N-(**1-Benzyl-5-tetrazol-5-ylimidazol-4-yl)formamide** (1) **and 5-(4-Amino-5-benzylimidazol-5-yl)tetrazole** (2).—A suspension of **6** (0.5 g) in 1 *N* sodium hydroxide (2.1 ml) and water (3 ml) was stirred at room temperature for 18 hr. The resulting solution was filtered, and the filtrate was neutralized with 1.1 *N* hydrochloric acid (2 ml) to give **1**. When **6** (1.5 g) was treated with 2 *N* sodium hydroxide (20 ml) for 18 hr, neutralization of the solution deposited **2**. The latter was also obtained by treatment of **1** with 20% methanolic hydrogen chloride for 18 hr, or **6** with concentrated hydrochloric acid for 60 hr.

Preparation of System 6. A.—A solution of 7-benzyl-6-chloro-7*H*-purine[5a] (2.0 g) in *N*,*N*-dimethylformamide (20 ml) containing sodium azide (1.0 g) was heated at 100° for 3 hr. The mixture was evaporated to dryness under reduced pressure, and the residue was washed with water (40 ml) and recrystallized from tetrahydrofuran–petroleum ether (bp 85–105°).

B.—To a suspension of 7-benzyl-6-hydrazino-7*H*-purine[5a] (1.0 g) in water (10 ml) containing sodium nitrite (300 mg) was added 1 *N* hydrochloric acid (4.3 ml). The mixture was stirred at room temperature for 60 hr; the solid was collected by filtration, washed with water, and recrystallized as above.

C.—A solution of **1** (0.2 g) in diethoxymethyl acetate (20 ml) was heated in an oil bath at 100° for 3 hr and evaporated to dryness *in vacuo*, and the resulting residue was recrystallized as above.

Preparation of System 13.—A suspension of 2-chlorohypoxanthine[5a] (0.9 g) and sodium azide (0.4 g) in 1:1 ethanol–water (20 ml) was refluxed for 1.5 hr. The resulting solution was cooled, and the product was collected and recrystallized from water. This sample darkened but did not lose its water of hydration after drying *in vacuo* at 110°. The one-half acetate was obtained by recrystallization of the hydrate from glacial acetic acid.

Acknowledgment.—The authors are indebted to Dr. W. C. Coburn, Jr., and Mrs. Martha C. Thorpe for their aid in the interpretation of the pmr spectra and to Dr. W. J. Barrett and the members of the Analytical Chemistry Section of Southern Research Institute for the spectral and microanalytical determinations. Some of the analyses reported were performed by the Galbraith Microanalytical Laboratories, Knoxville, Tennessee.

Synthesis of 5-Substituted Pyrimidines[1]

WADY ASBUN AND S. B. BINKLEY

Department of Biochemistry, College of Medicine, University of Illinois, Chicago, Illinois

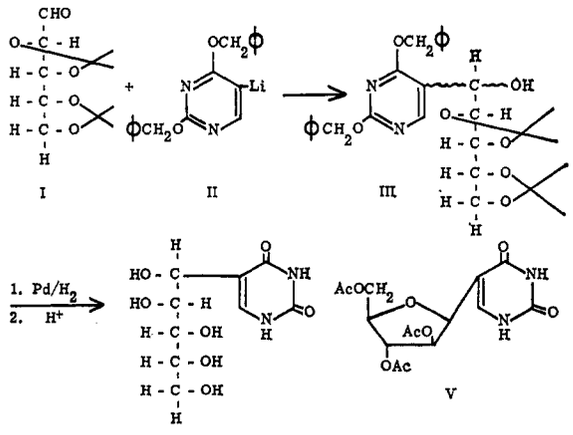
Received November 22, 1965

5-Substituted pyrimidine nucleosides were synthesized in practical yields by the reaction of diisopropylidene-*aldehydo*-pentoses with 2,4-dibenzyloxy-5-lithiopyrimidine. Thus, 5-α-D-arabinitoluracil, 5-β-D-xylofuranosyluracil, and 5-α-D-ribitoluracil were obtained. The configuration at the anomeric carbon was determined by optical rotatory dispersion studies.

The study of the chemistry and of the biological significance of pseudouridine has attracted the attention of many investigators. Particularly interesting is the fact that this nucleoside is found in soluble RNA, which is known to be an important factor in protein synthesis.[2,3] The isolation and structure elucidation of pseudouridine[4,5] was followed by its synthesis in low yields.[6] While this work was in progress, an improved procedure for the preparation of this compound was reported.[7]

The search for potential antimetabolites active in amino acid incorporation has led to the preparation of analogs in which a noncarbohydrate moiety was introduced at position 5 of the pyrimidine nucleus.[8] We report here a facile synthesis of pseudouridine analogs containing a five-carbon sugar. Diisopropylidene-*aldehydo*-D-arabinose, diisopropylidene-*aldehydo*-D-xylose, and diisopropylidene-*aldehydo*-D-ribose were condensed with 2,4-dibenzyloxy-5-lithiopyrimidine and the condensation products were converted to the desired compounds.

