N-Hydroxymethyl Derivatives of Nitrogen Heterocycles as Possible Prodrugs I: *N*-Hydroxymethylation of Uracils

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Abstract □ Solid samples of 1,3-dihydroxymethyluracil, 3-hydroxymethyl-1-methyluracil, and 1-hydroxymethyl-3-methyluracil were prepared, and their structures were confirmed by spectroscopic analysis. The thermodynamics and kinetics of the formation of *N*-hydroxymethylated uracils in aqueous formaldehyde solutions also were studied. The equilibrium constants for formation of *N*-1-hydroxymethyl derivatives were approximately twice those for formation of *N*-3-hydroxymethyl derivatives, and they were formed more rapidly throughout the pH 3–8 range. Substituents at C-5 of uracil had little effect on the thermodynamics of *N*-hydroxymethylation. The potential usefulness of *N*-hydroxymethyl compounds as prodrugs is discussed.

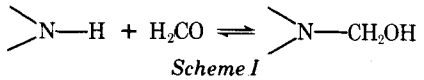
Keyphrases □ Uracil—*N*-hydroxymethyl derivatives synthesized, structures confirmed by NMR and elemental analyses, potential prodrugs □ *N*-Hydroxymethyl derivatives—of uracil, synthesis, structures confirmed by NMR and elemental analyses, potential prodrugs □ Prodrugs, potential—*N*-hydroxymethyl derivatives of uracil synthesized, structures confirmed by NMR and elemental analyses

The reversible reactions of formaldehyde with amines (1–7), amides (8, 9), and imides (10–15) in aqueous solution to form the corresponding *N*-hydroxymethyl derivatives have been studied extensively, and numerous rate and equilibrium constants have been reported. An *N*-hydroxymethylation reaction is represented in Scheme I. Reactions of this type are important in biological systems (16–19) and, because they can alter hydrogen-bonding possibilities in a molecule, they have been employed (20–22) to modify the secondary structure of nucleic acids in solutions. This paper is concerned with the kinetics and thermodynamics of *N*-hydroxymethylation of uracil (I) and several substituted uracils.

BACKGROUND

Uracil is not a drug, but its structure is representative of many molecules that are drugs. It is a heterocyclic molecule that contains both an amide and an imide group. Molecules that contain these groups frequently are very insoluble in water and organic solvents because of large crystal lattice energies due to intermolecular hydrogen bonds. There are two acidic protons in uracil, each of which can form a hydrogen bond with a carbonyl group in another molecule. Evidence that such bonds contribute to the stability of uracil crystals comes from comparison of the melting point and the solubility of uracil with those of *N*-alkyl uracils (Table I). The melting point of uracil is decreased and the solubility is increased when the N-1 and/or N-3 protons are replaced by a methyl group. IR and X-ray structure studies of uracil support the conclusion that, in the uracil crystals, molecules are linked by intermolecular hydrogen bonds (23–25).

The replacement of the N–H proton by a methyl group is not normally an allowable substitution in drug molecules, because it is a permanent change and can significantly change the pharmacological properties of the drugs. A better approach toward reducing the extent of intermolecular hydrogen bonds in a molecule like uracil would be the synthesis of transient chemical derivatives, *i.e.*, prodrugs. In prodrug molecules, the N–H protons could be replaced by groups such as the hydroxymethyl, which would be removed rapidly in water.



Scheme I

When the prodrug reverts to the parent drug, formaldehyde is released. Formaldehyde toxicity does not appear to be of great concern since pivampicillin and methenamine, which are marketed as safe drugs, also release formaldehyde in the body upon administration (26–29). However, formaldehyde toxicity may depend on the frequency of dose and the duration that the drug has to be taken.

This approach should be applicable to other heterocyclic drug molecules that are poorly soluble in water and other solvents by virtue of intermolecular hydrogen bonds in their crystalline phase. The application of some of these concepts to drug molecules recently was reported (30, 31).

