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Keyphrases

Sulfonamides—structure, activity relationship
Biological action, sulfonamides—chemical structure
PAB antagonists—chemical reactivity
Dipole moment—sulfonamides
Spectral properties—sulfonamides, precursors
Mechanism of action—sulfonamides

Research Articles

Solvolysis of 5-Halouridines and Related Nucleosides

By EDWARD R. GARRETT and GERALD J. YAKATAN*

The neutral and alkaline solvolyses of uridine (UD) derivatives substituted in the 5 position with I (IUD), Br (BUD), Cl (CUD), and CH_3 (MUD) were followed spectrophotometrically. The order of reactivity was IUD > BUD >CUD > UD >>> MUD (essentially stable). Ribosylbarbituric acid (RBA) was identified as a product of solvolysis of IUD and BUD in strong alkali and is itself unstable. The halouridines also degrade to nonchromophoric compounds. The rate-pH profiles for all the halouridines are similar and can be explained by hydroxyl ion attack on both the undissociated and dissociated halouridine. Hydroxyl ion attack on the undissociated species leads to the formation of nonchromophoric products. Hydroxyl ion attack on the dissociated species forms RBA. 5-Hydroxyuridine (OHUD) and dihydropyrimidines are postulated as intermediates. The lability of the halogen substituent enhances the ease of solvolysis. The rate of alkaline solvolysis of BUD is the same as the rate of bromide ion production and shows that the reaction intermediates are highly unstable. The arabino and lyxo derivatives of 5-fluorouracil are solvolyzed faster than all of the halouridines. The Arrhenius parameters for all compounds were determined.

SEVERAL NUCLEOSIDES have been shown to be active chemotherapeutic agents in the treatment of viral diseases (1, 2). Quantitative studies on the stability of nucleosides and the effects of structure and substituents should provide insight into their possible metabolic transformations, and they are of pharmaceutical importance for the estimation of maximum stability and inhibition of the formation of toxic side products (3,

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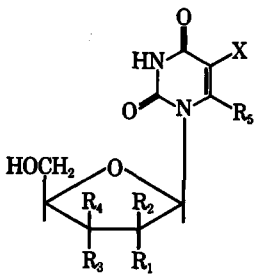
TABLE I—COMPOUNDS USED IN THIS STUDY



Compd.	X	R_1	R_2	R_3	R_4	R_5
Uridine (UD)	H	OH	H	OH	H	H
5-Iodouridine (IUD)	I	OH	H	OH	H	H
5-Bromouridine (BUD)	Br	OH	H	OH	H	H
5-Chlorouridine (CUD)	Cl	OH	H	OH	H	H
5-Methyluridine (MUD)	CH_3	OH	H	OH	H	H
5-Hydroxyuridine (OHUD)	OH	OH	H	OH	H	H
1-β-D-Ribofuranosylbarbituric acid (RBA)	H	OH	H	OH	H	OH
1-β-D-Lyxofuranosyl-5-fluorouracil (FUL)	F	H	OH	H	OH	H
1-β-D-Arabinofuranosyl-5-fluorouracil (FUA)	F	H	OH	OH	H	H

4, 6, 7). These studies may also provide information regarding new synthetic routes for derived compounds.

This quantitative investigation of the chemical transformations of the 5-halouridines is one aspect of the authors' continuing study of the solvolytic reactions of pyrimidine and purine nucleosides and their derivatives (4–10). The purpose of this paper is to report on the kinetics and possible mechanisms of solvolysis of the 5-substituted halouridines and the determination of the pertinent thermodynamic parameters. The major emphasis was on derivatives of uridine (UD) substituted in the 5 position with I (IUD), Br (BUD), Cl (CUD), and CH_3 (MUD). The structures of some of the pyrimidine nucleosides discussed are given in Table I.

EXPERIMENTAL

Materials—IUD, BUD, CUD, MUD, and 5-hydroxyuridine (OHUD) were purchased from Calbiochem and were all certified as chromatographically homogeneous by the company. UD was purchased from Nutritional Biochemicals Corporation, an elementary analysis, C, H, and N was provided, and the compound was used without further purification. Dr. T. Ukita of the University of Tokyo kindly provided a sample of the sodium salt of 1-β-D ribofuranosylbarbituric acid which was used in the paper chromatography studies. Dr. J. J. Fox of the Sloan-Kettering Research Institute supplied 1-β-D-ribofuranosylbarbituric acid monoalcoholate (SKI No. 30,057) for use in the kinetic studies on this compound. Dr. Fox also provided samples of 1-(β-D-lyxofuranosyl)-5-fluorouracil(FUL) and 1-(β-D-arabinofuranosyl)-5-fluorouracil(FUA). All other materials employed in this study were of analytical reagent grade.

Kinetic Procedures—Appropriate quantities of the nucleosides to produce final concentrations of 10^{-2} *M* were weighed into volumetric flasks and diluted to volume with nitrogen-purged distilled water. From these stock solutions, aliquots were taken and diluted with alkali of appropriate concentration or with an appropriate buffer solution to produce, in general, a final nucleoside concentration of 10^{-4} *M*. The solutions of alkali and buffer were previously equilibrated at the temperature of the study.

The reactions were maintained in constant-temperature baths controlled within 0.1° at selected temperatures between 60 and 80° and samples withdrawn at suitable time intervals. Spectra were recorded on the Cary model 15 recording spectrophotometer or spectrophotometric readings were taken on the Beckman model DU-2 spectrophotometer. Matched spectrophotometric cells (1.00 cm.) were employed for all measurements and a slit width of 0.10 mm. was used. The principal ultraviolet spectral data for the 5-halouridines have been reported previously (8, 11). Alkali solutions were prepared by dilution of a standardized NaOH stock solution with nitrogen-purged distilled water. The NaOH solution was standardized by titration with a standard perchloric acid solution on the Sargent model D recording titrator. All reactions run in buffer solutions were maintained at constant ionic strength of 0.15.

pH and pK_a Measurements—The pH values of the NaOH solutions at the temperatures of study were calculated from activity coefficient data available in the literature (12). The pH values of the buffer solutions were measured directly on a Beckman Zeromatic pH meter using Beckman high temperature electrodes and a thermostated cell to contain the buffer solution at the temperature of study. The pH meter was standardized with standard buffers at the same temperature. The pK_a's of the 5-halouridines were determined by titration of 0.05 meq. of the nucleoside with a standard NaOH solution on the Sargent model D recording titrator. The measurements were carried out in a thermostated cell at 80°.

