose 1-phosphate was converted to D-glucose 6-phosphate and determined spectrophotometrically by measuring the reduction of triphosphopyridine nucleotide concomitant with the oxidation of D-glucose 6-phosphate to 6-phospho-D-gluconic acid catalyzed by D-glucose 6-phosphate dehydrogenase.[13]

The reaction mixture consisted of 3 μM of dicyclohexylammonium β-D-glucopyranosylphosphoric acid (0.03 ml. of a 0.10 M solution), 0.05 ml. of a 2% solution of D-glucose 6-phosphate dehydrogenase, 0.05 ml. of a 0.1% solution of crystalline phosphoglucomutase, 0.03 ml. of 0.1 M $MgCl_2$, 0.10 ml. of a 0.5% solution of triphosphopyridine nucleotide and 0.8 ml. of 0.1 M Tris buffer, pH 7.5. During the first 6 minutes incubation at 25° the digest showed an increase in optical density at 340 mμ of 0.01 optical density unit. No further change was observed in an ensuing period of 27 minutes. Since 0.01 μM of authentic α-D-glucose 1-phosphate caused an increase in O.D. at 340 mμ of 0.06 unit by this assay method, no more than 0.0017 μM of α-D-glucose 1-phosphate was shown to be present in the 3.0 μM sample of β-D-glucose 1-phosphate. It was therefore assumed that anomeric contamination of the other preparations had been reduced to an equally low level of the order of 0.1% or less.

The cyclohexylammonium phosphate esters were dried in air at room temperature and submitted for chemical analysis.

Analytical Results

α-D-**Glucopyranosyl 1-(dicyclohexylammonium phosphate**): *Anal.* Calcd. for $C_6H_{13}O_9P(C_6H_{11}NH_2)_2$: C, 47.20; H, 8.58; N, 6.12; P, 6.77. Found: C, 47.03; H, 8.49; N, 5.83; P, 6.82.

β-D-**Glucopyranosyl 1-(dicyclohexylammonium phosphate**): *Anal.* Calcd. for $C_6H_{13}O_9P(C_6H_{11}NH_2)_2C_2H_5OH\cdot H_2O$: C, 46.00; H, 9.06; N, 5.35; P, 5.92. Found: C, 45.88; H, 8.84; N, 4.98; P, 5.91.

α-D-**Xylopyranosyl 1-(dicyclohexylammonium phosphate**): *Anal.* Calcd. for $C_5H_{11}O_8P(C_6H_{11}NH_2)_2$: C, 47.70; H, 8.72; N, 6.54; P, 7.23. Found: C, 47.32; H, 8.16; N, 6.55; P, 7.27.

β-D-**Xylopyranosyl 1-(dicyclohexylammonium phosphate**): *Anal.* Calcd. for $C_5H_{11}O_8P(C_6H_{11}NH_2)_2$: C, 47.70; H, 8.72; N, 6.54; P, 7.23. Found: C, 46.45; H, 8.39; N, 6.60; P, 7.12.

α-D-**Galactopyranosyl 1-(dicyclohexylammonium phosphate**): *Anal.* Calcd. for $C_6H_{13}O_9P(C_6H_{11}NH_2)_2$: C, 42.20; H, 8.58; N, 6.12; P, 6.77. Found: C, 47.35; H, 8.49; N, 5.96; P, 6.79.

β-D-**Galactopyranosyl 1-(dicyclohexylammonium phosphate**): *Anal.* Calcd. for $C_6H_{13}O_9P(C_6H_{11}NH_2)_2\cdot H_2O$: C, 45.30; H, 8.69; N, 5.88; P, 6.52. Found: C, 44.94; H, 8.44; N, 6.04; P, 6.66.

α-L-**Arabinopyranosyl (1-dicyclohexylammonium phosphate**): *Anal.* Calcd. for $C_5H_{11}O_8P(C_6H_{11}NH_2)_2$: C, 47.70; H, 8.72; N, 6.54; P, 7.23. Found: C, 47.35; H, 8.63; N, 6.62; P, 7.14.

β-L-**Arabinopyranosyl (1-dicyclohexylammonium phosphate**): *Anal.* Calcd. for $C_5H_{11}O_8P(C_6H_{11}NH_2)_2$: C, 47.70; H, 8.72; N, 6.54; P, 7.23. Found: C, 47.81; H, 8.80; N, 6.64; P, 7.09.