RESULTS AND DISCUSSION

Structure of Hydroxymethyl Derivatives of Uracil (I), 3-Methyluracil (III), and 1-Methyluracil (V)—Elemental analysis of the crystalline compound that precipitated from uracil solution in aqueous formaldehyde was consistent with the composition of 1 equivalent of uracil and 2 equivalents of formaldehyde. Evidence that the compound had Structure II rather than Structure VIII came from IR analysis (potassium bromide disks) and NMR spectra. The IR spectrum exhibited a strong peak at 1720 cm^{-1}, indicating the molecule contained at least one carbonyl group. The two doublets at 7.70 and 5.72 ppm in the NMR spectrum of the compound in dimethyl sulfoxide-d_6 (Table II), which were assigned (32) to the C-6 and C-5 protons, are much closer in chemical shift to those of the same protons of 1,3-dimethyluracil (VII, a model for Structure II, 7.68 and 5.67 ppm in dimethyl sulfoxide-d_6) than they are to those of 2,4-dimethoxypyrimidine (IX, a model for Structure VIII, 8.31 and 6.53 ppm).

Assignment of the other signals in the NMR spectrum of 1,3-dihydroxymethyluracil (II) was achieved by comparison with the spectra of 1-hydroxymethyl-3-methyluracil (IV) and 3-hydroxymethyl-1-methyluracil (VI) in dimethyl sulfoxide-d_6. The latter compounds were prepared from III and V.

Features of the NMR spectrum of a 50-mg/ml solution of IV in dimethyl sulfoxide-d_6 are described in Table II. The broad singlet at 6.62 ppm disappeared when water was added to the solution and can be assigned to the OH proton. The broad singlet at 5.10 ppm enclosed an area that was double that of the OH proton, and it sharpened when either deuterium oxide or trifluoroacetic acid was added (to increase exchange

Table I—Solubility and Melting Point of Uracil and *N*-Alkylated Uracils

Compound	Melting Point	Solubility in Water, mg/ml
I	340°	3
III	180°	200
V	232°	20
VII	123°	500

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Table II—NMR Data for Uracil and Substituted Uracils in Aprotic Solvents

Compound	Solvent[a]	Concentration, mg/ml	Chemical Shifts, ppm[b] C-6 H	C-5 H	N-1 CH_2	OH	N-3 CH_2	OH
I	A	50	7.65 d	5.56 d	—	—	—	—
II	A	50	7.70 d	5.72 d	5.13 bs	6.72 bs	5.25 d	6.20 t
III	A	50	7.40 d	5.57 d	—	—	—	—
IV	A	50	7.65 d	5.70 d	5.10 bs	6.62 bs	—	—
	A	10	7.72 d	5.76 d	5.13 d	6.66 dt	—	—
	B	50	7.30 d	5.76 d	5.20 bs	4.70 bs	—	—
	B	25	7.26 d	5.76 d	5.20 bs	4.36 dt	—	—
	B	12.5	7.26 d	5.76 d	5.20 d	4.08 t	—	—
	B	6.25	7.24 d	5.75 d	5.18 d	3.87 t	—	—
VI	A	50	7.63 d	5.62 d	—	—	5.20 d	6.13 t
	A	10	7.63 d	5.62 d	—	—	5.20 d	6.13 t
	B	50	7.20 d	5.78 d	—	—	5.50 d	4.12 t
	B	12.5	7.20 d	5.78 d	—	—	5.50 d	4.12 t

[a] Solvent A was dimethyl sulfoxide-d_6, and Solvent B was deuterochloroform. [b] Symbols are: d, doublet; bs, broad singlet; t, triplet; and dt, diffuse triplet.

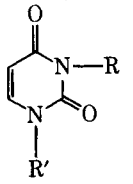
rates) or when the OH proton was irradiated. These results indicate that the signal at 5.10 ppm can be assigned to the N-1 CH_2O protons in IV and that, in this relatively concentrated solution, they are only weakly coupled to the OH proton.

The multiplicity of the signals at 6.62 and 5.10 ppm was different in less concentrated solutions. When the concentration of IV in dimethyl sulfoxide-d_6 was 10 mg/ml, the OH proton was a diffuse triplet at 6.66 ppm and the N-1 CH_2O protons were a doublet centered at 5.13 ppm. Similar changes in multiplicity with change in concentration were observed for spectra in deuterochloroform (Table II). These results suggest that the OH proton is more strongly coupled to the N-1 CH_2O protons in dilute rather than in concentrated solutions. The OH proton probably participates in intermolecular hydrogen-bonding reactions with other IV molecules in concentrated solutions.