Paper Chromatography—Paper chromatograms were prepared from Whatman No. 1 chromatography paper. The chromatograms were usually spotted with 25 μl. of sample and developed for more than a 12-cm. travel of solvent front in a *t*-butyl alcohol–methyl ethyl ketone–water–ammonium hydroxide (40:30:20:10) solvent which had previously been reported as useful for the separation of nucleosides and related compounds (13). They were then dried, and the resulting separation was viewed under a short wavelength ultraviolet light. The chromatograms were also placed in an iodine chamber to visualize spots which did not possess an ultraviolet chromophore. The nucleosides were identified by comparison of R_f values of the unknown with known standards spotted on the same chromatogram. Freshly prepared Ehrlich's reagent (14) was used to identify 1-β-D-ribofuranosylbarbituric acid (15).

Bromide Ion Measurement—An Orion bromide ion activity electrode, model 94–35, was used with a calomel reference electrode and a Beckman Zeromatic pH meter to follow bromide ion production from solvolysis of 10^{-2} *M* BUD in 0.40 *N* NaOH at

80°. Aliquots of the degrading solution were taken as a function of time and diluted with 0.5 *N* $HClO_4$ to give a pH of about 1. The resultant potential was used to determine bromide ion concentration. The calibration curve was constructed from the measured potentials of known amounts of NaBr prepared in a similar manner with the same ionic strength and pH values.

RESULTS

Rate Constants—The kinetic parameters describing the solvolysis of the 5-halouridines in alkali and the alkaline buffer region were determined by following the loss of absorbance, A, of the ultraviolet chromophore as a function of time. The spectra of IUD and BUD showed a decided hypsochromic shift in $\lambda_{max.}$ as solvolysis proceeded in strong alkali. A small residual absorbance, A_∞, was noted in some cases at the completion of the reaction. Typical spectral changes as a function of time are given for IUD, BUD, and CUD in 0.40 *N* NaOH in Figs. 1–3. Plots of ln $(A - A_\infty)$ at the $\lambda_{max.}$ *versus* time were reasonably linear for all studies according to the first-order expression:

$$\ln (A - A_\infty) = \ln (A_0 - A_\infty) - kt \quad \text{(Eq. 1)}$$

where A_0 is the absorbance at zero time and k is the apparent first-order rate constant. However, similar plots at lower wavelengths for IUD and BUD indicated the possibility of a sequential process where the intermediate possessed an ultraviolet chromophore.

Subsequent experiments, to be discussed later in this paper, revealed the identity of this intermediate and permitted the determination of the spectrum of this compound. Once this was known, it was possible to choose a wavelength to obtain the apparent first-order loss of the original nucleoside without spectral interference from the intermediate. The rate constants obtained by this method and the conditions under which they were obtained are listed in Tables II and III.

Several typical first-order plots at various alkali concentrations according to Eq. 1 at a wavelength where only IUD absorbs, *i.e.*, 290 mμ, are shown in Fig. 4. Reactions run with a fourfold increase in substrate concentration, in the absence of oxygen, and protected from light showed no significant changes from the apparent first-order rate constants obtained when these parameters were not controlled (see footnotes to Table II). No significant ionic strength effects or buffer catalysis were observed in any of the buffer regions for the solvolysis of the halouridines (see footnotes to Table II).

UD, FUL, and FUA degraded in alkali with no apparent perturbation of the $\lambda_{max.}$ values 263, 268, and 272 mμ, respectively. These compounds solvolyze more rapidly than the halouridines and plots according to Eq. 1 where $A_\infty \sim 0$ are linear and parallel for data obtained at several wavelengths. The obtained rate constants are given in Table III.

The alkaline solvolysis of 5-hydroxyuridine was complex. The $\lambda_{max.}$ of 303 mμ in alkali (or the $\lambda_{max.}$ of 278.5 mμ of the acidified samples) decreased with time with complete destruction of the chromophore. First-order plots of the data showed good linearity at lower temperatures, but apparent sequential rates were observed at higher temperatures (Table III).

Rate-pH Profiles—The rate-pH profiles for all of the 5-halouridines were similar. The profiles were constructed from the apparent first-order rate constants, k, and the pH values at 80° (Table II) and are shown in Fig. 5. The rate-pH profiles indicate specific hydroxyl-ion catalysis as well as a pH independent region of solvolysis. The apparent first-order rate constant at a given pH can be defined as in Eq. 2.

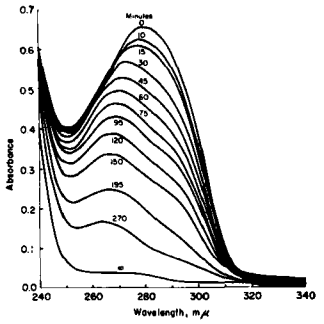


Fig. 1—Typical spectral changes for the solvolysis 10^{-4} M 5-iodouridine in 0.40 N NaOH at 80°. The curves are labeled as to minutes after the start of the reaction.

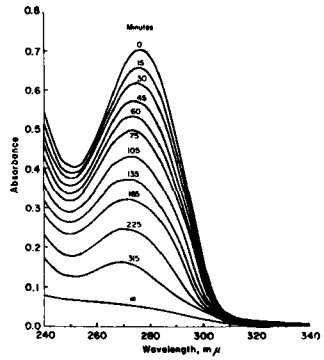


Fig. 2—Typical spectral changes for the solvolysis of 10^{-4} M 5-bromouridine in 0.40 N NaOH at 80°. The curves are labeled as to minutes after the start of the reaction.

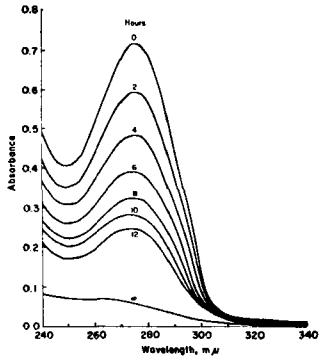


Fig. 3—Typical spectral changes for the solvolysis of 10^{-4} M 5-chlorouridine in 0.40 N NaOH at 80°. The curves are labeled as to hours after the start of the reaction.