D-Arabinose was converted to the diethyl dithioacetal derivative.[9] The diisopropylidene diethyl dithioacetal derivative of arabinose was prepared and the mercaptal groups were cleaved by a modification of a known procedure[10,11] to give diisopropylidene-*aldehydo*-D-arabinose (I). 2,4-Dibenzyloxy-5-bromopyrimidine[12] prepared in a manner analogous to that of 2,4-diethoxy-5-bromopyrimidine[13] was converted to the pyrimidyllithium compound[14] (II) at a temperature of −70° by treating it with *n*-butyllithium. The operation was successful only when *n*-butyllithium was precooled to −70° before adding it to the reaction vessel; under this condition the solution became a pale yellow color; an orange color[15] was observed only



5 - α - D - ARABINITOL URACIL

IV

(1) (a) Supported by Grant CY 3231 from the U. S. Public Health Service. (b) Presented at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965.

(2) (a) J. D. Smith and D. B. Dunn, *Biochem. J.*, **72**, 294 (1959); (b) D. B. Dunn, *Biochim. Biophys. Acta*, **34**, 286 (1959).

(3) P. L. Bergquist and R. E. F. Matthews, *ibid.*, **34**, 567 (1959).

(4) W. E. Cohn, *ibid.*, **32**, 569 (1959).

(5) W. E. Cohn, *J. Biol. Chem.*, **235**, 1488 (1960).

(6) R. Shapiro and R. W. Chambers, *J. Am. Chem. Soc.*, **83**, 3920 (1961).

(7) D. M. Brown, M. G. Burdon, and R. P. Slatcher, *Chem. Commun.*, **77** (1965).

(8) T. V. Rajkumar and S. B. Binkley, *J. Med. Chem.*, **6**, 550 (1963).

(9) E. Fischer, *Ber.*, **27**, 673 (1894).

(10) J. English, Jr., and P. H. Griswold, Jr., *J. Am. Chem. Soc.*, **67**, 2039 (1945).

(11) M. L. Wolfrom, S. M. Olin, and E. F. Evans, *ibid.*, **66**, 204 (1944).

(12) T. V. Rajkumar, Ph.D. Thesis, University of Illinois, 1963.

(13) G. E. Hilbert and E. F. Jansen, *J. Am. Chem. Soc.*, **56**, 134 (1934).

(14) B. W. Langley, *ibid.*, **78**, 2136 (1956).

(15) T. L. V. Ulbricht, *Tetrahedron*, **6**, 225 (1959).

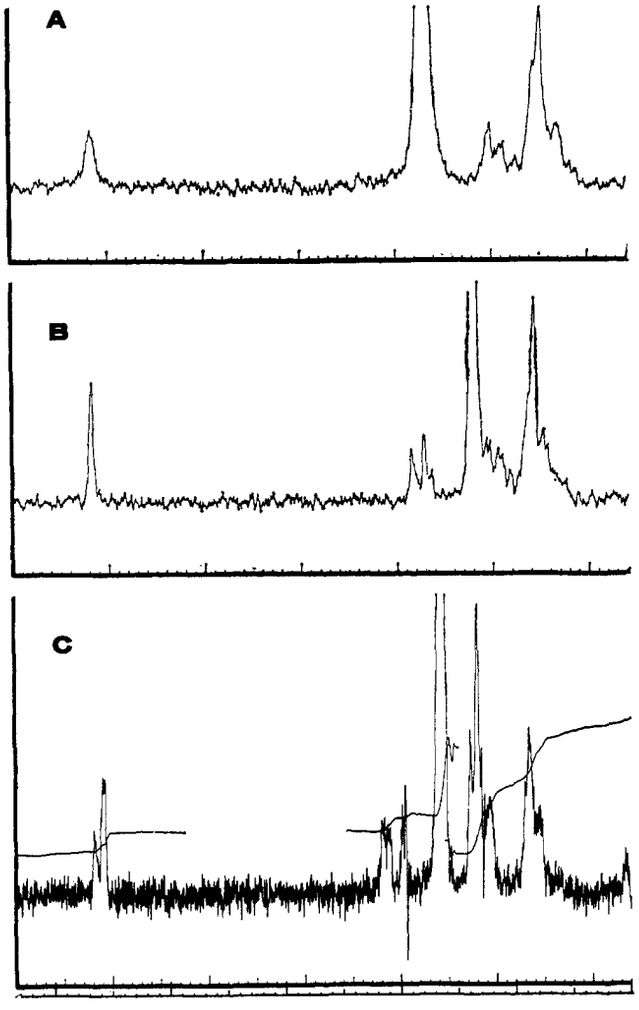


Figure 1.—The nmr spectra of 5-α-D-arabinitoluracil measured at room temperature (A); of the same compound at 90° (B); and of 5-β-D-xylofuranosyluracil taken at room temperature (C).