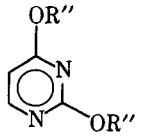
The spectra of VI solutions in dimethyl sulfoxide-d_6 are described in Table II. One difference between VI and IV is that its spectra in dimethyl sulfoxide-d_6 and in deuterochloroform were insensitive to concentration changes between 50 and 6.25 mg/ml. The OH resonance was a triplet centered at 6.12 ppm and was strongly coupled with the N-3 CH_2O protons that appeared as a sharp doublet centered at 5.26 ppm. The N-3 CH_2OH proton appears to have a much smaller tendency than the N-1 CH_2OH proton to participate in intermolecular hydrogen bond formation.

Comparison of the NMR spectral characteristics of II with those of IV and VI enabled the following peak assignments to be made: C-6 H, 7.70 ppm; N-1 CH_2OH, 6.72 ppm; N-3 CH_2OH, 6.20 ppm; C-5 H, 5.72 ppm; N-3 CH_2OH, 5.25 ppm; and N-1 CH_2OH, 5.13 ppm.

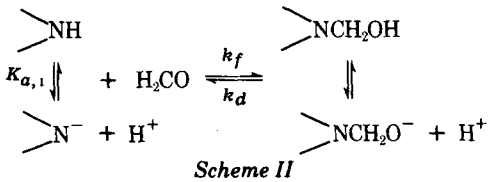
Thermodynamics and Kinetics of *N*-Hydroxymethylation of III and V in Water—The addition of 2.5 *M* formaldehyde to solutions of III or V (pH 4–10) produced changes in UV and NMR spectra that were complete within 4 hr at pH 4 and in <30 min at pH >7. The resulting spectra did not change during 7 days at 25°. UV spectral characteristics of reactants and products are in Table III, and NMR data (in deuterium oxide) are in Table IV.



I: R = R′ = H
II: R = R′ = CH_2OH
III: R = CH_3, R′ = H
IV: R = CH_3, R′ = CH_2OH
V: R = H, R′ = CH_3
VI: R = CH_2OH, R′ = CH_3
VII: R = R′ = CH_3



VIII: R″ = CH_2OH
IX: R″ = CH_3



Scheme II

Confirmation that the products were IV and VI, respectively, came from the fact that the final solutions had identical spectra to solutions of authentic material in the same solvents.

Rate and equilibrium constants were calculated from UV changes. A pseudo-first-order rate constant, k_{obs}, was calculated from plots of log $(A - A_\infty)$ *versus* time for reactions in solutions containing excess formaldehyde. Values of k_{obs} were related linearly to the initial formaldehyde concentration, $[H_2CO]_0$, and the line had a positive intercept on the y axis when $[H_2CO]_0 = 0$. Thus, it was concluded that k_{obs} values were related to values of $[H_2CO]_0$:

$$k_{obs} = k_f[H_2CO]_0 + k_d \qquad \text{(Eq. 1)}$$

where k_f and k_d are the second- and first-order rate constants, respectively, for the reaction depicted in Scheme II. Values of k_f and k_d calculated from plots of k_{obs} against $[H_2CO]_0$ are in Table V.

The pH dependence of k_f and k_d is consistent with the conclusion that the rate-determining step in the forward reaction involves the uracil anion and formaldehyde and that for the reverse reaction involves the anion of the *N*-hydroxymethyl derivative. The mechanism proposed for the reverse reaction is identical to the one that was suggested (31, 32) to account for the decomposition of *N*-hydroxymethyl derivatives of other amides and imides.

