zenesulfonamido)-isoquinoline as fine white crystals; yield 43%; m.p. 270° with darkening (*Anal.* Calcd. for $C_{15}H_{11}N_3O_4S$: N, 12.78; S, 9.72. Found: N, 12.9; S, 9.34).

5-Sulfanilamidoisoquinoline was obtained in 80% yield by the hydrolysis of the acetyl compound. Recrystallization gave fine white needles melting at 224° (reported, 223–224.5°[3]). Reduction of 5-(*p*-nitrobenzenesulfonamido)-isoquinoline gave only a 34% yield of crude product.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, SCHOOL OF MEDICINE AND THE LABORATORIES OF THE ALLAN HANCOCK FOUNDATION, UNIVERSITY OF SOUTHERN CALIFORNIA]

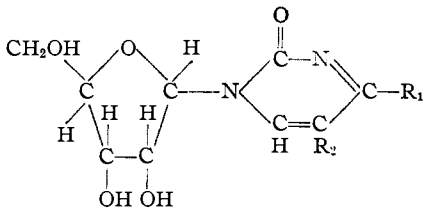
Uridine and Cytidine Derivatives[1]

BY MARTIN ROBERTS AND DONALD W. VISSER

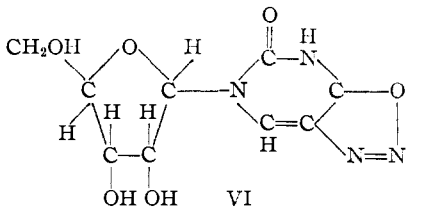
The preparation of the new compounds 5-methyluridine, 5-methylcytidine, 5-aminouridine, diazouridine and an improved method for the preparation of 5-hydroxyuridine is described. These compounds are of interest because of their structural similarity to uridine and cytidine which are utilized for nucleic acid biosynthesis.

It has been shown previously[2] that 5-chlorouridine (I) reversibly inhibits the growth response of *Neurospora* mutant, 1298, produced by uridine or cytidine. It was of interest, therefore, to prepare other derivatives of the pyrimidine nucleosides and test them in biological systems. Compounds of this type may provide a means of studying nucleic acid metabolism and may also have chemotherapeutic applications.

A method for the synthesis of 5-methylcytidine (II), 5-methyluridine (III), 5-aminouridine (IV), 5-hydroxyuridine (V) and diazouridine (VI) are reported in this paper. Results of the biological studies will be reported elsewhere.



I, R_1 = OH, R_2 = Cl
II, R_1 = NH_2, R_2 = CH_3
III, R_1 = OH, R_2 = CH_3
IV, R_1 = OH, R_2 = NH_2
V, R_1 = OH, R_2 = OH



VI

5-Methylcytosine has been reported as a constituent of nucleic acids,[3,4] and a thymine pentoside has been isolated from sponges.[5] It seemed desirable, therefore, to synthesize the related compounds, 5-methylcytidine (II) and 5-methyluridine (III), especially since these nucleosides are also structurally similar to thymidine. 5-Methylcytidine (II) was prepared from 2,4-diethoxy-5-methylpyrimidine using a modification of the procedure of Howard, *et al.*[6] Acid hydrolysis of the intermediate condensation product yielded 5-methyluridine (III).

The synthesis of 5-aminouridine (IV) was particularly desirable since the compound was not only of interest as a possible antimetabolite, but also could be used to synthesize other compounds by substitution through diazotization. Coupling with diazotized *p*-nitroaniline in the 5-position of uridine and then reduction of the resulting azo dye to the amine was a possible scheme of synthesis[7] which proved to be impractical. An orange azo dye could be separated from the other products of the reaction by means of chromatography using alumina as an absorbent, but the yield was very low. Johnson, *et al.*,[8] comment that substitution on the N_1 of pyrimidines prevents diazo compounds from coupling.

5-Aminouracil has been prepared from 5-bromouracil by heating with aqueous ammonia at 180°,[9] however, under these conditions 5-bromouridine decomposed excessively. When the temperature was lowered to 55° very little decomposition resulted and 5-aminouridine (IV) was readily isolated in good yield.