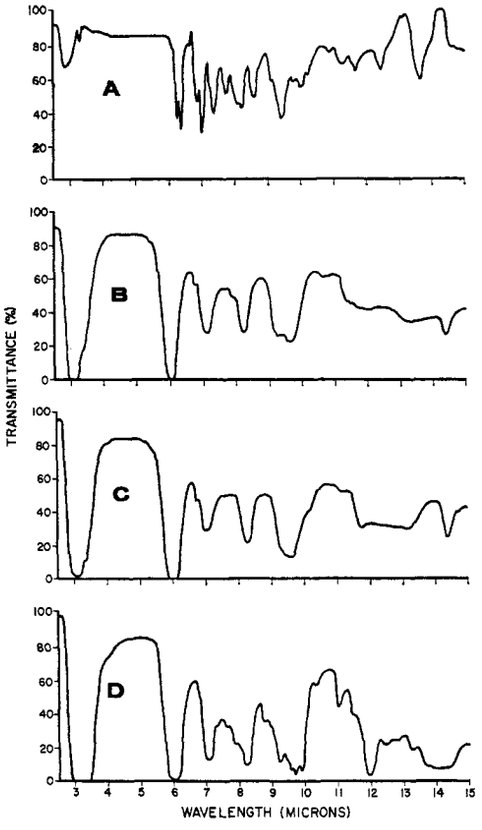


Figure 2.—Infrared spectra of β-2,4-dibenzyloxy-5-(2′,3′,4′,5′-diisopropylidene)xylitoluracil (A); of 5-α-D-arabinitoluracil (B); of 5-β-D-xylofuranosyluracil (C); and of 5-α-D-ribitoluracil (D).

when *n*-butyllithium was not precooled. Condensation of 2,4-dibenzyloxy-5-lithiopyrimidine (II) with diisopropylidine-*aldehydo*-D-arabinose yielded, after chromatography on alumina, a material which resisted all attempts at crystallization, and it is assumed to be mainly a mixture of α- and β-2,4-dibenzyloxy-5-(2′,3′,4′,5′-diisopropylidene)arabinitoluracil (III). The protective benzyl substituents were removed by catalytic hydrogenation and the isopropylidene groups by mild acid hydrolysis. Crystallization from methanol–ethanol yielded 5-α-D-arabinitoluracil (IV) in 18.5% yield; the nmr spectra at 90° showed the C_1' hydrogen at τ 5.28 (doublet, J = 7 cps); this signal was hidden behind the HDO peak when the nmr spectra was taken at room temperature. A similar situation was observed with pseudouridine.[5] Acetylation of the mother liquor and chromatography on alumina gave tri-*O*-acetyl-β-D-arabinofuranosyluracil (V) in 1.5% yield.

The presence of the furanose ring in this compound was determined by the method of Viscontini.[16] Periodate oxidation of the free nucleoside, followed by sodium borohydride reduction and acid hydrolysis, gave glycerine. The nmr spectra showed a signal at τ 5.74, corresponding to the methylene of the 5′-CH_2OCOCH_3 group, thus suggesting that the 5′-methylene of the carbohydrate is not involved in ring formation. Therefore the ring is most likely in the furanose form. It is known that the nmr signal for this group is not visible in the spectra when it is part of the ring.[17]

During the course of this work it was observed that the 5-substituted uracil nucleosides having an open chain gave a yellow spot when the paper chromatograms were sprayed with sodium periodate and benzidene solutions,[16] whereas compounds in which the sugar moiety was in the ring form gave white spots. In the first case sodium periodate cleaves the sugar chain to give 5-formyluracil which reacts with benzidene to form a Schiff base having a yellow color. A similar reaction was reported to take place when 5-formyluracil reacts with dianisidine to give a yellow imine.[18] Furthermore, 5-α-D-arabinitoluracil after periodate oxidation had an ultraviolet absorption peak at 275 mμ, the same as reported for 5-formyluracil.[18]

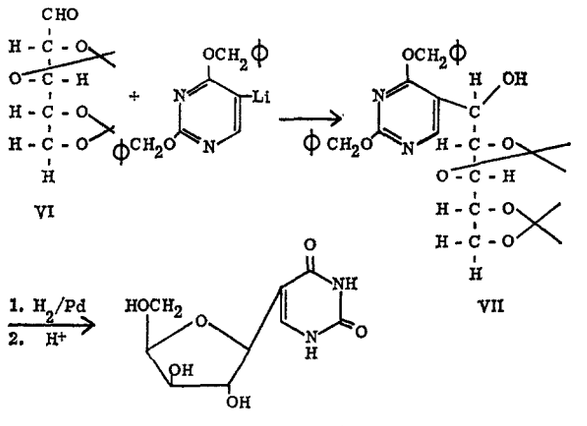
Diisopropylidene-*aldehydo*-D-xylose (VI) was prepared by a sequence of reactions similar to the ones used for arabinose. The reaction of the xylose derivative with 2,4-dibenzyloxy-5-lithiopyrimidine gave a mixture. Chromatography on alumina yielded a pale yellow gummy material which crystallized overnight to give β-2,4-dibenzyloxy-5-(2′,3′,4′,5′-diisopropylidene)xylitoluracil in 29.5% yield (VII). Cleavage of the protective groups and crystallization from methanol gave 5-β-D-xylofuranosyluracil (VIII) in 78% yield (23% from diisopropylidene-*aldehydo*-D-xylose). The nmr spectra showed a signal for the 5′-hydroxy-

(16) M. Viscontini, D. Hoch, and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).