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[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION, CORNELL UNIVERSITY MEDICAL COLLEGE]

Pyrimidine Nucleosides. III. On the Syntheses of Cytidine and Related Pyrimidine Nucleosides[1]

BY JACK J. FOX, NAISHUN YUNG, IRIS WEMPEN AND IRIS L. DOERR

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Procedures are described for the synthesis of cytidine (and thus of uridine) by condensation of mercury derivatives of certain pyrimidines with tri-*O*-benzoyl-D-ribofuranosyl halides. The synthesis of 1-β-D-xylofuranosylcytosine is also described.

Metabolic studies have shown that, with the mammal, exogenously supplied uracil, thymine and cytosine are not extensively incorporated into polynucleotides; whereas cytidine, and to a lesser extent uridine and thymidine, is extensively incorporated into pentose and deoxypentose nucleic acids.[2] These studies point to the desirability of developing, for the synthesis of cytidine, methods adaptable for the incorporation of radioisotopes and for the synthesis of analogs of cytidine for study as potential chemotherapeutic agents or as metabolite antagonists.

Howard, *et al.*,[3] synthesized cytidine by the Hilbert–Jansen procedure[4] by the condensation of 2,4-diethoxypyrimidine with tri-*O*-acetyl-D-ribofuranosyl bromide. The yields, however, were quite low (0.35 g. of cytidine sulfate from 15 g. of the pyrimidine and 5 g. of halogenose). In part I of this series[5] it was shown that thymine nucleosides may be prepared in good yields *via* the condensation of a mercury derivative of thymine with poly-*O*-acylglycopyranosyl *or* poly-*O*-acylglycofuranosyl

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(2) See chapter by G. B. Brown and P. M. Roll in E. Chargaff and J. N. Davidson, "The Nucleic Acids," Vol. II, Academic Press, Inc., New York, N. Y., 1954, p. 341, for a comprehensive review of these studies.

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

(4) G. E. Hilbert and E. F. Jansen, THIS JOURNAL, **58**, 60 (1936).

(5) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, *ibid.*, **78**, 2117 (1956).

halides followed by removal of the protecting acyl groups. In this paper we report the application of this procedure to the synthesis of cytosine nucleosides, including cytidine.

Results and Discussion

Mercuri-pyrimidines.—In previous studies[5] it was shown that improved yields were obtained in condensation reactions between halogenoses (poly-*O*-acylglycosyl halides) and dithyminylmercury when the latter compound was pure. Since the mercury derivatives of pyrimidines are usually insoluble substances, it is advisable to use stoichiometric amounts of the pyrimidine, mercuric chloride and alkali in order to obtain the desired metal derivative free from impurities. *Such a product is obtained when the yield of the preparation is essentially quantitative.* Obviously, it is essential to know which particular mercury derivative of a given pyrimidine is formed (*i.e.*, a monochloromercuripyrimidine, a dipyrimidylmercury or a pyrimidylmercury) so that the appropriate stoichiometric proportions of reactants to be used may be planned accordingly. The type of mercury derivative formed must, therefore, be determined experimentally for each pyrimidine. This knowledge of the type of metal pyrimidine is also of value in the determination of the amount of halogenose to be used in the subsequent condensation reaction for nucleoside synthesis.

Initial experiments were tried with free cytosine from which a monochloromercuri derivative was prepared. However, no identifiable nucleoside derivative could be isolated from the condensation reaction with poly-*O*-acetylglycosyl halides. This approach was abandoned in favor of the blocked pyrimidine,[6] N-acetylcytosine,[7] (4-acetamido-2(1H)-pyrimidinone), which is easily prepared by acetylation of cytosine.

Unlike thymine, N-acetylcytosine does not form a di-pyrimidylmercury or a monochloromercury derivative but a product containing mercury and pyrimidine in a 1:1 ratio. With the proper proportion of reactants and when care is taken to avoid removal of the acetyl group,[8] pure N-acetylcytosinemercury (I) is obtained quantitatively.

4-Ethoxy-2(1H)-pyrimidinone,[9] on the other hand, reacts with equimolar proportions of mercuric chloride and alkali to form a monochloromercury derivative in quantitative yield.

Condensation Reactions.— When N-acetylcytosinemercury (I) is treated with one molar proportion of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (II, acetobromoglucose) in boiling toluene, little or no acetylated nucleoside is obtained. With two molar proportions of II the reaction proceeds smoothly affording III, 1-(tetra-*O*-acetyl-β-D-glucopyranosyl)-4-acetamido-2(1H)-pyrimidinone in good yield. That two equivalents of halogenose are required for the removal of the pyrimidine-bound mercury in I is in accord with the 1:1 ratio between mercury and the pyrimidine and emphasizes the necessity of knowing precisely the type of metal-pyrimidine employed in the condensation reaction.