TABLE II—APPARENT FIRST-ORDER RATE CONSTANTS, 10^5k IN SEC.$^{-1}$, FOR THE SOLVOLYSIS OF SOME PYRIMIDINE RIBOSIDES AT 80.0°

pH[a]	Medium	IUD Calcd.	IUD Exptl.	BUD Calcd.	BUD Exptl.	CUD Calcd.	CUD Exptl.	RBA Calcd.	RBA Exptl.	UD[f] Exptl.	UD[f] Calcd.
6.00	Phosphate buffer	—	—	0.080	0.125	0.052	0.086	—	—	—	—
6.87[b]	Phosphate buffer	0.539	0.570	0.478	0.546	0.299	0.311	1.40	1.41	—	—
7.09	Phosphate buffer	0.730	0.823	0.688	0.757	0.426	0.443	1.40	1.47	—	—
7.69	Phosphate buffer	1.83	1.82	1.39	1.34	0.825	0.779	1.40	1.40	—	—
7.91	Phosphate buffer	2.30	2.19	1.60	1.69	0.924	0.859	1.41	1.36	—	—
8.28	Phosphate buffer	2.94	2.69	1.86	1.96	—	—	—	—	—	—
9.08	Bicarbonate buffer[g]	3.42	3.28	2.07	2.01	1.18	1.16	1.51	1.57	—	—
9.40	Bicarbonate buffer[g]	3.66	3.50	2.09	2.12	1.19	1.19	1.63	2.13	—	—
9.65[c]	Bicarbonate buffer[g]	3.67	3.60	2.13	2.17	1.21	1.23	1.81	3.45	—	—
9.90	Bicarbonate buffer[g]	3.74	3.80	2.14	2.32	1.22	1.28	2.13	3.70	—	—
10.54	0.01*N* NaOH	3.95	3.72	2.36	2.94	1.28	1.52	4.48	4.11	—	—
11.20	0.05*N* NaOH	4.76	5.01	3.59	4.18	1.56	1.94	13.5	15.8	0.189	0.156
11.49[d]	0.10*N* NaOH	6.50	6.00	4.51	4.81	1.89	2.49	21.4	24.1	0.326	0.270
11.75	0.20*N* NaOH	7.89	8.00	7.28	7.18	2.46	2.70	30.7	31.6	0.491	0.421
12.05[e]	0.40*N* NaOH	13.1	11.0	11.9	9.49	3.71	3.51	41.9	37.2	0.601	0.630
12.23	0.60*N* NaOH	16.2	16.1	14.2	14.3	—	—	—	—	—	—
12.35	0.79*N* NaOH	—	—	—	—	—	—	—	—	0.701	0.840

[a] The pH values of the phosphate and bicarbonate buffer solutions were read at 80° on a pH meter standardized at that temperature. The other pH values were calculated from $pH = pK_w - p\text{OH}$ where $p\text{OH} = -\log\gamma\,[\text{NaOH}]$ where the activity coefficient, γ, and the pK_w values were obtained from the literature (12). [b] Additional values at this pH were: BUD, 0.20 *M* buffer, $\mu = 0.36$, $10^5k = 0.566$; 0.30 *M* buffer, $\mu = 0.36$, $10^5k = 0.530$. [c] Additional values at this pH were: BUD, $\mu = 0.10$, $10^5k = 2.12$; 0.10 *M* buffer, $\mu = 0.21$, $10^5k = 2.21$: IUD, $\mu = 0.21$, 0.10 *M* buffer, $10^5k = 2.21$: IUD, $\mu = 0.21$, 0.10 *M* buffer, $10^5k = 3.70$: CUD, $\mu = 0.10$, $10^5k = 1.28$; $\mu = 0.21$, 0.10 *M* buffer, $10^5k = 1.28$: RBA, $\mu = 0.21$, 0.10 *M* buffer, $10^5k = 3.70$; $\mu = 0.32$, 0.15 *M* buffer, $10^5k = 4.50$. [d] IUD protected from light, $10^5k = 6.10$; BUD with solution purged with nitrogen for 3 min. after each sample was removed, $10^5k = 4.71$. [e] Fourfold increase in initial concentration (4×10^{-4} *M*) gave $10^5k = 9.91$ for BUD and 11.5 for IUD. [f] The experimental data are subject to some error resulting from silica formation in reaction vessels which prevented the accurate determination of any A_∞ values. [g] All bicarbonate–carbonate buffers in rate-pH profile were 0.05 *M*. $[CO_3^=]$ at the various pH values were: 9.08, 0.004 *M*; 9.40, 0.016 *M*; 9.65, 0.028 *M*; 9.90, 0.039 *M*.

TABLE III—APPARENT FIRST-ORDER RATE CONSTANTS, 10^5k IN SEC.$^{-1}$, AND THERMODYNAMIC PARAMETERS FOR THE SOLVOLYSIS OF SOME PYRIMIDINE NUCLEOSIDES AT VARIOUS TEMPERATURES

°C.	IUD 0.4 *N* NaOH	IUD pH 9.81[c]	BUD 0.4 *N* NaOH	BUD pH 9.81[c]	CUD 0.4 *N* NaOH	CUD pH 9.81[c]	RBA 0.4 *N* NaOH	UD 0.4 *N* NaOH	FUL[a] 0.4 *N* NaOH	FUA[b] 0.4 *N* NaOH	OHUD 0.4 *N* NaOH[f]
80	11.0	3.70	9.49	2.30	3.51	1.28	37.2	0.601	—	—	101 14.8
75	7.90	2.26	5.76	1.45	1.92	0.789	—	0.382	—	—	—
70	4.61	1.44	3.47	0.865	1.14[d]	0.484	17.4[e]	0.256	27.8	77.7	67.6 6.92
60	1.94	—	—	—	0.414	—	6.37	—	14.0	38.0	24.7 4.98
50	—	—	—	—	—	—	—	—	5.54	16.1	1.97
40	—	—	—	—	—	—	—	—	2.30	6.12	—
ΔH_a (kcal./mole)	20.4	22.7	24.4	23.7	24.3	23.7	20.5	21.2	18.8	18.8	15.2
Log *P*	8.67	9.61	11.1	10.0	10.6	9.77	9.26	7.90	8.48	8.91	5.52

[a] Additional values were: 0.20 *N* NaOH, 25°, 0.303; borate buffer, pH 9.45, 80°, 15.2. [b] Borate buffer, pH 9.55, 80°, 89.0. [c] Bicarbonate–carbonate buffer. [d] 69.4°. [e] 71.0°. [f] OHUD is susceptible to oxidative degradation and oxygen was not excluded in these experiments. Higher temperatures gave apparent sequential rates and estimates of the rate constants obtained by polyexponential analysis are given. ΔH_a and log *P* values are for the slower reaction.