(17) R. W. Lenz, *J. Polymer Sci.*, **51**, 247 (1961).

(18) R. E. Cline, R. M. Fink, and K. Fink, *J. Am. Chem. Soc.*, **81**, 2521 (1959).

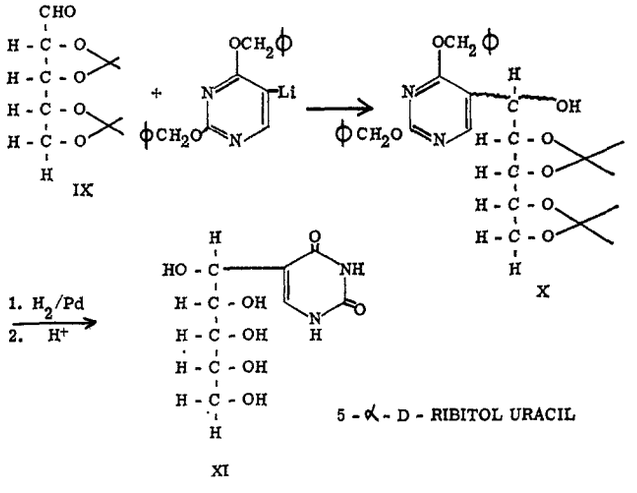
methylene group at about τ 6.15, thus indicating that the 5′-methylene group of the carbohydrate is not involved in ring formation.[17] Confirmation of the ring size was obtained by the method of Viscontini[16] as described above. Ring closure therefore takes place between carbons 1′ and 4′ of the xylitol molecule. Chromatography of the mother liquor on paper revealed the presence of 5-xylitoluracil as a minor component.



VIII

See Figures 1–3 for nmr, infrared, and ultraviolet spectra.

The reaction of diisopropylidene-aldehydo-D-ribose (IX) with 2,4-dibenzyloxy-5-lithiopyrimidine afforded, after purification and cleavage of the protective groups, 5-α-D-ribitoluracil (XI) in 10.3% yield.



XI

The configuration at the anomeric carbon was determined on the basis of optical rotatory dispersion studies.[19] It has been shown that pyrimidine N^1-β-D-nucleosides give positive Cotton effects and the anomers negative Cotton effects.[20] The same holds true for pyrimidine N^3-nucleosides.[21] These compounds do not obey Hudson's isorotation rules,[22] which fact indicates that of the two anomeric D-

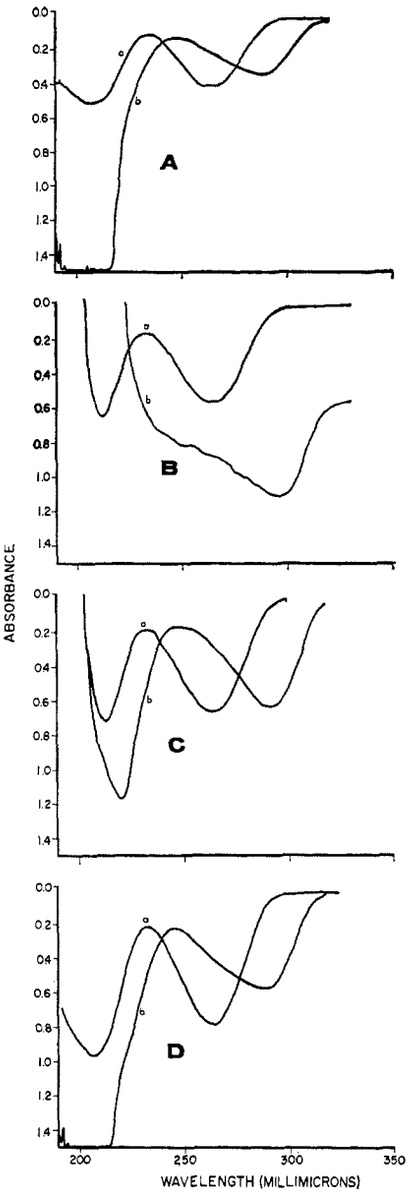


Figure 3.—Ultraviolet absorption spectra measured at pH 7 (a) and at pH 14 (b) of 5-α-D-arabinitoluracil (A); of tri-*O*-acetyl-β-D-arabinofuranosyluracil (B); of 5-β-D-xylofuranosyluracil (C); and of 5-α-D-ribitoluracil (D).