According to the model, the rate law for the rate-determining step in the reactions would be:

$$k_{obs} = \frac{k_f' K_a^u}{K_a^u + [H^+]}[H_2CO]_0 + \frac{k_d' K_a^{HU}}{K_a^{HU} + [H^+]} \qquad \text{(Eq. 2)}$$

where K_a^u and K_a^{HU} are the acid dissociation constants of the uracil and the *N*-hydroxymethyl derivative (*i.e.*, to yield $>NCH_2O^-$), respectively, and k_f' and k_d' are the rate constants for reactions of the anionic species. The values of K_a^u for III and V are 1×10^{-10} and 2×10^{-10}, respectively (33). The K_a^{HU} values are not known, but they are expected to be larger than the K_a^u values because the negative charge would be localized on the oxygen atoms in the *N*-hydroxymethyl anions whereas it would be delocalized in the uracil anions. Hence, under the experimental conditions, both K_a^{HU} and K_a^u are less than $[H^+]$ and can be neglected from the de-

Table III—UV Spectral Characteristics of Aqueous Solutions of Uracil Derivatives

Compound	Solvent	λ_{max}, nm	ϵ_{max}
V	Buffer, pH 7	265.5	9900
	Buffer, pH 7, + 2.5 *M* H_2CO	268.5	9300
III	Buffer, pH 7,	257.5	7800
	Buffer, pH 7, + 2.5 *M* H_2CO	258	8900
I	Buffer, pH 7	257.5	8200
	Buffer, pH 7, + 2.5 *M* H_2CO	261	8400
VII	Buffer, pH 7	265	9200
	Buffer, pH 7, + 2.5 *M* H_2CO	265	9200

Table IV—NMR Data for Uracil and Substituted Uracils in Deuterium Oxide

Compound	Solvent[a]	Chemical Shifts, ppm[b] C-6 H	C-5 H	N-1 CH_2OD	N-3 CH_2OD
I	A	7.55 d	5.83 d	—	—
	B	7.68 d	5.88 d	5.23 s	5.33 s
III	A	7.50 d	5.86 d	—	—
	B	7.68 d	5.89 d	5.24 s	—
V	A	7.63 d	5.80 d	—	—
	B	7.57 d	5.82 d	—	5.39 d

[a] Solvent A was deuterium oxide, and Solvent B was 2.5 *M* H_2CO in deuterium oxide. [b] Symbols are: d, doublet; and s, singlet.

nominators in Eq. 2, which becomes:

$$k_{obs} = \frac{k_f' K_a^u}{[H^+]}[H_2CO]_0 + \frac{k_d' K_a^{HU}}{[H^+]} \quad \text{(Eq. 3)}$$

By definition:

$$k_f' = \frac{k_f[H^+]}{K_a^u} \quad \text{(Eq. 4)}$$

$$k_d' = \frac{k_d[H^+]}{K_a^{HU}} \quad \text{(Eq. 5)}$$

Confirmation that the model is consistent with the data comes from the fact that values of $k_f[H^+]/K_a^u$ (*i.e.*, k_f') and $k_d[H^+]$ (*i.e.*, $k_d' K_a^{HU}$) were essentially constant between pH 3 and 6.

The anion of III is a stronger base than that of V, and it reacts ~40 times more rapidly with formaldehyde. Both properties are likely to arise because the anion of V is stabilized by delocalization of its electrons among the adjacent oxygen atoms.

Equilibrium constants for the *N*-hydroxymethylation reactions of III and V were calculated both from values of the quotient k_f/k_r and from the slopes of plots of $(A_0 - A)/[H_2CO]_0$ *versus* A, where A_0 is the initial absorbance of uracil solution and formaldehyde and A is the absorbance of a similar solution in which hydroxymethylation has reached equilibrium. Values of the equilibrium constants for III and V are in Table VI.

The results indicate that the magnitude of the equilibrium constant for formation of the >N-1 CH_2OH derivative is double that for formation of the >N-3 CH_2OH derivative. Thus, IV is formed more rapidly and to a greater extent than VI.

No *N*-hydroxymethylation of III and V was observed to occur at pH values two or more units above their pKa values.