Repeated attempts to prepare 5-hydroxyuridine (V), as described by Levene and LaForge,[10] failed to give a crystalline product. A modification of the procedure, however, resulted in 5-hydroxyuridine (V) which melted 20° higher than reported by these workers.

Diazouridine (VI) was prepared from 5-aminouridine using the method of Johnson, *et al.*,[11] for the synthesis of diazouracil from aminouracil. The structure of VI is assumed to be similar to the one postulated for diazouracil.

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Experimental

5-Methylcytidine (II).—A modification of the method of Howard, *et al.*,[6] was used. Anhydrous hydrogen bromide was liquefied in a bomb tube containing 2.2 g. (0.007 mole) of tetraacetofuranosyl-D-ribose[12] until 8 ml. was obtained. The tube was sealed and warmed to 20° by immersing in water. After ten minutes the tube was cooled in a Dry Ice–acetone-bath, opened, and the hydrogen bromide allowed to boil off. About 50 ml. of dry benzene was added and the mixture lyophilized. This was repeated three times without allowing the solution to warm to room temperature. 2,4-Diethoxy-5-methylpyrimidine[13] (6.7 g., 0.036 mole) in 50 ml. of benzene was added and the mixture lyophilized to remove the benzene. The residue was heated overnight in an oil-bath at 60° to couple the sugar and pyrimidine. The unreacted 2,4-diethoxy-5-methylpyrimidine was removed by distillation at 100° (10^{-4} mm.) yielding 4.4 g. (0.24 mole). The residue was dissolved in 15 ml. of benzene and transferred to a bomb tube and the benzene removed by lyophilization. Ten ml. of methanol was added to the residue and the mixture saturated with ammonia at −20°. The tube was sealed and heated at 50° for five days. The ammonia and methanol were removed from the resulting light brown solution by distillation under reduced pressure (30°) using a water aspirator. About 50 ml. of methanol was added and the distillation repeated. Fifty ml. of absolute ethanol was added and the solution boiled with a small amount of activated charcoal. The charcoal was filtered from the hot solution and IV crystallized from the filtrate upon standing overnight at −10°. Recrystallization from 95% ethanol yielded 300 mg. (17%) of IV. An alternate method of purification used was to absorb IV on a column of Amberlite IR-120[14] and elute with 0.5 *N* ammonia water. The eluant was lyophilized and the residue was crystallized from 95% ethanol, yielding IV, m.p. 238–240°.[15] *Anal.* Calcd. for $C_{10}H_{15}N_3O_6$: C, 46.71; H, 5.84; N, 16.35. Found: C, 46.95; H, 5.76; N, 16.37.

5-Methyluridine (III).—The procedure was the same as that described for 5-methylcytidine except that after removing the excess 2,4-diethoxy-5-methylpyrimidine the residue was dissolved in 70 ml. of methanol and 30 ml. of methanol containing 27% (by weight) of hydrogen chloride. The mixture was allowed to stand at room temperature for three days. The solution was concentrated under reduced pressure (water aspirator) at 40° to a thick sirup. About 50 ml. of absolute ethanol was added and the solution was again concentrated to a thick sirup. Charcoal treatment of this hot solution removed most of the color. Ether was added until a slight precipitate formed. On cooling in the refrigerator an oil separated which crystallized slowly. The product was recrystallized from 95% ethanol. The yield was 180 mg. (10%), m.p. 175–177°. *Anal.* Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.86; H, 5.17; N, 10.90.