Compound III was identical in melting point, mixed melting point and spectral behavior with the compound obtained by the acetylation of 1-β-D-glucopyranosylcytosine prepared *via* the Hilbert–Jansen procedure.[4]

Condensation of I with 2 molar proportions of 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride (IV), in refluxing xylene (see Flow Chart) affords a 52% yield of 1-(tri-*O*-benzoyl-β-D-ribofuranosyl)-4-acetamido-2(1H)-pyrimidinone (V). Treatment of V with alcoholic ammonia in a sealed tube at 100° gives synthetic cytidine (isolated as the sulfate) in 72% yield. The identity of this material with the naturally-occurring cytidine (converted to the sulfate) isolated from ribonucleic acid was established by melting point, mixed melting point, elemental analyses, optical rotations and detailed spectral behavior.

Similarly, condensation of I with tri-*O*-benzoyl-D-xylofuranosyl chloride[5] (VI) gave good yields of VII (the xylosyl analog of V). A similar product, though in somewhat lower yield, is obtained when the bromo analog of VI[5,10] is employed. Deacylation of VII with alcoholic ammonia affords 1-β-D-xylofuranosylcytosine (VIII). When treated with metaperiodate, VII consumed one mole of oxidant per mole *slowly*[11] without the liberation of formic acid, in accord with a pentofuranosyl structure. The ultraviolet absorption spectrum of VIII at various *p*H values was practically identical with that reported for cytidine,[12] which shows that the sugar moiety is linked to the 1-position of the pyrimidine ring.

The optical rotation of the dialdehyde solution resulting from the oxidation of VIII with aqueous metaperiodate was similar to the rotation of the solution resulting from similar treatment of cytidine, showing that the configuration at the glycosyl centers of VIII and VII is of the β-D-form. This conclusion was further substantiated by the results of a comparison of the molecular rotations of related nucleoside pairs (see Table I).

A somewhat similar series of reactions was carried out with mono-chloromercuri-4-ethoxypyrimidone-2 (IX). Condensation of IX in hot toluene with acetobromoglucose yields 1-(tetra-*O*-acetyl-β-D-glucopyranosyl)-4-ethoxy-2(1H)-pyrimidinone (X), which is identical with the product obtained by Hilbert[13] from 2,4-diethoxypyrimidine and II. Treatment of X with methanolic hydrogen chloride according to Hilbert's procedure gives 1-β-D-glucopyranosyluracil.

(6) It is not to be assumed that prior blocking of the amino function of aminoöxypyrimidines is always necessary for the preparation of mercury derivatives suitable for nucleoside condensations. For instance, a mercury derivative prepared directly from 5-carbethoxycytosine has been condensed with 2,3,5-tri-O-benzoyl-D-ribosyl chloride to yield the 2′,3′,5′-tri-*O*-benzoyl derivative of 5-carbethoxycytidine (J. J. Fox and N. Yung, unpublished observations).

(7) H. L. Wheeler and T. B. Johnson, *Amer. Chem. J.*, **29**, 492 (1903); D. M. Brown, A. R. Todd and S. Varadarajan, *J. Chem. Soc.*, 2384 (1956).

(8) There is no evidence of deacetylation of N-acetylcytosine in aqueous solution at 40–50° at *p*H 12 during a period of 15 minutes.

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

(10) H. G. Fletcher, Jr., *ibid.*, **75**, 2624 (1953).

(11) It has been shown previously[5] that nucleosides containing *trans*-hydroxyls in the sugar moiety require 1–2 days for the oxidation with metaperiodate, whereas ribosyl nucleosides (*e.g.*, uridine, cytidine or 1-β-D-ribofuranosylthymine) are completely oxidized to the dialdehydes within 5 minutes.

(12) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

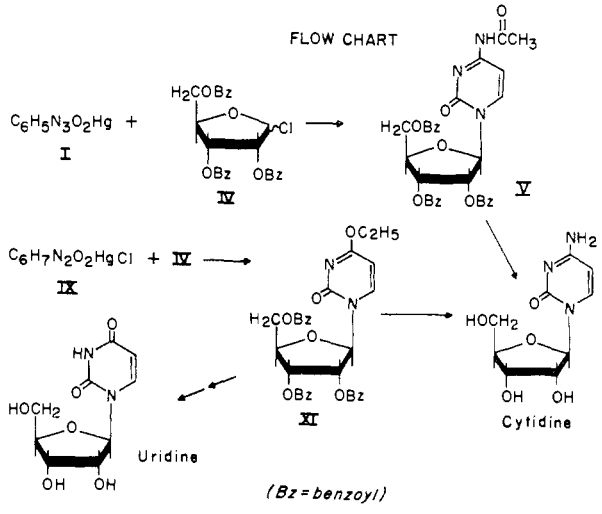
(13) G. E. Hilbert, THIS JOURNAL, **52**, 4489 (1930).