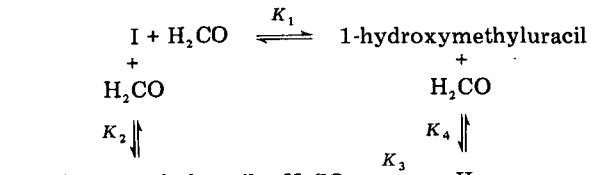
Thermodynamics of Uracil *N*-Hydroxymethylation—Addition of 2.5 *M* H_2CO to uracil solutions in water (or deuterium oxide) caused the UV and NMR spectra to change to those produced by equimolar solutions of II. Relevant data are shown in Tables III and IV.

Knowledge of the reactions of III and V in aqueous formaldehyde, together with the knowledge that II also is formed, leads to the conclusion that the relevant reactions of uracil are those shown in Scheme III. The magnitudes of the equilibrium constants in Scheme III were evaluated both from changes in UV absorbance and from phase solubility diagrams. All experiments were carried out at pH 3–7, a range in which the reactants and products were not appreciably ionized.

The absorbance of an equilibrated solution of uracil in aqueous formaldehyde, A, can be related to the initial absorbance of the solution, A_0:

$$\frac{A}{A_0} = \frac{1 + K_1[H_2CO]_0\epsilon_1 + K_2[H_2CO]_0\epsilon_2 + K_1K_4[H_2CO]^2\epsilon_3}{1 + K_2[H_2CO]_0 + K_2[H_2CO]_0 + K_1K_4[H_2CO]^2} \quad \text{(Eq. 6)}$$

where ϵ_1 is the ratio of molar extinction coefficients for 1-hydroxymethyluracil and uracil, ϵ_2 is the ratio for 3-hydroxymethyluracil and uracil, and ϵ_3 is the ratio for II and uracil. An iterative procedure that utilized Eq. 6 and experimental data yielded values of $K_1 + K_2$ and K_1K_4.



Scheme III

By definition:

$$K_1/K_2 = K_3/K_4 \quad \text{(Eq. 7a)}$$

The value of K_1/K_2 was evaluated using Eq. 7a on the basis that the value of K_3/K_4 would be the same as that for the ratio of equilibrium constants for *N*-hydroxymethylation of III and for V. In other words:

$$\frac{K_1}{K_2} = 2.26 \quad \text{(Eq. 7b)}$$

When this assumption had been made, values of K_1, K_2, K_3, and K_4 were computed from the values of $K_1 + K_2$, K_1K_4, and K_3/K_4 (Table VI).

The second method of calculating equilibrium constants was based on phase solubility analysis. Experiments involved measuring the total amount of uracil that dissolved $[I]_T$ in solutions containing different amounts of formaldehyde. The solubility of uracil in solutions that did not contain formaldehyde was $[I]_0$. Equilibrium constants were calculated by:

$$\frac{[I]_T - [I]_0}{[I]_0([I]_T - [I]_0 - [H_2CO]_0/2)} = \frac{4K_1K_4([I]_T - [I]_0 - [H_2CO]_0/2)}{\{(K_1 + K_2)[I]_0 - 1\}^2} + \frac{2(K_1 + K_2)}{(K_1 + K_2)[I]_0 - 1} \quad \text{(Eq. 8)}$$

Figure 1 shows a plot of $[I]_T$ *versus* $[H_2CO]_0$. The plot is an Ap type (34) of phase solubility diagram, which indicates a 1:1 interaction of ligand (formaldehyde) with the substrate (I). Figure 2 shows a linear plot of $([I]_T - [I]_0)/\{[I]_0([I]_T - [I]_0 - [H_2CO]_0/2)\}$ *versus* $([I]_T - [I]_0 - [H_2CO]_0/2)$. Values of K_1K_4 and $(K_1 + K_2)$ were calculated from the slope of this plot and the intercept at $[H_2CO]_0 = 0$, respectively. The magnitudes of the individual equilibrium constants were calculated by utilizing the relationship $K_3/K_4 = K_1/K_2$ and making the approximation that $K_3/K_4 = 2.26$ (Table VI).

The similarity between K values calculated from both UV spectrophotometric methods and phase solubility studies adds support to the validity of the model.