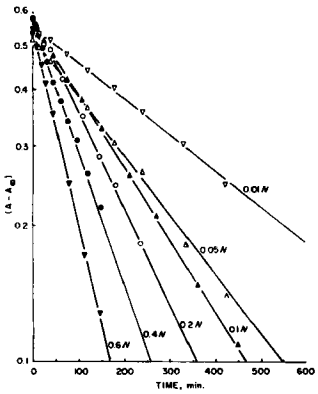


Fig. 4—Typical apparent first-order plots for the solvolysis of 5-iodouridine in various NaOH concentrations at 80°. The absorbance values, A, were measured at 290 mμ and A_∞ is the final asymptotic absorbance.

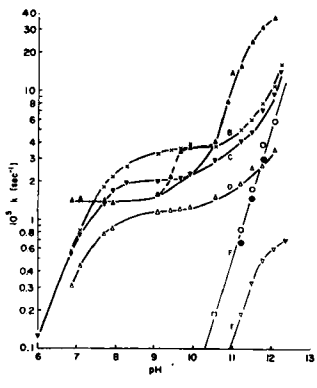


Fig. 5—Rate-pH profiles for the solvolysis of some pyrimidine nucleosides at 80°. Key: A, RBA; B, IUD; C, BUD; D, CUD; E, UD. The open and closed circles for line F show the dependence on pH of the apparent first-order rate constants for the production of RBA from IUD and BUD, respectively.

$$k = \{k_0 + k_{OH}[OH^-]\} f_u + \{k''_0 + k'_{OH}[OH^-]\} f_{u^-} \quad \text{(Eq. 2)}$$

where k_{OH} and k'_{OH} are the bimolecular rate constants for hydroxyl ion attack on the undissociated (f_u) and dissociated (f_{u^-}) halouridine, respectively, and k_0 and k''_0 are the apparent first-order rate constants for water attack on the undissociated and dissociated species, respectively. Hydroxyl ion attack on the undissociated species is kinetically equivalent to water attack on the monoanion (16), and water attack on the undissociated species is either nonexistent or negligible, so that Eq. 2 can be written:

$$k = k_{OH}[OH^-] f_u + k'_{OH}[OH^-] f_{u^-} \quad \text{(Eq. 3)}$$

or as its kinetic equivalent:

$$k = \{k'_0 + k'_{OH}[OH^-]\} f_{u^-} \quad \text{(Eq. 4)}$$

The Eq. 3 can be rewritten by substituting for f_u and f_{u^-} in terms of the equilibrium constant, K_a, for the dissociation of the halouridine to the monoanion (14):

$$k = \{k_{OH}[OH^-]\} \frac{1}{1 + K_a/[H^+]} + \{k'_{OH}[OH^-]\} \frac{1}{1 + [H^+]/K_a} \quad \text{(Eq. 5)}$$

at pH values <9.5, the profiles can be fit by the first term of Eq. 5, *i.e.*,

$$k = \{k_{OH}[OH^-]\} \frac{[H^+]}{[H^+] + K_a} \quad \text{(Eq. 6)}$$

At pH values >9.5, the second term of Eq. 5 contributes to the observed first-order rate constant. The bimolecular rate constants which produce the best fits of the rate-pH profiles and the kinetic pKa values determined from the profiles are given in Table IV. The rate constants calculated from the values of Table IV and Eq. 3 are shown in Table I. The calculated fits obtained for the 5-halouridines are consistent with the experimental data.

The rate-pH profile for uridine (Fig. 5) is different from those for the 5-halouridines. The profile definitely bends over at high pH values and indicates that another pKa′ (attributable to the sugar moiety) influences the rate of reaction. An expression that fits the uridine data is

$$k = k'_{OH}[OH^-] f_{u^-} \quad \text{(Eq. 7)}$$

or its kinetic equivalent

$$k = k''_0 f_{u^-} \quad \text{(Eq. 8)}$$

Table IV—Catalytic Rate Constants for the Solvolysis of Some Pyrimidine Nucleosides at 80°

	IUD	BUD	CUD	RBA	UD
pKa′[a]	7.75	7.40	7.35	11.85[b]	12.1[c]
k_{OH}	3.16	3.56	2.29	—	—
$10^4 k'_{OH}$	2.82	3.02	0.955	39.8	0.479
$10^5 k'_0$	4.15	2.09	1.20	1.40	—

[a] Values reported are determined from best fit of rate-pH profile. pKa′ values determined by titration without control of ionic strength at 80° were: IUD, 7.98; BUD, 7.65; CUD, 7.63. [b] Value reported is for second pKa′ at 80°. Literature value at 25° is 12.4 (15). [c] Value is attributed to sugar pKa′ at 80°.

The catalytic constant and an estimate of the sugar pKa′ of uridine are given in Table IV.

The rate-pH profile for RBA is also different from those of the halouridines. In this case, the second pKa′ is implicated in the rate-pH profile since the second pKa′ for this compound has been reported to be 12.4 at 25° (15) and would be even lower at 80°. A definite bending of the profile in strong alkali can be observed. One of the kinetically equivalent expressions that fit the RBA data for pH values >6.8 >pKa′$_1$ = 3.6 at 25° is

$$k = \{k'_0 + k'_{OH}[OH^-]\} f_{u^-} \quad \text{(Eq. 9)}$$

The Eq. 9 can be rewritten in terms of the second pKa of RBA as:

$$k = \left\{k'_0 + k'_{OH}[OH^-]\right\} \frac{1}{1 + K_{a_2}/[H^+]} \quad \text{(Eq. 10)}$$

Values for the best fit of this profile and the appropriate constants are also found in Tables IV and II, respectively. The discrepancies observed in the bicarbonate buffer region could only be due to catalysis by buffer species since RBA has no pKa in this pH region. The observed buffer catalysis is denoted by a dashed line in the rate-pH profile for RBA (Fig. 5). Buffer catalysis was confirmed by observing an increase in the apparent first-order rate constant with increasing buffer concentration at constant pH (footnote to Table II). The intercept of a plot of $k_{app.}$ *versus* buffer concentration is the apparent first-order rate constant in the absence of buffer effects according to:

$$k_{app.} = k_{hyd.} + k_{buff.}[\text{BUFFER}] \quad \text{(Eq. 11)}$$

The value obtained for $k_{app.}$ was consistent with the calculated value for RBA solvolysis at this pH obtained from Eq. 9. The smooth curve in the rate-pH profile in the bicarbonate buffer region is drawn in agreement with this information.

