2 hr. The imide was isolated by distillation *in vacuo* to yield 12.4 g., 78%, of product, b.p. 87–90°/0.1 mm., n_D^{20} 1.4940. The *hydrochloride* was prepared as described, m.p. 173–174°. The *methiodide* was prepared as described, m.p. 230–231°.

3-Dimethylaminopropyl-3-azabicyclo[3.3.0]octane. The *N*-dimethylaminopropyl base was obtained in a manner analogous to that of the simple *N*-methyl base on reduction of the *N*-dimethylaminopropyl imide with lithium aluminum hydride in anhydrous ether. From 18 g. (0.080 mole) of the imide there was obtained 14 g., 89% of the base with b.p. 102–104°/5 mm., n_D^{20} 1.4742. The *dihydrochloride* was prepared in alcohol in the usual way and on recrystallization melted at 261–262°. The *bis-methiodide* was prepared by refluxing the base with a 10% excess of methyl iodide in methanol for 1 hr. The bis-methonium salt was collected on cooling and recrystallized from methanol-ether, m.p. 256–257°.

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[CONTRIBUTION FROM THE PHYSIOLOGY DEPARTMENT, TUFTS UNIVERSITY SCHOOL OF MEDICINE]

Chemistry of Pyrimidines. I. The Reaction of Bromine with Uracils[1-3]

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Uracils react with bromine in aqueous solution to form 5-bromo-6-hydroxyhydro derivatives. Upon dehydration in solution the latter form 5-bromo derivatives, which in turn with excess bromine, form 5,5-dibromo-6-hydroxyhydro derivatives. The mechanism of the formation of the 5-bromo derivatives from the latter has been elucidated.

In our study of the effects of ultraviolet irradiation on nucleic acids, we have used a number of pyrimidine derivatives as model compounds. Several common reactions have been studied and certain inconsistencies in the literature have been noted. It was the purpose of the present work to clarify these inconsistencies.

In 1907, Wheeler and Johnson[4] first observed the colored reaction product obtained by the action of bromine on uracil and cytosine and since then this reaction has become a well known color test for uracils and cytosines. More recently, a colorimetric method for the determination of uracil and cytosine[5] has been based upon this bromine reaction. The reaction also played an important part in the structural determination of pyrimidine nucleosides and the preparation of derivatives.[6] In 1940. Johnson *et al.* concluded that "5,5-dibromo-4(6)-hydroxyhydrouracil (IVA) decomposed spontaneously and quantitatively to 5-bromouracil and HOBr."[7] In order to prove their statement that HOBr was formed and subsequently served as an oxidizing agent, Johnson *et al.* allowed the dibromo compound to react with thiourea, ethylenethiourea, malonic acid, and barbituric acid and ketene.[7,8] Some interesting observations on the reaction of bromine with pyrimidine compounds have also been reported by Cohn.[9] In the present report we wish to propose a possible mechanism for this bromine reaction.

RESULTS AND DISCUSSION

The addition of one mole equivalent of bromine to uracil (IA) or 1,3-dimethyluracil (IB) in aqueous solution resulted in the formation of 5-bromo-6-hydroxyhydro derivatives (IIA or IIB). The existence of (IIA) or (IIB) was indicated by the loss of the ultraviolet spectrum of IA or IB, the isolation of 6-hydroxyhydro-1,3-dimethyluracil[10] by hydrogenolysis and the formation of 5-bromo-derivatives (IIIA or IIIB). The dehydration of IIA or IIB to form IIIA or IIIB proceeded spontaneously and quantitatively and thus provided an excellent method for the preparation of III. Therefore, it seemed that Johnson's statements[7] to the effect that IVA decomposed spontaneously and quantitatively to III could actually hold for II. A thorough study of IV was made in order to clarify these different views. IV was prepared by the addition of one mole equivalent of bromine to 5-bromo- derivatives (III) in aqueous solution. However, the bromine analysis of the product obtained after repeated crystallization from hot water were low (IVA, found: 54.05, 54.38; IVB, found: 49.15)

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(3) A preliminary report has been published in *Nature*, **180**, 91 (1957).

(4) H. L. Wheeler and T. B. Johnson, *J. Biol. Chem.*, **3**, *Am. Chem. J.*, **42**, 30 (1909); H. L. Wheeler and L. M. Liddle, *J. Am. Chem. Soc.*, **30**, 1152 (1908).

(5) M. Soodak, A. Pircio, and L. R. Cerecedo, *J. Biol. Chem.*, **181**, 712 (1949).

(6) P. A. Levene, *J. Biol. Chem.*, **63**, 653 (1925); "Nucleic Acids", ACS Monograph No. 56, pp. 54 and pp. 148 (Chemical Catalog Co., Inc., New York, 1931).