glycosides the more dextrorotatory is the α isomer. Pseudouridine C, having a β configuration,[23] presents a negative Cotton effect ($[\phi]_{255}$ −5120°, $[\phi]_{230}$ −356°). 5-D-Xylofuranosyluracil (VIII) also gives a negative ORD curve ($[\phi]_{256}$ −23,036°, $[\phi]_{230}$ +1030°); therefore it has a β configuration. On the other hand 5-D-arabinitoluracil (IV) presents a positive Cotton effect ($[\phi]_{280}$ +2180°, $[\phi]_{223}$ +1735°), the same as 5-ribitoluracil ($[\phi]_{256}$ + 10,561°), suggesting an α configuration for both of these compounds. It is apparent that 5-pyrimidine nucleosides differ in their ORD behavior from N^1- and N^3-pyrimidine nucleosides.

In the case of 5-β-pyrimidine nucleosides the mild acidic conditions necessary to cleave the protective isopropylidene groups causes simultaneous ring closure of the sugar chain; however, this reaction does not affect the open chain of 5-α-pyrimidine nucleosides even with stronger acidic conditions and prolonged

(19) We thank Professors Takeru Higuchi and Edward Kremers of the University of Wisconsin for the ORD determinations.

(20) T. L. V. Ulbricht, J. P. Jennings, P. M. Scopes, and W. Klyne, *Tetrahedron Letters*, 695 (1964).

(21) T. R. Emerson and T. L. V. Ulbricht, *Chem. Ind.* (London), **26**, 2129 (1964).

(22) C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

(23) A. M. Michelson and W. E. Cohn, *Biochemistry*, **1**, 490 (1962).

reaction times. This finding supports the configurations assigned to these compounds on the basis of optical rotatory dispersion.

Experimental Section

Melting points were taken on a Melt-Temp unit and are corrected. Infrared spectra were determined on a Perkin-Elmer Infracord spectrophotometer. Ultraviolet absorption spectra were obtained on a Perkin-Elmer recording spectrometer. Rotations were in 0.1 *N* aqueous sodium hydroxide solution, unless otherwise stated, and taken with a Rudolph polarimeter. Values of $[\alpha]_D$ have been approximated to the nearest degree. Nuclear magnetic resonance spectra were determined at 60 Mc on a Varian Associates A-60 recording spectrometer. Optical rotatory dispersion was determined at the University of Wisconsin, at 25° (*c* 0.006, warer). Paper chromatography was conducted by the descending technique on Whatman No. 1 paper in methanol–ammonium hydroxide solution (99:1, v/v) with indication by ultraviolet light and the sodium metaperiodate test.[16] Thin layer chromatography was used after each condensation reaction prior to the application of column chromatography on alumina. It was carried out on 0.25-mm-thick layers of Whatman silica gel SG_{41} on plate glass using chloroform as solvent. The spots were located by means of a Mineralight ultraviolet lamp and by spraying with "chromic acid" mixture to make visible the nonultraviolet absorbing spots.

Ether and tetrahydrofuran were distilled from lithium aluminum hydride and stored over sodium wire. The experiments with organolithium compounds were performed in an atmosphere of argon. *n*-Butyllithium was prepared by the method of Gilman[24] and it was estimated by differential titration.[25]

Diisopropylidene-*aldehydo*-D-arabinose (I).—D-Arabinose diethyl mercaptal (16.9 g) was treated with acetone (300 ml) and sulfuric acid (12 ml); after stirring at room temperature for 2.5 hr, the solution was cooled and neutralized with dry ammonia; the inorganic precipitate was removed by filtration; and the filtrate was evaporated to dryness to yield a yellow gummy like material (28 g). The mercaptal group was cleaved as follows. To a three-necked flask containing a solution of mercuric chloride (60 g) in acetone (200 ml), cadmium carbonate (33 g), and water (15 ml) was added diisopropylidene-D-arabinose diethyl mercaptal in acetone (200 ml). The flask was warmed in a water bath at 40° before and during the reaction. After stirring for 10 min the reaction mixture became thick. It was cooled immediately in an ice bath and filtered with the aid of suction; cadmium carbonate (6 g) was added to the filtrate and the acetone was removed under vacuum at room temperature. The contents of the flask were extracted with warm chloroform, and the chloroform solution was washed with 10% aqueous potassium iodide solution (three 50-ml portions), followed by water; then it was dried over anhydrous sodium sulfate and evaporated to dryness to give a gummy material (10.3 g). The product was purified by distillation at 0.9-mm pressure, the fraction boiling at 86° corresponding to the diisopropylidene *aldehydo* compound (6.072 g, 59% yield from arabinose diethyl mercaptal, 19.5% yield from arabinose): $[\alpha]^{25}D$ +2.73° (*c* 0.6, chloroform); infrared peak at 5.80 μ (strong).