Dependence of Rate on Temperature—Estimates of the Arrhenius parameters for the solvolysis of several pyrimidine nucleosides were obtained from the slopes and intercepts of plots of the logarithm of the apparent first-order rate constants, k, *versus* the reciprocal of the absolute temperature, T, in accordance with the expression:

$$\log k = \log P - \Delta H_a/2.303\,RT \quad \text{(Eq. 12)}$$

where R is 1.987 cal. deg.$^{-1}$ mole^{-1} and the various k, ΔHa, and log P values at several pH values are given in Table III. Typical Arrhenius plots are shown in Fig. 6.

Identification of Reaction Intermediate—The possibility existed that an intermediate from the alkaline solvolysis of halouridines might be a barbituric acid derivative such as 1-β-D-ribofuranosylbarbituric acid which had been recently synthesized (15). Halouracils do form barbituric acids on attack by alkali (17, 9). Evidence that this compound was an intermediate in the alkaline solvolysis of the halouridines was obtained in the following manner:

(*a*) Ukita and co-workers (15) reported that RBA had pKa's of 3.6 and 12.4 and a $\lambda_{max.}$ in alkali at 263 mμ. By performing *in situ* spectrophotometric pKa measurements on samples of IUD and BUD degraded in 0.40 *N* NaOH at 80° for 1 hr., the authors observed a pKa of 3.7 and another pKa′

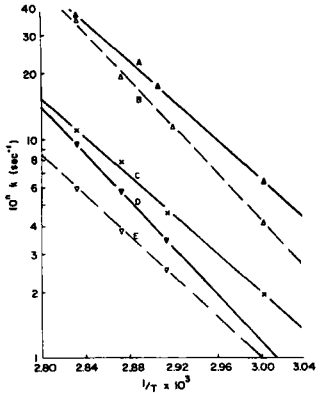


Fig. 6—Typical Arrhenius plots for several pyrimidine nucleosides in 0.40 N NaOH. Key: A, RBA; B, CUD; C, IUD; D, BUD; E, UD; n = 5 for lines A, C, and D; n = 6 for B and E.

attributable to the undegraded halouridine. The degraded mixture also showed a $\lambda_{max.}$ at 264 mμ in strong alkali.

(*b*) The concentration of IUD at any time could be determined from its absorptivity value, ϵ, at a wavelength where the products of degradation did not absorb. The calculated absorbance due to IUD at any time can be subtracted from the total absorbance (Fig. 2) at each wavelength to permit the plotting of these differences against wavelength. The plots for several time intervals at every 5 mμ from 330 to 230 mμ showed the presence of a chromophore with a sharp maximum at 264 mμ consistent with the RBA and different from the spectrum of barbituric acid. The ratios of the absorbances of these difference spectra at any two wavelengths gave constant values which were consistent with the spectra of RBA obtained with the same solvent conditions.

(*c*) The molar absorptivity of RBA in acid solution is negligible (18) but is high in alkaline solution (15,470 in *N* NaOH) (18). This was consistent with the experimental observation that acidified alkaline-degraded BUD showed only the spectrum of BUD with no perturbation of the $\lambda_{max.}$ of BUD in acid solution. Conversion of the acid solution back to alkaline pH values regained the original spectra shown in Fig. 3 with definitive indications of the RBA chromophore. The low ϵ value of RBA in acid solution does not permit its spectral observance in low concentrations.

(*d*) Paper chromatography of alkaline degraded IUD and BUD demonstrated two spots having ultraviolet absorption. One spot was identified as the undegraded halouridine by comparison with a standard. The other spot immediately turned orange on spraying with Ehrlich's reagent, a characteristic reaction for barbituric acid derivatives (15). If left untreated overnight, the RBA spot turned yellow on the chromatogram, another characteristic reaction of barbituric acid derivatives (19). The spot attributed to the undegraded nucleoside neither turned yellow on standing nor was colored orange by Ehrlich's reagent. The R_f value for RBA in the developing system used was 0.40 and a standard spot of pure RBA had exactly the same value. The R_f value for barbituric acid under the same conditions was 0.30. No chromatographic evidence for the formation of the corresponding halouracils was obtained and indicated that lysis of the sugar moiety did not occur.

(*e*) Fox and his co-workers have reported that they have recently succeeded in preparing RBA from the alkaline treatment of BUD (18). The sample of RBA he provided was used in the authors' kinetic studies on this compound.

(*f*) The ultraviolet spectra of degrading IUD and BUD indicate only a hypsochromic shift which never reaches the $\lambda_{max.}$ reported for RBA. This is entirely consistent with an RBA intermediate since it has been shown that RBA degrades in alkali faster than the halouridines and there can never be a very large amount of RBA present at any time. Only the hypersensitivity of the spectrum to RBA caused by the large ϵ value of RBA in relation to those of the halouridines allows the intermediate to be seen at all.

(*g*) The halouracils degrade under similar conditions (9) at rates only one-fifth of those for the halouridines. There was no spectral evidence of halouracil appearance and thus indicated that lysis of the sugar moiety did not occur, at least until after the pyrimidine ring had been cleaved.

Estimation of Rate Constants for Parallel Reactions—As was described previously, plots of ln $(A - A_\infty)$ *versus* time at 265 mμ indicated a sequential process was taking place during solvolysis of the halouridines. This suggested that a model describing the kinetics of solvolysis of the halouridines, XUD, might be:

$$\text{XUD} \xrightarrow{k_1} \text{RBA} \xrightarrow{k_2} \text{Nonchromophoric or rapidly degrading products (NC)} \qquad \text{(Eq. 13)}$$

The apparent first-order rate constants, k_2, were obtained by following the loss of the chromophore due to RBA under conditions equivalent to those used to study the halouridine solvolysis. These values appear in Table II. If the only hydrolysis product of the halouridines is RBA, then the concentration of RBA at any time can be predicted from the obtained values of the rate constants, k_1, for the loss of XUD; k_2 for the solvolysis of RBA, and the initial concentration of the halouridine, $[\text{XUD}]_0$, by (9):

$$\text{RBA}_{\text{PRED}} = [\text{XUD}]_0 \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \qquad \text{(Eq. 14)}$$

When these calculations were carried out over a wide range of sample times and the calculated concentration of RBA converted to absorbance due to RBA, it was observed that the predicted value for the absorbance of RBA at any time was *greater* than the actual absorbance due to RBA as calculated from spectral observations by:

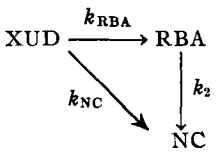
$$A_{264}^{\text{RBA}} = A_{264}^{T} - A_{264}^{\text{IUD}} \qquad \text{(Eq. 15)}$$

where A_{264}^{T} is the total absorbance at 264 mμ at any time and A_{264}^{IUD} is the absorbance at 264 mμ due to IUD at any time. A_{264}^{IUD} can be obtained from:

$$A_{264}^{\text{IUD}} = A_{290}^{T} \times \frac{\epsilon_{264}^{\text{IUD}}}{\epsilon_{290}^{\text{IUD}}} \qquad \text{(Eq. 16)}$$

This information indicated that not all of the

solvolyzed halouridine was going through the RBA intermediate. Since RBA was the only chromophoric product observed, it has been postulated that parallel reactions exist according to:



(Eq. 17)

For this case, the actual concentration of RBA present at any time, RBA_{ACT}, can be formulated (9):

$$RBA_{ACT} = [XUD]_0 \frac{k_{RBA}}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad \text{(Eq. 18)}$$

where $k_1 = k_{RBA} + k_{NC}$. The ratio of the values RBA_{ACT}/RBA_{PRED} at any time from Eqs. 18 and 14 is

$$\frac{RBA_{ACT}}{RBA_{PRED}} = \frac{k_{RBA}}{k_1} \quad \text{(Eq. 19)}$$

The actual absorbance, $A_{264}^{RBA} = \epsilon_{RBA} RBA_{ACT}$, can be calculated from Eq. 15 and the predicted absorbance, ϵ_{RBA} RBA_{PRED}, can be calculated from Eq. 12 since the ϵ value for RBA is known. Thus the ratio of Eq. 19 can be determined. From this ratio and the known apparent first-order rate constant, k_1, for the loss of XUD, k_{RBA} can be calculated. The apparent first-order rate constant to nonchromophoric products, $k_{NC} = k_1 - k_{RBA}$ can also be estimated.

The values of these ratios were obtained over wide intervals of *A versus* time plots. The resultant rate constants and the yields of RBA at different pH values are presented in Table V. The apparent first-order rate constants for RBA solvolysis were more than 10 times greater than the apparent first-order rate constants for CUD solvolysis in strong alkali. This explains why no RBA was observed and why there were no spectral shifts during CUD solvolysis.

It is interesting to note that the rate constants for the production of RBA from both IUD and BUD follow the same kinetic dependency,

$$k_{RBA} = k'_{OH}[OH^-] f_{u^-} \quad \text{(Eq. 20)}$$

TABLE V—RATE CONSTANTS AND YIELDS IN THE SOLVOLYSIS OF 5-IODO AND 5-BROMO URIDINE TO RIBOSYLBARBITURIC ACID AND NONCHROMOPHORIC PRODUCTS AT 80.0°

pH[a]	$10^5 k_1$[b]	$10^5 k_2$[c]	% RBA	%NC	$10^5 k_{RBA}$	$10^5 k_N$[c]
IUD						
10.54	3.72	4.11	5	95	0.19	3.53
11.20	5.01	15.8	17	83	0.85	4.16
11.49	6.00	24.1	29	71	1.74	4.26
11.75	8.00	31.6	49	51	3.92	4.08
12.05	11.0	37.2	53	47	5.83	5.17
BUD						
10.54	2.94	4.11	0	100	—	—
11.20	4.18	15.8	14	86	0.67	4.14
11.49	4.81	24.1	31	69	1.49	3.32
11.75	7.18	31.6	42	58	3.02	4.16
12.05	9.49	37.2	28	72	2.66	6.83

[a] pH values are calculated from $pH = pKw - pOH$ where $pOH = -\log \gamma NaOH[NaOH]$ using appropriate values at 80°. [b] Overall apparent first-order rate constants for solvolysis of IUD and BUD. [c] Apparent first-order constants for the solvolysis of RBA to nonchromophoric products.

The value obtained for k'_{OH} was 2.47×10^{-4} l./mole-sec. at 80.0°.

Preliminary Studies on Buffer Catalysis of RBA Degradation—Preliminary investigations on the buffer catalysis of RBA observed in the bicarbonate buffer region (Table II, Fig. 5) indicated that carbonate ion is the catalytic species while bicarbonate ion has an inhibitory effect on catalysis. If the difference between the experimental rate constant and the rate constant calculated on the basis of Eqs. 3 or 5 (Table II) is due only to bicarbonate and/or carbonate catalysis,

$$k_{exptl.} - k_{calcd.} = k_{diff.} = k_{CO_3^-}[CO_3^-] + k_{HCO_3^-}[HCO_3^-] \quad \text{(Eq. 21)}$$

Since,

$$[HCO_3^-] = [CO_3^-][H^+]/K_a \quad \text{(Eq. 22)}$$

then,

$$k_{diff.} = \{k_{CO_3^-} + k_{HCO_3^-}[H^+]/K_a\}[CO_3^-] \quad \text{(Eq. 23)}$$

and,

$$\frac{k_{diff.}}{[CO_3^-]} = k_{CO_3^-} + k_{HCO_3^-}[H^+]/K_a \quad \text{(Eq. 24)}$$

Thus, a plot of $k_{diff.}/[CO_3^-]$ *versus* $[H^+]$ should be a straight line with a *positive* slope equal to $k_{HCO_3^-}/K_a$ and an intercept of $k_{CO_3^-}$.

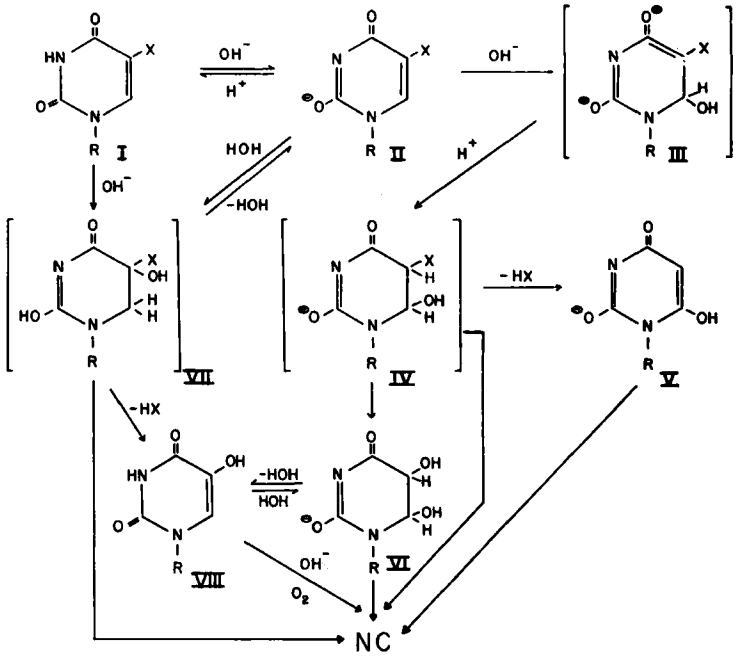
Such a plot was constructed from the six bicarbonate buffer studies of Table II and its footnotes. Five of these studies lay on a straight line of negative slope. This was indicative of the fact that CO_3^- was a catalytic species and that the presence of the undissociated acid, HCO_3^-, inhibited rather than catalyzed the reaction. Estimates at 80° of $k_{CO_3^-}$ and $k_{HCO_3^-}/Ka$ were 45.3×10^{-5} and -3.64×10^5, respectively. The phenomenon of inhibition of solvolysis by weak acids has been observed previously in such cases as acetic acid inhibition of anhydride solvolysis (20).