TABLE I

	$[\alpha]_D$[a]	$[M]_D$	Difference in $[M]_D$
1-β-D-Xylofuranosylcytosine (VIII)	+48°	+12,380	+12,900
1-β-D-Xylofuranosylthymine[5]	− 2	− 520	
1-β-D-Ribofuranosylcytosine (cytidine)	+31	+ 8,000	+10,580
1-β-D-Ribofuranosylthymine[5]	−10	− 2,580	
1-(Tri-*O*-benzoyl-β-D-xylofuranosyl)-thymine[5]	+58	+33,060	+80,370
1-(Tri-*O*-benzoyl-β-D-ribofuranosyl)-thymine[5]	−83	−47,310	
1-(Tri-*O*-benzoyl-β-D-xylofuranosyl)-4-acetamido-2(1H)-pyrimidinone hydrate (VII)	+70	+43,050	+77,680
1-(Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-acetamido-2(1H)-pyrimidinone (V)	−58	−34,630	

[a] The rotations of the free nucleosides were determined in distilled water; those of the benzoylated nucleoside intermediates were determined in chloroform.

Reaction of IX with tri-*O*-benzoyl-D-ribofuranosyl chloride (see Flow Chart) yields XI (the ribofuranosyl analog of X) which may be converted with alcoholic ammonia to cytidine in high yield.



That XI may also serve as an intermediate in the synthesis of uridine was demonstrated by debenzoylation of XI catalytically with sodium alkoxide in ethanol followed by acidification of the reaction product. The presence of uridine was shown by ultraviolet absorption spectra and chromatographic behavior.

General Considerations.—These syntheses show that the mercuri procedure can be extended to the preparation of nucleosides (including glycofuranosyl nucleosides) of other pyrimidines. Both methods for the preparation of cytidine from mercury-pyrimidine derivatives described herein should be adaptable to the synthesis of isotopically-labeled nucleoside. In this regard, consideration should be given to the portion of the nucleoside molecule in which the isotope label is to reside. Since the N-acetylcytosinemercury approach is rather "wasteful" of sugar, this method would be the more desirable for the preparation of cytidine bearing radioisotope in the pyrimidine moiety. The second approach, which involves the use of equimolar proportions of halogenose and IX, requires the not easily prepared 4-ethoxy-2(1H)-pyrimidinone as starting material.

By virtue of the fact that cytidine may be converted into uridine by treatment with nitrous acid,[14] these syntheses of cytidine also serve as preparative methods for the synthesis of uridine.

To date, no exceptions have been noted to the previously-made observation,[5] namely, that pyrimidine nucleosides of identical glycosylic configuration are obtained from the Hilbert method and the mercuri procedure when identical halogenoses are employed.

Experimental[15]

N-Acetylcytosinemercury (I).—Acetylcytosine[7] (3.06 g., 0.02 mole) was added to 1500 ml. of water. Sodium hydroxide (20 ml. of 1 *N* solution) was added and the mixture warmed with stirring until solution occurred. The temperature should be maintained below 50° to avoid deacetylation of starting material. The solution was filtered from trace amounts of undissolved material (when necessary). Mercuric chloride (5.43 g., 0.02 mole) dissolved in ethanol was added to the stirred solution whereupon precipitation occurred. The *neutral* solution was warmed to approximately 70°. (This treatment caused the finely-divided precipitate to become granular and settle rapidly.) After cooling to 40°, the mixture was treated with an additional 0.02 mole of alkali and the neutral solution again warmed to 70°. Upon cooling, the mixture was filtered and the product washed repeatedly with water until the filtrate was free of halide ion. After washing with alcohol and ether, the precipitate was dried, 6.9 g. (theory 7.0 g.). The product was chlorine-free and analyzed for an N-acetylcytosinemercury.

Anal. Calcd. for $C_6H_5N_3O_2Hg$: C, 20.44; H, 1.42; N, 11.90. Found: C, 20.10; H, 1.68; N, 11.64.

If the alkali is added in one portion, filtration becomes difficult and laborious. Attempts to prepare a monochloromercuri-N-acetylcytosine using a 1:1:1 proportion of N-acetylcytosine, mercuric chloride and alkali were unsuccessful. Under these conditions, a 50% yield of I was obtained and the remainder was recovered as N-acetylcytosine. When Compound I was treated with concentrated alkali, mercuric oxide precipitated.