5-α-D-Arabinitoluracil (IV).—In a 500-ml three-necked flask equipped with a dropping funnel, an argon inlet tube, and a low-temperature thermometer was put 2,4-dibenzyloxy-5-bromopyrimidine (14.8 g, 40 mmoles), tetrahydrofuran (170 ml), and ether (170 ml); the flask was cooled to −70° using a Dry Ice–acetone bath. *n*-Butyllithium (42 mmoles), precooled at −70°, was added in two parts while stirring, avoiding a rise of temperature above −60°. A pale yellow solution was formed after 3 min. Diisopropylidene-*aldehydo*-arabinose in ether solution (20 ml) was added in one portion and the Dry Ice–acetone bath was removed after 10 min. When the temperature reached −10°, 2 *N* sulfuric acid (50 ml) was added, and the stirring action was continued for 2 more min. The organic layer was separated and washed with water; the aqueous layers were backwashed with chloroform; the organic layers were combined and evaporated to semidryness under vacuum; and the residue was redissolved in chloroform and dried over anhydrous sodium sulfate. Evaporation of the organic solution gave a pale yellow gum-like material (22.4 g). This product was chromatographed on alumina (440 g) (Merck acid washed) and the column was eluted with benzene (400 ml), benzene–chloroform (5:5, 400 ml) and chloroform (1600 ml). The eluents were collected in 200-ml portions. Fractions 9–12 were similar on thin layer chromatography and were combined (9.987 g), dissolved in ethanol (300 ml), and reduced by hydrogenation in the presence of palladium–carbon (10%, 2 g). Hydrogenation was interrupted after 16 hr and the catalyst separated by filtration; 0.2 *N* sulfuric acid (70 ml) was added to the ethanolic solution. After stirring for 16 hr at room temperature, barium hydroxide solution was added to pH 5; the barium sulfate formed was separated by centrifugation and the supernatant solution after evaporation yielded a crystalline solid residue (3.126 g). This compound was redissolved in methanol, ethanol was added, and the solution was concentrated under vacuum until crystallization started. After cooling in the refrigerator for 20 hr, the crystalline material was filtered (1.962 g, 18.5% yield): mp 173.5–174.5; $[\alpha]^{25}D$ +95° (*c* 0.4, 0.1 *N* NaOH); $\lambda_{max}^{H_2O}$ 262 mμ (ε 4763, pH 7), 287 mμ (ε 4230, pH 12).

Anal. Calcd for $C_9H_{14}O_7N_2$: C, 41.30; H, 5.35; N, 10.69. Found: C, 41.33; H, 5.34; N, 10.79.

Tri-*O*-acetyl-β-D-arabinofuranosyluracil (V).—The mother liquor from 5-α-D-arabinitoluracil shows three ultraviolet absorbing spots on paper chromatography, R_f 0.19 (5-α-D-arabinitoluracil, yellow color with periodate test), 0.38 (5-β-D-arabinofuranosyluracil, white spot with the same test), and 0.53 (same R_f value as uracil). The mother liquor was evaporated to dryness and treated with pyridine (10 ml) and acetic anhydride (20 ml); after 24 hr ice (10 g) was added, and the mixture was extracted with chloroform (three 20-ml portions), washed with water (50 ml), dried over sodium sulfate, and evaporated to dryness to give a gummy residue (1.661 g). The product was chromatographed on alumina (30 g) (Merck acid washed), and the column was eluted with benzene, benzene–chloroform (5:5), and chloroform–methanol (96:4). Evaporation of the chloroform–methanol eluent gave a gummy material (0.868 g) which crystallized from ethyl acetate–petroleum ether mixture in granules (0.224 g). Heated in a capillary tube it softened at about 180° and it melted at 201–202°. It was recrystallized from ethyl acetate to give granules (0.046 g): mp 212°; $[\alpha]^{25}D$ −36.92° (*c* 0.46, MeOH); λ_{max}^{MeOH} 262 mμ (ε 8050, pH 7), 293 mμ (ε 13,400, pH 12).

Anal. Calcd for $C_{15}H_{18}O_9N_2$: C, 49.20; H, 4.92; N, 6.55. Found: C, 49.11; H, 5.06; N, 6.54.

Acetylation of 5-α-D-arabinitoluracil, gives a noncrystalline compound, $[\alpha]^{25}D$ +75°.

Diisopropylidene-*aldehydo*-D-xylose (VI).—D-Xylose (Pfanstiehl, 20 g) was dissolved in concentrated hydrochloric acid (20 g) and cooled to 0°, and cold ethanethiol was added (20 ml); after stirring for 10 min, the ice bath was removed and the stirring action was maintained for 5 min more. The solution was neutralized with alcoholic potassium hydroxide, the inorganic salts were filtered off, the solution was evaporated to dryness, and the mercaptal derivative was dissolved in acetone, leaving an inorganic residue of potassium chloride. Evaporation of the solvent gave D-xylose diethyl mercaptal (34.215 g) free from inorganic compounds. It is noncrystalline. By the same sequence of reactions described for D-arabinose diethylmercaptal, it was converted to diisopropylidene-*aldehydro*-D-xylose (7.043 g, 22.4% yield from xylose): $[\alpha]^{25}D$ −184.61° (*c* 0.52, chloroform); bp 79–81° (0.5 mm).