DISCUSSION

The order of reactivity of the halouridines to alkaline solvolysis is IUD > BUD > CUD. Uridine (UD) is solvolyzed about 5–6 times more slowly than CUD in strong alkali while MUD is stable for 5 days in 0.40 *N* NaOH at 80.0°. These observations would indicate that the lability of the halogen substituent in the 5-position of the pyrimidine ring enhances the ease of the solvolysis. The ability of the halogen to act as a leaving group under these conditions rather than its electronegative effect on making the 6-position susceptibile to hydroxyl ion attack is supported by the fact that the rate constants for the formation of RBA are of the same magnitude for BUD as for IUD (Table IV and Fig. 5). This was also observed for barbituric acid formation from bromo and iodouracil (9).

The fact that barbituric acid and halouracil formation is not observed definitely indicates that the lysis of the sugar moiety from halouridine does not occur, at least until after degradation or cleavage of the pyrimidine ring.

The possible routes of solvolysis of the halouridines consistent with the kinetic and spectra data are given in Scheme I. The observed production (only in strong alkali) of RBA (V) from IUD and BUD must be formulated in agreement with

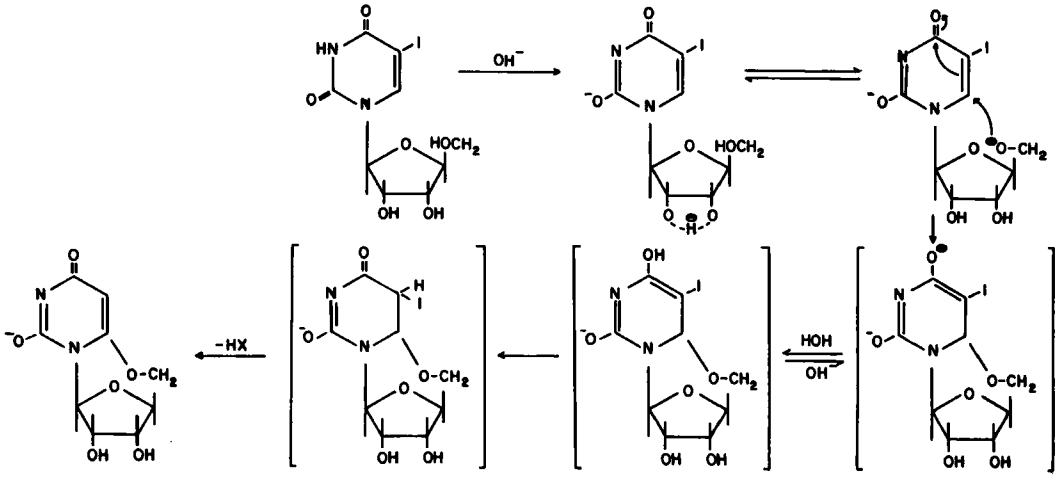


Scheme I

the derived kinetic dependency (Eq. 20) as a direct hydroxyl ion attack at C_6 of the monoanion and subsequent dehydrohalogenation (II → III → IV → V).

The kinetic dependency for RBA formation (Eq. 20) denies the rate-determining hydration of the 5,6 double bond on the monoanion. It would not, however, deny water attack on the dianion which would be kinetically indistinguishable from hydroxyl ion attack on the monoanion, if the halouridine may exist as a dianion at high pH values (*i.e.*, at pH values where a sugar hydroxyl can be ionized) (21). The fact that the rate-pH profiles for IUD and BUD do not bend over at high pH values indicates that an ionized sugar hydroxyl is not involved in an intramolecular nucleophilic attack at C_6 of the pyrimidine base. However, Lipkin and co-workers (22) have reported the formation of a 6,5′-anhydro-ribosylbarbituric acid by "alkaline hydrolysis of 5-iodouridines" which may be a consequence of a secondary dissociation of the sugar moiety (Scheme II).

Fox (17) has reported ~40% yield of a 6,5′-anhydro-2′,3′-*O*-isopropylidene-5-iodouridine with *N* alkali at 55°. It has been hypothesized that the isopropylidene group forces the sugar ring into a conformation that favors cyclization to form 6,5′-anhydronucleosides (23, 24, 17). When Fox treated BUD and IUD with alkali under the same conditions employed for their isopropylidenated derivatives, the reaction was much slower, but "UV data indicated that some 6,5′-anhydro-ribosylbarbituric acid had formed" (17). The authors have no information on the spectral characteristics or alkaline



Scheme II

stability of 6,5′-anhydro-ribosylbarbituric acid. Thus, it cannot be denied that there is the possibility that RBA may be formed by solvolysis of the 6,5′ anhydro intermediate whose formation results on intramolecular condensation of the dianion in the rate-determining step. This reaction rate constant for this process would have to be very large since the second pKa′ of the halouridine is not kinetically observed.

Hydration of the 5,6 double bond of the halouridine monoanion and subsequent dehydrohalogenation could produce compound VIII, 5-hydroxyuridine (OHUD). This compound is extremely unstable in the presence of alkali and is susceptible to oxidative degradation. OHUD degrades rapidly in 0.40 *N* NaOH at 80° showing a nonlinear semilogarithmic plot of absorbance *versus* time which may be interpreted as a sequential reaction, A → B → C, where the estimated rate constants are given in Table III. There is no apparent shift in the $\lambda_{max.}$ of the OHUD chromophore with time. The halouridines degrade much more slowly under equivalent conditions and any OHUD produced could not be observed spectrally. It is not unreasonable to assume that some OHUD is formed in this system, since 5-hydroxydeoxyuridine has been observed as a product of the alkaline solvolysis of iododeoxyuridine (7) and the isopropylidenated derivatives of IUD and BUD (17). Wang (25) also states that OHUD could be formed from BUD.