Chloromercuri-4-ethoxy-2(1H)-pyrimidinone (IX).—4-Ethoxy-2(1H)-pyrimidinone[9] (0.05 mole) was dissolved in 500 ml. of water containing 0.05 mole of sodium hydroxide. Mercuric chloride (0.05 mole) in alcohol was added to the stirred solution whereupon a white solid precipitated. Upon filtration and washing with cold water, cold ethanol and ether, 17.8 g. (theory 18.7 g.) was obtained. This mercuri derivative may be recrystallized from ethanol. It analyzed for a monochloromercuri derivative of 4-ethoxy-2-(1H)-pyrimidinone.

Anal. Calcd. for $C_6H_7N_2O_2HgCl$: N, 7.47; Cl, 9.46. Found: N, 7.38; Cl, 9.27.

1-(Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-acetamido-2(1H)-pyrimidinone (III).—N-Acetylcytosinemercury (2.0 g., 0.0057 mole) was added to 150 ml. of toluene. The vigorously-stirred suspension was dried by azeotropic distillation of approximately one-fourth of solvent. Acetobromoglucose (II, 2.3 g., 0.0057 mole) was added to the hot, stirred mixture. After several minutes at reflux temperature, solution occurred whereupon an additional charge of 0.0057 mole of sugar was added. After a total of 30 minutes at reflux temperature, the reaction flask was cooled and the contents treated with petroleum ether (500 ml.). After cooling, the precipitate was separated by filtration and taken up in chloroform. The chloroform-insoluble residue (0.25 g.)

(14) P. A. Levene and W. A. Jacobs *Ber.*, **43**, 3159 (1910).

(15) All melting points are uncorrected unless specified otherwise. Analyses by Dr. J. F. Alicino, Metuchen, N. J.

was discarded and the filtrate washed with 30% aqueous potassium iodide followed by a wash with water and dried over sodium sulfate. Removal of the chloroform *in vacuo* left a viscous sirup which was taken up in a minimum of hot alcohol and placed in the ice-chest. After 2 days the sirup which had formed slowly crystallized, 2.2 g., m.p. 212–216°. Recrystallization from ethanol gave a pure material, 1.4 g., which melted at 150° to a thick sirup which solidified and remelted at 217–218°. Similar melting point behavior was observed with an authentic specimen of III prepared *via* the Hilbert procedure by the acetylation of 1-β-D-glucopyranosylcytosine,[4] and a mixed melting point of the two specimens gave no depression. Both specimens showed similar ultraviolet absorption properties (max. at 249 and 298 mμ; min. at 275 mμ in alcohol).

1-(Tri-*O*-benzoyl-β-D-ribofuranosyl)-4-acetamido-2(1H)-pyrimidinone (V).—1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-ribose[16] (0.01 mole) was added to 150 ml. of anhydrous ether previously saturated with hydrogen chloride at 0°. After 4 days at 5–10°, the solvent was removed *in vacuo*, and to the sirup 50 ml. of anhydrous benzene was added and removed under vacuum three times. The benzene solution of the halogenose (2,3,5-tri-*O*-benzoyl-D-ribosyl chloride, IV) was added to an azeotropically-dried and stirred mixture containing 0.005 mole of I in hot xylene. After several minutes, the mixture became homogeneous. After a total of 25 minutes at reflux temperature, the reaction was cooled and treated with petroleum ether. The precipitate was removed and taken up in chloroform and washed with 30% aqueous potassium iodide, water and dried. After removal of solvent, the residual sirup was dissolved in a minimum of hot ethyl acetate and treated with petroleum ether to incipient cloudiness and cooled. After crystallization had begun, more petroleum ether was added slowly to complete the crystallization. Upon filtration, 1.5 g. (51%) of product was obtained, m.p. 179–180°. Two recrystallizations from ethanol gave the pure material, m.p. 191–192° (cor.), $[\alpha]^{25}_{589m\mu}$, −58°, $[\alpha]^{25}_{546m\mu}$, −67°; spectral properties: maxima at 230 and 282 mμ, shoulder at 295 mμ, in ethanol.

Anal. Calcd. for $C_{32}H_{27}N_3O_9$: C, 64.32; H, 4.56; N, 7.03. Found: C, 64.33; H, 4.49; N, 6.81.