5-Aminouridine (IV).—Two grams (0.0062 mole) of 5-bromouridine[2] was dissolved in 20 ml. of liquid ammonia in a bomb tube. The tube was sealed and heated to 50–55° for five days. The tube was then opened and the ammonia allowed to boil off. Most of the remaining ammonia was removed at reduced pressure using a water aspirator. A minimum amount of hot water was added (about 5 ml.) and then 75 ml. of hot isopropyl alcohol. The hot solution was treated with charcoal and filtered. Upon cooling, 5-aminouridine crystallized. The compound was dissolved in a minimum amount of hot water and 3 volumes of isopropyl alcohol were added. Crystals of IV (1 g., 63%) formed on cooling, m.p. 214–216°. *Anal.* Calcd. for $C_9H_{13}N_3O_6$: C, 41.69; H, 5.06; N, 16.21. Found: C, 41.78; H, 5.14; N, 16.19.

A mixture of potassium hydroxide and potassium ferricyanide added to IV gives a blue color.[16]

5-Hydroxyuridine (V).—Bromine water (about 60 ml.) was added to 2.5 g. (0.010 mole) of uridine until the solution was yellow. Air was bubbled through the solution until the solution became colorless. Three grams of lead oxide (litharge) was added and the mixture was heated with stirring for 40 minutes on a steam-bath, during which time the solution was evaporated to 1/3 its original volume. The mixture was chilled to the freezing point in a Dry Ice–acetone-bath and the precipitate of lead oxide and lead bromide was removed by filtering and washed with a small quantity of ice-water. Amberlite IR-120[14] was added in small quantities to the filtrate until a negative test for lead was obtained with sulfide. The solution was filtered and the filtrate was lyophilized. About 100 ml. of absolute ethanol was added to the residue and the solution was concentrated to about 50 ml. The solution was placed in the refrigerator overnight. The crystals which formed were recrystallized by dissolving in a minimum of hot water, filtering and adding four volumes of hot absolute ethanol. Crystals of V formed on cooling overnight at −10°. The yield was 1 g. (38%), m.p. 242–245°. *Anal.* Calcd. for $C_9H_{12}N_2O_7$: C, 41.54; H, 4.65; N, 10.77. Found: C, 41.54; H, 4.46; N, 10.75.

A dilute solution of ferric chloride[17] gives a blue color with V. No color develops when a mixture of potassium hydroxide and potassium ferricyanide[17] are added to V.

Diazouridine (VI).—A modification of the method of Johnson, *et al.*,[11] was used. 5-Aminouridine (2.59 g., 0.01 mole) was dissolved in 25 ml. of 1 *N* hydrochloric acid and cooled to 0°. Ten ml. of a 6.9% (0.01 mole) solution of sodium nitrite was added at 0° and crystals formed in less than five minutes. Two volumes of 95% ethanol at −5° were added, and the solution was cooled to −10°. Rapid filtration and recrystallization from methanol gave 2 g. (77%) of a light yellow crystalline product, which melted with decomposition at 178–182°. *Anal.* Calcd. for $C_9H_{10}N_4O_6$: C, 40.00; H, 3.73; N, 20.72. Found: C, 39.72; H, 3.94; N, 20.44.

When an aqueous solution of VI is added to α-naphthol and boiled, or when VI is added to a weakly alkaline solution of α-naphthol, a red color develops. No reaction is obtained upon the addition of VI to a slightly alkaline sucrose solution.[18]

TABLE I

ULTRAVIOLET ABSORPTION OF SOME PYRIMIDINE BASES AND NUCLEOSIDES AT *p*H 7

	$\lambda_{max.}$, mμ	$\epsilon_{max.} \times 10^{-4}$
5-Aminouracil[19]	290	0.86
5-Aminouridine	294	.74
5-Hydroxyuracil[19]	280	.64
5-Hydroxyuridine	280	.82
5-Methylcytosine[20]	274	.62
5-Methylcytidine	278	.87
Thymine[21]	264	.86
5-Methyluridine	268	1.08
5-Bromouridine	279	0.96
5-Chlorouridine	276	1.00
Diazouridine	237	1.17

Spectrophotometric Data.—The ultraviolet adsorption maxima of these and similar compounds are given in Table I. Each compound was dissolved in distilled water at a concentration of about 20 micrograms per ml. and measured in the Beckman spectrophotometer, model DU.

LOS ANGELES 7, CALIFORNIA RECEIVED JULY 27, 1951

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