The formation of unstable dihydropyrimidine compounds may also be responsible for the fraction of the reaction that does not go through RBA. Cleavages of 5,6 dihydropyrimidine nucleosides in alkali at the 3,4 position are well-documented (26) and may lead to nonchromophoric products similar to one isolated from the alkaline treatment of arabinopyrimidine nucleosides (27). The dihydropyrimidines degrade so rapidly in alkali that it would be impossible to observe these intermediates spectrally. Sander has shown, for example, that dihydrouracil degrades about 500 times faster at 25° than IUD does at 80° (28), whereas uracil has high stability (9). It would also be possible for VI to yield VIII by dehydration (25). The postulated intermediates III, IV, and VII must be highly reactive since the rate constant for the loss of BUD as obtained from the production of bromide ion as a function of time is essentially the same as that obtained spectrally for the loss of BUD. Lozeron and co-workers (29) have shown that 5-fluoro-6-hydroxyuracil, a product isolated from the photochemical degradation of 5-fluouracil, is extremely unstable in strong alkali at low temperatures and opens at the 3,4 position.

The possible formation of RBA from the solvolysis of CUD in strong alkali could not be monitored since RBA degrades so much faster than CUD (Fig. 5). However, the shape of the rate-pH profile for CUD is the same as that for IUD and BUD although the rate constants are of a lower order of magnitude.

If one looks at the rate-pH profiles in terms of the formation of RBA and nonchromophoric products, an interesting phenomenon can be observed. The profile for the formation of nonchromophoric products seems to follow the values predicted by Eq. 6, *i.e.*, hydroxyl ion attack on the undissociated molecule or its kinetic equivalent, water attack on the monoanion. The profile describing the formation of RBA (Fig. 5) calls for hydroxyl ion attack on the halouridine monoanion. Thus, the rate-pH profile of the halouridines seems to represent the sum of two different reactions, one of which occurs throughout the entire pH range studied and the other (formation of RBA) occurring only in strong alkali.

RBA is not formed in the bicarbonate or phosphate buffer regions. It was not observed spectrally even though it is solvolyzed in these regions at the same or slower rates than the parent halouridine (Fig. 5), and it has a much higher absorptivity coefficient than the halouridines.

The formation of OHUD and/or unstable dihydropyrimidine derivatives probably accounts for the solvolysis of the halouridines in the buffer regions. Isobarbituric acid has been prepared from bromouracil by treatment with sodium bicarbonate (25, 9). Wang (25) also reported that OHUD was prepared from BUD in over 60% of the theoretical yield using lead oxide and postulated VII as an intermediate. OHUD degrades very rapidly in the bicarbonate buffer region at 80° and would not be observed spectrally.

The fact that UD and MUD are highly stable to alkaline solvolysis does not deny the possibility of a reversible hydration of the 5,6 double bond but further emphasizes the need for an electronegative 5-substituent that can take part in an elimination or substitution reaction, and aid in facilitating the ring opening at the 3,4 position.

The authors have also presented some data on the alkaline solvolysis of FUA and FUL, the 5-fluoro arabino and lyxo analogs of uridine. These compounds solvolyze much more quickly than all the halouridines which can be explained on the basis of Fox's mechanism (27) where intramolecular nucleophilic attack by the 2′-hydroxyl anion is facilitated sterically to give the 6,2′-anhydronucleoside. Subsequent tautomerization and ring cleavage at the 3,4-position of the pyrimidine base (27) produces a compound with no ultraviolet absorption spectrum. This compound can be converted by a much slower process to 1-(β-D-arabinofuranosyl)-2-oxo-4-imidazoline-4-carboxylic acid which does have a chromophore (30). FUA degrades faster than FUL even though FUL has both the 2′ and 3′ sugar hydroxyls in the "up" position and lyxose has a lower pK than arabinose (21). It may be argued that the lyxose anion, where the 2′ and 3′ hydroxyls are vicinal, may be stabilized by a hydrogen-bonded 5-membered ring (31). Thus, the lyxo analog may have a less concentrated negative charge on a specific alkoxide ion and not react as rapidly as the arabino analog in which the negative charge may be more localized at the 2′ position.

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Keyphrases

5-Halouridines, related nucleosides—solvolysis
Solvolysis—halogen-substituted uridines
UV spectrophotometry—solvolysis monitoring
Paper chromatography—separation
UV light—nucleoside visualization
Bromide ion measurement—bromide ion activity electrode

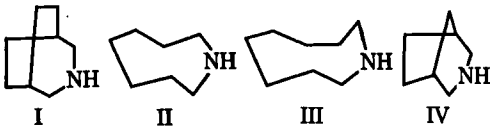
Use of 3-Azabicyclo[3.2.1]octane in the Mannich Reaction

By N. D. POTTI* and W. LEWIS NOBLES

The preparation of a group of ketonic Mannich bases utilizing 3-azabicyclo(3.2.1)-octane as the amine component is described. Also reported are the syntheses of several γ-amino secondary and tertiary alcohols obtained from these Mannich bases by sodium borohydride reduction and the Grignard reaction, respectively. These compounds are being screened for possible pharmacodynamic and chemotherapeutic activity.

AZABICYCLIC RING SYSTEMS are often found in alkaloids, many of which are medicinally useful, *e.g.*, morphine, atropine, *etc.* During the past two decades numerous 3-azabicyclic compounds were synthesized and tested for useful therapeutic activities (1–12). Many of them possessed hypotensive and antibacterial activities.

Nobles and his associates found interesting pharmacological activities associated with derivatives of complex amines like 3-azabicyclo[3.2.2]nonane I (6–8, 12); heptamethyleneimine II (13); and octamethyleneimine III (14).



I II III IV

Certain ketonic Mannich bases derived from such complex amines possessed an unexpected high order of antibacterial activity (15). The object of the present investigation was to extend the study with the complex amine, 3-azabicyclo-[3.2.1]octane (IV). A convenient laboratory method of preparation of this amine is reported elsewhere (16).

Promising pharmacological properties of β-aminoketones prompted several groups of workers to continue their studies with several of the derivatives of β-aminoketones (7–8, 13, 14, 17–19). Among these derivatives were included several γ-amino secondary and tertiary alcohols. In general, these alcohols were reported to be more

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