Synthesis of Cytidine (1-β-D-Ribofuranosylcytosine).—Three grams of crude V was placed in 60 ml. of ethanol previously saturated with ammonia at 0° and heated overnight in a sealed tube at 100°. The tube was opened and the amber-colored solution was concentrated *in vacuo* to a sirup to which water was added and the ethyl benzoate removed by distillation under vacuum. The residue was taken up in water and extracted several times with small portions of chloroform in order to remove benzamide. The aqueous phase was Norited and the filtrate was concentrated to a sirup. This sirup was difficultly soluble in absolute ethanol and was dissolved in a minimum of hot, 95% alcohol. Four drops of concentrated sulfuric acid was then added to the hot solution followed by absolute ethanol to incipient turbidity. Crystallization of cytidine (as the sulfate salt) occurred almost immediately; yield 1.07 g. (73%), m.p. 224–225° (dec. with efferv.). The mixed melting point with authentic cytidine sulfate prepared from naturally-occurring cytidine was 222.5–223.5°. The nucleoside was recrystallized from dilute alcohol for analyses.

Anal. Calcd. for $[C_9H_{13}N_3O_5]_2 \cdot H_2SO_4$: C, 37.00; H, 4.82; N, 14.37; S, 5.47. Found: C, 37.26; H, 4.72; N, 14.55; S, 5.29.

The ultraviolet absorption spectrum of the synthetic nucleoside at various *p*H values including the high alkaline range was essentially identical with the detailed spectrum previously reported for cytidine,[12] ϵ_{max} in 0.1 *N* HCl = 13.0 × 10³. A comparison of the optical rotational properties of synthetic cytidine sulfate with a sample prepared from naturally-occurring cytidine gave these values:

	$[\alpha]^{25}$ (in water) 589 mμ	546 mμ
Cytidine sulfate (natural)	+34°	+43°
Cytidine sulfate (synthetic)	+33	+41

Howard and co-workers[3] report $[\alpha]^{16}D$ +35° (in 1% H_2SO_4).

(16) R. K. Ness, H. W. Diehl and H. G. Fletcher, Jr., THIS JOURNAL, **76**, 763 (1954); H. M. Kissman, C. Pidacks and B. R. Baker, *ibid.*, **77**, 18 (1955).

1-(Tri-*O*-benzoyl-β-D-xylofuranosyl)-4-acetamido-2(1H)-pyrimidinone (VII) Method A.—A mixture containing 5.0 g. of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α-D-xylose[5] in 200 ml. of anhydrous ether was saturated with hydrogen chloride at 0°. After 4 days at 5° the solution was concentrated *in vacuo* to a yellow sirup to which anhydrous benzene was added and removed several times under vacuum. A solution of this halogenose (2,3,5-tri-*O*-benzoyl-D-xylosyl chloride) was added to a previously dried, stirred mixture of 1.75 g. of I in hot xylene, and after 25 minutes at reflux temperature complete solution had occurred. The solution was cooled and treated with petroleum ether. The amorphous precipitate was separated from the solvent and taken up in chloroform. The chloroform solution was washed successively with 30% aqueous potassium iodide and water and dried. Upon concentration of the chloroform solution a sirup was obtained which was taken up in 1–2 ml. of hot ethyl acetate. Ethanol was then added to a point of incipient turbidity. Upon cooling, needle clusters separated, 2.0 g. (65% based upon I), m.p. 155–158°. After two recrystallizations from ethyl acetate–ethanol, 1.6 g. of pure material was collected, m.p. 172–173° (cor.), $[\alpha]^{25}_{589\ m\mu}$ +70°, $[\alpha]^{25}_{546\ m\mu}$ +88° (*c* 1.5 in $CHCl_3$).

Anal. Calcd. for $C_{32}H_{27}N_3O_9 \cdot H_2O$: C, 62.44; H, 4.72; N, 6.83. Found: C, 62.67; H, 5.03; N, 6.50.

Method B.—As shown previously,[5] the easily accessible tetra-*O*-benzoyl-α-D-xylofuranose[10] (XII) may be used instead of the 1-*O*-acetyl-tri-*O*-benzoyl analog in these condensations. XII (5.6 g., 0.01 mole) was added to 100 ml. of anhydrous methylene dichloride and the solution saturated with hydrogen bromide at 0°. The tightly-stoppered flask remained at room temperature for 30 hr. after which the yellow solution was poured in a thin stream into vigorously stirred ice-water. The organic layer was separated quickly and washed *rapidly* with *ice-cold* bicarbonate solution to remove the acids and finally with ice-water. After drying quickly with sodium sulfate, the chloroform solution was concentrated to a sirup and treated with anhydrous benzene as in method A above. The benzene solution of the halogenose was then added to an azeotropically-dried mixture containing 0.005 mole of I in hot toluene. Method A was then followed; yield 0.7 g. (23%), m.p. 163–165° (uncor.). Light absorption properties were identical with those given by the product obtained from method A: maxima 234 and 283 mμ, shoulder at 300 mμ.