β-2,4-Dibenzyloxy-5-(2′,3′.4′,5′-diisopropylidene)xylitoluracil (VII).—Diisopropylidene-*aldehydo*-D-xylose (7.043 g, 30 mmole) was condensed with 2,4-dibenzyloxy-5-lithiopyrimidine (II, 32 mmole) using the same reaction conditions indicated above. The reaction mixture (19.9 g) was chromatographed on alumina (400 g) (Merck acid washed); the column was eluted with benzene (400 ml), benzene–chloroform (5:5, 400 ml) and chloroform (1500 ml). The eluents were collected in 200-ml volumes. Thin layer chromatography associated with infrared spectra revealed the presence of the condensed product in fractions 6 to 10. These fractions were combined and evaporated to dryness. Upon standing overnight, crystals were formed. The mixture was suspended in ether–petroleum ether mixture (5:5) and filtered to give compound VII (3.455 g), mp 107–109°; recrystallization from ethyl acetate–petroleum ether gave needles, mp 112–113°. The mother liquor was rechromatographed on alumina to give an additional amount of the same crystalline material (1.162 g, total yield 29.5%). In successive preparations

(24) H. Gilman, J. A. Beel, C. G. Brannen, M. W. Bullock, G. E. Dunn, and L. S. Miller, *J. Am. Chem. Soc.*, **71**, 1499 (1949).

(25) H. Gilman and A. H. Heubein, *ibid.*, **66**, 1515 (1944).

this compound was crystallized directly by dissolving the reaction mixture in petroleum ether and allowing it to stand overnight at room temperatures; yields in both cases were comparable; $[\alpha]^{25}D$ −42.20° (*c* 0.40, chloroform).

Anal. Calcd for $C_{29}H_{34}O_7N_2$: C, 66.30; H, 6.51; N, 5.37. Found: C, 66.50; H, 6.53; N, 5.26.

5-β-D-Xylofuranosyluracil (VIII).—The protective groups of compound VII were cleaved using exactly the same reaction conditions employed for the preparation of 5-α-D-arabinitoluracil. Fractional crystallization from methanol–ethanol afforded 5-β-D-xylofuranosyluracil (1.702 g, 78% yield, 23% yield from diisopropylidene-*aldehydo*-D-xylose): mp 202–204°; $[\alpha]^{25}D$ −27.77° (*c* 0.54, 0.1 *N* NaOH); λ_{max}^{MeOH} 262 mμ (ε 6720, pH 7), 289 mμ (ε 6610, pH 12).

Anal. Calcd for $C_9H_{12}O_6N_2$: C, 44.30; H, 4.92; N, 11.48. Found: C, 44.04; H, 5.07; N, 11.44.

5-α-D-Ribitoluracil (XI).—Diisopropylidene-*aldehydo*-D-ribose (IX, 3.317 g, 14.5 mmoles), prepared by the same sequence of reactions shown for compound VI, was condensed with 2,4-dibenzyloxy-5-lithiopyrimidine (16 mmoles). The condensed product was chromatographed on alumina (Merck acid washed, 200 g), and the column was eluted with benzene (200 ml), benzene–chloroform (5:5, 200 ml), and chloroform (600 ml). The desired compound was eluted with chloroform. After cleaving the protective groups and fractional crystallization from ethanol, 5-α-D-ribitoluracil was obtained in 10.3% yield: mp 195–197°; $[\alpha]^{25}D$ −19.7° (*c* 0.37, 0.1 *N* NaOH); $\lambda_{max}^{H_2O}$ 262 mμ (ε 5610, pH 7), 287 mμ (ε 3740, pH 12).

Anal. Calcd for $C_9H_{14}O_7N_2$: C, 41.30; H, 5.35; N, 10.69. Found: C, 41.50; H, 5.61; N, 10.16.

Micromethod for the Determination of Ring Size of Sugars in Nucleosides.[16]—The same procedure was used to determine the ring size of 5-β-D-arabinofuranosyluracil and 5-β-D-xylofuranosyluracil. The compound (40 μmoles) and sodium periodate (80 μmoles) were dissolved in 0.8 ml of water. After 4 hr, 16 mg of sodium borohydride in 0.8 ml of water was added and the solution was allowed to stand overnight. The solution was acidified with 0.8 ml of 2 *N* HCl and heated in a steam bath for 30 min. The solution was evaporated to semidryness under vacuum and the residue was extracted with ethanol. The ethanolic extract was concentrated and spotted on Whatman No. 1 filter paper (6 × 22 in.). Ethylene glycol and glycerol were used as reference compounds. The chromatograms were developed for 8 hr with a solvent mixture of ethyl acetate, pyridine, and water (70:20:10). The papers were dried and sprayed with a 0.5% solution of sodium periodate. After 3 min they were sprayed with a 5% solution of benzidine in a ethyl acetate–ethanol mixture (2:8). The compounds appeared as white spots against a blue background. Both sugar nucleosides gave single spots which had R_f values identical with that of glycerol.