(7) T. B. Johnson, *J. Am. Chem. Soc.*, **62**, 2269 (1940); also G. E. Hilbert and E. F. Jansen, *J. Am. Chem. Soc.*, **56**, 136 (1934).

(8) T. B. Johnson and C. O. Edens, *J. Am. Chem. Soc.*, **63**, 1058 (1941); T. B. Johnson and M. J. Winton, *J. Am. Chem. Soc.*, **63**, 2379 (1941); M. Fytelson and T. B. Johnson, *J. Am. Chem. Soc.*, **64**, 306 (1942).

(9) W. E. Cohn, *Biochem. J.*, **64**, 28P (1956).

(10) S. Y. Wang, M. A. Apicella, and B. R. Stone, *J. Am. Chem. Soc.*, **78**, 4180 (1956).

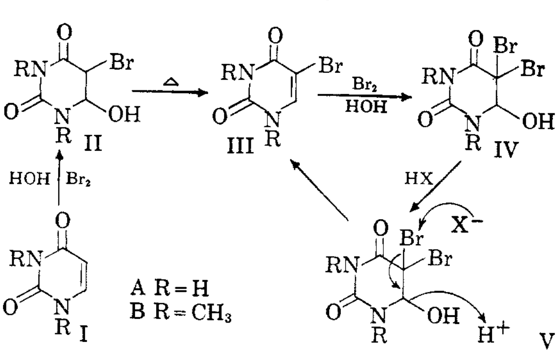
for both compounds. These results led us to recrystallize the compounds from nonaqueous solution because the above bromine analysis checked very well with $^1/_2H_2O$ of crystallization (IVA, calcd. 54.02; IVB, calcd. 49.18). Both compounds dissolved easily in acetone and were crystallized out by the addition of petroleum ether (30–60°) and the bromine analyses were found to be correct. After drying, the compounds which were crystallized from organic solvents could be kept for a long period of time without decomposition. Therefore, this suggested that the instability of IV is probably due to the presence of water of crystallization. (Levene and Johnson did not observe this.)

As for the mechanism of the formation of 5-bromo-derivatives (III) from the dibromo derivatives (IV), the following experiments were carried out. IVA and IVB in 2×10^{-2} *M* aqueous solution were heated to reflux for 8 hr. or more. A solution of IVA gave a maximum at 275 mμ (ϵ 3.9×10^3) and a solution of IVB gave a maximum at 280 mμ (ϵ 3.4×10^3), thus indicating about 50% conversion to IIIA and IIIB, which was confirmed by the isolation and identification of IIIA and IIIB from the above experiments. This suggested that hydrogen bromide, resulting from the hydrolysis of dibromo derivatives (IV), catalyzed the formation of III. When IVA and IVB in 1×10^{-4} *M* aqueous solution were heated under reflux for 24 hr. or more, no increase in ultraviolet absorption was observed at any time. This is probably due to the hydrolysis of IV to form isodialuric acid. However, when the reaction was carried out in 2 *N* HCl or HBr, the absorption at 275 mμ for IIIa ($\lambda_{max}^{2N\,HCl}$ 276 mμ, ϵ 7.2×10^3) and at 280 mμ for IIIB ($\lambda_{max}^{2N\,HCl}$ 283 mμ, ϵ 7.8×10^3) gradually increased to more than 80% of that for IIIA and IIIB at the end of 8 hr. The above observations indicate that III is formed from IV only when HX is present. The probable reason for Levene's[6] statement that "the dibromo compound is undoubtedly the original product which formed immediately on addition of bromine" is that he overlooked the existence of II, which he did not observe because of the inability to isolate these derivatives.

Most workers have been under the impression that IV is highly unstable (which belief now appears untenable), since they used the method of Levene[11] for the preparation. This method requires the addition of bromine until the color of the solution remains permanently yellow. However, we observed that if the reaction was carried out at or below room temperature, the yellow color appeared after one mole equivalent of bromine had been added to the solution. Thus, the isolation of IV is not possible under these conditions. The formation of dibromo compounds (IV) or the disappearance of the yellow color for the second mole equivalent of bromine is dependent on the rate of the formation of III and thus is determined by the rate of dehydration of II. The rate of dehydration seems to be influenced by the bulkiness of neighboring groups (X_1) to the 6-hydroxy group. For example, at room temperature and *p*H 2, IIA is dehydrated in less than 10 min., IIB is completed in 2 hr., but for uridine no more than 10% dehydration was observed in 24 hr. However, when heated on a steam bath for 10 min., these three compounds were all completely dehydrated.