1-β-D-Xylofuranosylcytosine—Crude VII (0.70 g.) was added to 30 ml. of methanolic ammonia previously saturated at 0° and the solution heated overnight at 100° in a sealed tube. The reaction was treated in a manner similar to the synthesis of cytidine (see above). The free nucleoside crystallized from ethanol, 220 mg. (77%), m.p. 237–238°. The ultraviolet absorption spectrum was similar to that for cytidine[15] (*p*H 1, maxima at 212.5 and 280 mμ, minimum at 241 mμ; *p*H 7, maximum at 271 mμ, shoulder at 229 mμ, minimum at 250 mμ).

Anal. Calcd. for $C_9H_{13}N_3O_5$: C, 44.43; H, 5.38; N, 17.27. Found: C, 44.62; H, 5.20; N, 17.15.

When treated with metaperiodate, xylofuranosylcytosine consumed one mole of oxidant per mole without the liberation of formic acid. Two days were required for this uptake to reach constancy. For the determination of the rotations of the dialdehyde solutions resulting from oxidation with metaperiodate, both natural cytidine and xylofuranosylcytosine were compared. The results were as follows (*c* 0.7 in distilled water):

	$[\alpha]^{25}D$	$[\alpha]^{25}D$ of the dialdehyde soln.
Cytidine	+31°	+39°
1-β-D-Xylofuranosylcytosine	+48	+38

1-(Tetra-*O*-acetyl-β-D-glucopyranosyl)-4-ethoxy-2(1H)-pyrimidinone (X).—Chloromercuri-4-ethoxy-2(1H)-pyrimidinone (IX, 3.75 g., 0.01 mole) in 200 ml. of dry, hot xylene was treated with 4.1 g. of II and the mixture stirred under reflux temperature. Within a few minutes a clear solution resulted. After 40 minutes the reaction was cooled and treated with one liter of petroleum ether, filtered and the precipitate taken up in chloroform. After separation from some insoluble material, the chloroform solution was treated in the usual manner with aqueous potassium iodide, dried and concentrated to a sirup. Trituration of the sirup with alcohol–ether gave crystalline material, 1.1

g. (23%), m.p. 200–203°. One recrystallization from alcohol gave pure material, m.p. 203–204°. A mixed melting point with an authentic sample prepared according to Hilbert[13] gave 202–204°. The ultraviolet absorption spectra were also similar (maximum at 274 mμ, minimum at 243 mμ).

A small sample of X was treated with methanolic hydrogen chloride according to Hilbert, and 1-β-D-glucopyranosyluracil[13] was isolated, m.p. 199–201°. A mixed melting point with authentic material[13] gave no depression.

Synthesis of Cytidine.—1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-ribose[16] (0.02 mole) was added to 250 ml. of anhydrous ether saturated with hydrogen chloride at 0°. After 4 days at 5–10° the solvent was removed *in vacuo* as previously described, and a benzene solution of the sirupy halogenose was added to an azeotropically-dried mixture of 7.5 g. of IX in hot xylene. After 5 minutes under reflux the stirred mixture clarified. After an additional 20 minutes the reaction was cooled, treated with petroleum ether, filtered and the precipitate dissolved in chloroform. The chloroform solution was treated in the usual manner, and upon removal of the solvent a sirup was obtained which was taken up in a minimum volume of warm ethyl acetate and treated with ether. Upon cooling overnight some unreacted 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose separated out (0.3 g., m.p. 126–129°) and was removed. In the filtrate a mass of crystals appeared which, upon filtration, gave 3.7 g. (XI) of white powdery material, m.p. 96–106° (to a viscous liquid). An additional 3.2 g. of lower melting material precipitated from the mother liquor (total yield of crude material 60%). Both fractions were combined and used directly for conversion to free nucleosides.

Crude XI (1.5 g.) was treated with 50 ml. of alcoholic ammonia in a sealed tube at 100° overnight. The contents were worked up in the usual manner (*vide supra*) and gave 520 mg. of cytidine (as the sulfate), 70%. Recrystallization from ethanol–water did not raise the melting point, 222–223°, and a mixed melting point with the synthetic material prepared *via* the *N*-acetylcytosine route or with material prepared from natural cytidine gave no depression.