The Acid-Catalyzed Solvolysis of Pyrimidine Nucleosides[1]

EDWARD R. GARRETT, JOACHIM K. SEYDEL, AND ALAN J. SHARPEN

College of Pharmacy, University of Florida, Gainesville, Florida 32603

Received March 7, 1966

The kinetics of acid-catalyzed solvolysis of various pyrimidine nucleosides have been studied at various temperatures and specific acid catalysis was established for all compounds except 5-hydroxyuridine which exhibited pH-independent solvolysis. Acid stability is markedly affected by the number of hydroxyl groups in the sugar ring with complete hydroxylation of the sugar preventing degradation under the conditions used. The stereochemistry of the 3′-hydroxyl exerted a significant effect on the rate of solvolysis of 2′-deoxyribosides. Both electronegative and electropositive substituents in the 5 position of the pyrimidine ring increased the rate of acid-catalysed solvolysis relative to the unsubstituted 2′-deoxyuridine. The nucleosides solvolyzed at the glycoside link yielding the corresponding uracil and degraded sugar as products. An exception was 5-iodouridine which was transformed to uridine. The mechanism of N-glycoside hydrolysis in acid media is not clear; however, the substituent effects observed can be explained in terms of reactivity of various proposed intermediates in the degradation sequence.

The varying sensitivity of pyrimidine and purine nucleosides to hydrolysis is qualitatively well known.[2,3] It depends on both the nature of the nitrogen moiety, the nature and position of its substituents, and the structure of the sugar moiety.[4-9] Purine nucleosides are less stable to acid hydrolysis than pyrimidine nucleosides[4,5] and isocytidine is less stable than cytidine.[2] Hydrogenation of the 4,5 double bond in pyrimidine nucleosides causes an enormous increase in instability,[8b,10] a very important fact in the determination and characterization of the sugar moiety in different biological systems.

Some biological data can be interpreted by hypothesizing exchange of sugar moieties. The blocking of deoxyribonucleic acid (DNA) synthesis by 5-fluoro-2′-deoxyuridine (FDU) can be inhibited by 5-bromo-2′-deoxyuridine (BDU), thymidine, or by thymine and 5-bromouracil (BU) with the special purine nucleosides, deoxyguanosine or adenosine.[11] This could be explained by sugar transfer in biological systems since the rate of hydrolysis of purine nucleosides is known to be relatively fast.

The importance of nucleosides in biological systems has been well demonstrated by the discovery of their antitumor[12] and antiviral[13] properties. Quantitative studies on the stability of nucleosides and the effects of structure and substituents should provide insight into their metabolic transformations and are of pharmaceutical importance for the estimation of maximum stability and the inhibition of the formation of toxic side products.[14] The number of quantitative kinetic studies on nucleosides with determination of thermodynamic

(1) (a) Presented in part at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965; (b) supported by U. S. Public Health Service Grant GM-09864-03,04, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

(2) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press Inc., New York, N. Y., 1963, p 26.

(3) F. Micheel and A. Heesing, *Chem. Ber.*, **94**, 1814 (1961).

(4) C. Tamm, M. E. Hodes, and E. Chargaff, *J. Biol. Chem.*, **195**, 49 (1952).

(5) H. S. Shapiro, E. Chargaff, *Biochim. Biophys. Acta*, **23**, 451 (1951).

(6) P. A. Levene and W. A. Jacobs, *Chem. Ber.*, **44**, 1027 (1911); **42**, 2469, 2703 (1909).

(7) J. Baddiley, G. W. Kenner, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 657 (1944).

(8) (a) G. E. Hilbert and C. E. Rist, *J. Biol. Chem.*, **117**, 371 (1937); (b) G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, **52**, 4489 (1930).

(9) F. Shafizadeh, *Advan. Carbohydrate Chem.*, **13**, 24 (1958).

(10) P. A. Levene and F. B. La Forge, *Chem. Ber.*, **45**, 608 (1912).

(11) P. Langen and E. Liss, *Biochem. Z.*, **336**, 139 (1962).

(12) R. Papac, E. Jacobs, C. Wong, W. Silliphant, and D. A. Wood, *Proc. Am. Assoc. Cancer Res.*, **3**, 257 (1961).

(13) H. E. Kaufman, E. L. Martola, and C. Dohlman, *Arch. Opthalmol.*, **68**, 235 (1962).

(14) E. R. Garrett, T. Suzuki, and D. J. Weber, *J. Am. Chem. Soc.*, **86**, 4460 (1964).