From the above results, it appears that uracil derivatives, whether substituted at position 3 or not, show the same chemical behavior in reactions with bromine. There may be differences between them but they are only quantitative instead of qualitative.

In conclusion, we suggest that IV is formed from III, and III in turn is formed from II. The formation of III from IV is catalyzed by HX and may proceed *via* the transition state V.



EXPERIMENTAL[12]

5-Bromouracil (*IIIA*). Uracil (2×10^{-2} mole) was pulverized and suspended in 40 cc. of water. One mole equivalent of bromine (1.04 ml.) was added with swirling and a clear solution resulted. The reaction solution was then heated on a steam bath for 25 min. and a crystalline product started to appear after 10 min. of heating. After cooling, the product was collected and washed carefully with warm water. Over 90% yield of the compound was collected. It did not melt up to 300° but decomposed and sublimed.[4,6]

$\lambda_{max}^{H_2O}$ 276 mμ, ϵ 7.30×10^3; $\lambda_{min}^{H_2O}$ 241 mμ, ϵ 1.76×10^3; λ280/λ260 1.47, λ250/λ260 0.52; (*p*H 5.6)

Anal. Calcd. for $C_4H_3O_2N_2Br$: N, 14.67; Br, 41.84; Found: N, 14.81; Br, 41.51.

5-Bromo-1,3-dimethyluracil (*IIIB*). 1,3-Dimethyluracil (2×10^{-2} mole) was dissolved in 20 cc. of water and the above procedure for making IIIA was followed exactly. A yield of over 90% of IIIB, m.p. 184–185°, was obtained. Mixed melting point with an authentic sample 184–185°; $\lambda_{max}^{H_2O}$ 283 mμ, ϵ 8.57×10^3; $\lambda_{min}^{H_2O}$ 246 mμ, ϵ 1.57×10^3 [10,13]; λ280/λ260 0.24, λ250/λ260 0.49.

5,5-dibromo-6-hydroxyhydrocil (IVA). Pulverized 5-bromouracil ($2 \times 10^{-2}M$) was suspended in 40 cc. of water and 1.04 ml. of bromine was added. The reaction mixture,

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(11) P. A. Levene, *J. Biol. Chem.*, **63**, 683 (1925).

(12) All melting points are uncorrected and were taken with a Fisher-Johns melting point apparatus. The analyses were carried out by Dr. S. M. Nagy and his associates, Microchemical Laboratory, Massachusetts Institute of Technology. Ultraviolet spectra were determined with Beckman spectrophotometer, model DU.

(13) T. B. Johnson and S. H. Clapp, *J. Biol. Chem.*, **5**, 62 (1909).

in a stoppered flask, was stirred for about 6 hr. At the end white powder-like crystals were collected and washed with chilled water. Over 80% of the theoretical amount was obtained and an additional 10% more was obtained after the mother liquor had been concentrated to about one third of its original volume with a stream of nitrogen. This compound melted at 209–211° with strong effervescence, then resolidified.[4]

Anal. Calcd. for $C_4H_4N_2Br_2O_3.{}^1/_2H_2O$: C, 16.18; H, 1.70; N, 9.44; Br, 53.82. Found: C, 16.09; H, 1.64; Br, 54.05.

This compound was recrystallized from acetone–petroleum ether (30–60°), m.p. 210° with strong effervescence, then resolidified.

Anal. Calcd. for $C_4H_4O_3N_2Br_2$: Br, 55.51; Found: Br, 55.12.

5,5-Dibromo-6-hydroxy-1,3-dimethylhydrouracil (IVB). Pulverized 5-bromo-1,3-dimethyluracil (IIIB) was treated identically as the above procedure and gave over 80% of the theoretical yield. Crystallization from hot water yielded a product with a m.p. 136–137°.[12]

Anal. Calcd. for $C_6H_8O_3N_2Br_2.{}^1/_2H_2O$: Br, 49.18. Found: Br, 49.15.

Crystallization from acetone-petroleum ether (30–60°) instead of water, yielded a product with a m.p. 139–140°.

Anal. Calcd. for $C_6H_8O_3N_2Br_2$: Br, 50.58. Found: Br, 50.50

Effect of reflux on 5,5-dibromo-6-hydroxyhydrouracil (IVA) in 2 × $10^{-2}M$ aqueous solution. A 2 × $10^{-2}M$ solution of IVA (0.2879 g. in 50 cc.) was heated under reflux. At one hour intervals, a 0.5 ml. sample of the solution was withdrawn and diluted to 100 ml. in a volumetric flask. The ultraviolet spectrum of each sample was determined. Prior to reflux the solution had an initial *p*H of 5.88 and after, a *p*H of 3.90. The readings were obtained as shown in Table I.