Crude XI (0.4 g.) in 50 ml. of ethanol was treated with 2 ml. of sodium ethoxide (1 *N*) and the solution refluxed for 1 hr. The reaction was acidified with concentrated hydrochloric acid (0.5 ml.) and filtered from some sodium chloride. The acidic filtrate was warmed to reflux temperature for 10 minutes and then concentrated to a sirup which was taken into water and extracted with ether. The ether layer was discarded and the water layer was treated with charcoal and filtered. A spectral determination of the aqueous solution showed that uridine was present (no shift in the spectrum between *p*H 1 and 7, maximum at 262 mμ; in 0.1 *N* alkali, maximum at 263 mμ). When chromatographed in butanol–water (86/14) a major spot (similar in R_f to uridine) was obtained along with a minor component.

Polarimetric Investigations.—Optical rotations were determined with a polarimetric unit model D attachment[17] to the Beckman model DU spectrophotometer calibrated with standard sucrose solutions. For the determination of the rotations of the dialdehydes produced, solutions of known concentrations were treated in the polarimetric cell with excess sodium metaperiodate. Readings were taken at frequent intervals until a constant reading was attained. The specific rotations of the dialdehydes produced were based upon the original concentrations of the nucleoside solutions.

Metaperiodate Titration Studies.—Concentrations of nucleosides ranging between 0.0015 and 0.002 mmole per ml. were treated with excess sodium metaperiodate and titrated iodometrically according to the usual procedures.[18,19] The acidity produced was determined by the method of Jackson and Hudson.[20]

Acknowledgments.—The authors are indebted to Drs. A. Bendich and G. B. Brown for helpful discussions and continued interest.

(17) Standard Polarimeter Co., New York, N. Y.

(18) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937).

(19) B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 592 (1944).

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE U. S. VITAMIN CORPORATION]

Guanamine Diuretics

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A series of guanamines of the type I has been synthesized and evaluated as oral diuretics in rats. Diuretic activity is found to be critically dependent on structural characteristics of the group R. Certain of the variants of this series are the most active guanamine diuretics so far reported.

Although the diuretic activity of formoguanamine[1-4] (I, R = H) has been known for some time, it is only recently that structural variations with enhanced diuretic activity[5] have been characterized.

(1) (a) W. L. Lipschitz and E. Stokey, *J. Pharmacol.*, **83**, 235 (1945); (b) W. L. Lipschitz and Z. Hadidian, *ibid.*, **81**, 84 (1944) (diuretic studies in animals).

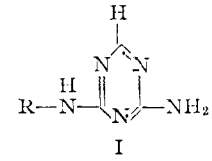
(2) (a) S. A. Freire, *Rev. brasil. biol.*, **8**, 1 (1948) [*C. A.*, **42**, 7876 (1948)]; (b) A. Turchetti, *Riforma med.*, **64**, 405 (1950) [*C. A.*, **44**, 10165 (1950)] (the mode of activity).

(3) (a) S. A. Freire, *Arquiv. biol.* (*São Paulo*), **31**, 141 (1947) [*C. A.*, **42**, 4672 (1948)]; (b) W. L. Lipschitz and E. Stokey, *J. Pharmacol. Exp. Therap.*, **92**, 131 (1948); (c) L. DeBellis, *Boll. soc. ital. biol. sper.*, **29**, 1224 (1953) [*C. A.*, **48**, 12306 (1954)] (use in humans).

(4) A. A. Kattus, E. V. Newman and J. Franklin, *Bull. Johns Hopkins Hosp.*, **89**, 1 (1951) [*C. A.*, **45**, 10401 (1951)] (use of acylated derivatives in human studies).

(5) (a) O. Ciamler and G. Bulesu, *Magyar Kém. Folyóirat*, **57**, 68 (1951) [*C. A.*, **46**, 1023 (1952)]; (b) Richter and Gedeon, *Vegyészeti Gyár Rt.*, (Hungarian Corp.), British Patent 676,024; (c) D. A. LeSher and F. E. Shideman, *J. Pharmacol. Exp. Therap.*, **116**, 38 (1956).

Maximum activity was noted in the compounds I, where R = phenyl, *p*-chlorophenyl, *p*-bromophenyl. Structures of this type have been reported[6] to yield remarkable results in cases of cardiac edema.



I

An additional variant,[7] claimed to yield diuretic activity, includes compounds of the type where R = H, and the hydrogen in the 6-position is replaced by a dialkylaminomethyl group.

(6) G. V. Austerweil, *Chemistry & Industry*, 372 (1952).

(7) V. Ettel and J. Nosek, *Chem. Listy*, **46**, 289 (1952) [*C. A.*, **47**, 3344 (1953)].