TABLE I

Time, Hr.	2 × 10^{-2} *M* in H_2O		1 × 10^{-4} *M* in 2*N* HCl	
	IVA, ϵ_{275}, × 10^{-3}	IVB, ϵ_{280}, × 10^{-3}	IVA, ϵ_{275}, × 10^{-3}	IVB, ϵ_{280}, × 10^{-3}
0	0.23	0.37	0.22	0.39
1	0.77	0.92	3.19	3.95
2	1.75	1.67	3.90	5.60
3	2.66	2.01	4.48	6.01
4	3.39	2.97	4.98	6.20
5	3.89	3.39	5.21	6.30
6	4.00	3.62	5.49	6.21
7	3.76	3.65	5.45	6.21
8	3.80	3.77	5.59	6.21

Effect of reflux on 5,5-dibromo-6-hydroxy-1,3-dimethylhydrouracil (IVB) *in 2 × $10^{-2}M$ aqueous solution.* A 2 × $10^{-2}M$ solution of IVB (0.3159 g. in 50 cc.) was treated in a similar manner as above. The readings are shown in the table.

Isolation of 5-bromouracil (IIIA) *from aqeuous solution of 2 × $10^{-2}M$ of* IVA *by reflux.* A solution containing 0.576 g. of IVA dissolved in 100 cc. H_2O (2 × $10^{-2}M$) was heated under reflux for 8 hr. The final *p*H reading was 1.8 and extinction at $\lambda_{275}^{H_2O}$ was ϵ 3.7 × 10^3. The solution was evaporated to dryness and gave a crude yield of 0.3143 g. After two recrystallizations from absolute methanol, a crystalline product which decomposed and sublimed without melting up to 300° was obtained. Its ultraviolet spectrum was identical with the standard curve of IIIA and the optical density ratios were λ280/λ260, 1.49 and λ260/λ240, 2.66 as compared to the standard values of 1.47 and 2.61, respectively.

Isolation of 5-bromo-1,3-dimethyluracil (IIIB) *from aqueous solution of 2 × $10^{-2}M$ of* IVB *by reflux.* A solution containing 0.316 g. of IVB dissolved in 50 cc. of H_2O (2 × $10^{-2}M$) was heated under reflux for 8 hr. The final reading of extinction at $\lambda_{280}^{H_2O}$ was ϵ 3.6 × 10^3. The solution was extracted six times with 25 cc. portions of chloroform and the organic layers were filtered through anhydrous sodium sulfate. The crude crystalline residue, after evaporation of chloroform, weighed 89 mg. One recrystallization from absolute ethanol gave a product with a m.p. 184–185° and mixed m.p. 184–185° (with authentic 5-bromo-1,3-dimethyluracil). Also identical ultraviolet spectra for both were obtained.

Effect of reflux on 1 × $10^{-4}M$ aqueous solution of 5,5-dibromo-6-hydroxyhydrouracil (IVA). A solution of 1 × $10^{-4}M$ of the compound IVA in water was heated under reflux. The ultraviolet spectrum of each sample which had been pipetted from the solution at certain time intervals was determined from 220–300 mμ. The readings at 275 mμ were as follows:

Time, hr.	0	3	6	12	24
ϵ_{275} × 10^{-3}	0.18	0.38	0.41	0.38	0.33

Effect of reflux on 1 × $10^{-4}M$ aqueous solution of 5,5-dibromo-6-hydroxy-1,3-dimethylhydrouracil (IVB). A solution of 1 × $10^{-4}M$ of the compound (IVB) in water was treated in a similar manner as above. The readings at 280 mμ were as follows:

Time, hr.	0	3	6	12	24
ϵ_{280} × 10^{-3}	0.27	0.14	0.18	0.11	0.21

Effect of reflux in acid (2N HCl or HBr) on 1 × $10^{-4}M$ solution of 5,5-dibromo-6-hydroxyhydrouracil (IVA). A solution of 1 × $10^{-4}M$ of the compound (IVA) in 2*N* HCl was heated under reflux, in a flask. The ultraviolet spectrum of each sample at certain time intervals was determined from 220–300 mμ. The readings at 275 mμ are shown in the table.

Effect of reflux in acid (2N HCl or HBr) on 1 × $10^{-4}M$ solution of 5,5-dibromo-6-hydroxy-1,3-dimethylhydrouracil (IVB). A solution of 1 × $10^{-4}M$ of the compound (IVB) in 2*N* HCl was heated under reflux, and the readings were taken in exactly the same manner as above and are shown in Table I.

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