Lewin (11) studied the interaction of uracil with aqueous formaldehyde by measuring changes that occur in pH when formaldehyde is added to partially neutralized uracil solutions. The pH changes occur because each *N*-hydroxymethylation reaction removes one acidic hydrogen atom from the uracil molecule. Lewin (11) interpreted his results as indicating that only IV was formed to any appreciable extent. The appropriate equilibrium constant was evaluated using:

$$\text{antilog } \Delta pH = 1 + K_2[H_2CO]_0 \quad \text{(Eq. 9)}$$

The model used in the present study requires that:

$$\text{antilog } \Delta pH = \frac{1 + (K_1 + K_2)[H_2CO]_0 + K_1K_4[H_2CO]_0^2}{1 + 0.32K_2[H_2CO]_0 + 0.63K_1[H_2CO]_0} \quad \text{(Eq. 10)}$$

where 0.63 is the ratio of acid dissociation constants for 3-hydroxymethyluracil and uracil and 0.32 is the ratio for 1-hydroxymethyluracil and uracil. These ratios were evaluated by assuming that the pKa values

Table V—Rate Constants for *N*-Hydroxymethylation of III and V at 25°

Compound	pH	$10^4 k_{obs}$[a], min^{-1}	$10^2 k_f$, $M^{-1}\,min^{-1}$	$10^{-5} k_f'$, $M^{-1}\,min^{-1}$	$10^2 k_d$, min^{-1}	$10^6 k_d' K_a^{HU}$, $min^{-1}\,M$
III	3.06	219	1.46	1.27	0.73	6.36
	4.01	1,760	12.8	1.25	6.82	6.66
	5.04	20,000	128	1.17	74	6.75
V	4.05	116	0.75	0.033	0.47	0.42
	5.01	1,220	6.92	0.034	5.97	0.55
	6.02	12,000	68.4	0.033	54.9	0.52

[a] Values of k_{obs} were calculated at $[H_2CO]_0 = 1$ *M*.

Table VI—Equilibrium Constants for *N*-Hydroxymethylation of Uracil and Uracil Derivatives at 25° and μ = 0.1 *M*

Compound	pH Range	Method	K, M^{-1}	K_1, M^{-1}	K_2, M^{-1}	K_3, M^{-1}	K_4, M^{-1}
V	4–7	UV	1.5				
	4–6	Kinetic	1.3				
III	4–7	UV	3.4				
	4–6	Kinetic	2.2				
I	5–7	Phase solubility		5.2	2.3	2.2	1.2
	5–7	UV		5.3	2.3	4.0	1.8
5-Methyluracil	4–6	UV		3.1	1.4	5.8	2.5
5-Fluorouracil	4	UV		4.2	1.9	4.4	1.9
5-Chlorouracil	4	UV		4.1	1.8	6.4	2.8
5-Bromouracil	4	UV		4.8	2.1	5.5	2.4
5-Iodouracil	4	UV		4.2	1.9	6.7	2.9

of the *N*-hydroxymethyluracil derivatives were equal to those of the respective *N*-methyluracils.

If $[H_2CO]_0$ is in excess and the values $K_1 = K_2 = K_3 = K_4 = L$, Eq. 10 reduces to:

$$\text{antilog } \Delta pH = \frac{(1 + L[H_2CO]_0)^2}{1 + 0.95L[H_2CO]_0} \simeq 1 + L[H_2CO]_0 \quad \text{(Eq. 11)}$$

Hence, Lewin's method was not sensitive enough to distinguish between the various *N*-hydroxymethyl derivatives of uracil.

Inspection of the *K* values in Table VI reveals that *N*-hydroxymethylation of N-1 of uracil is the most favored reaction whether or not an *N*-hydroxymethyl group is present on N-3.

The strength of the *N*-hydroxymethylation reactions is greater than would be immediately concluded from the magnitude of the constants in Table VI. Formaldehyde exists in water as an equilibrium mixture of anhydrous and hydrated formaldehyde. The equilibrium constant for the hydration reaction (35) is 41 M^{-1} at 25°. Hence, if the concentration of water is taken to be 55.5 *M*, the equilibrium constants for reaction of the uracils with anhydrous formaldehyde are the values in Table VI multiplied by the factor of 2277, which takes into account the relative concentration of anhydrous formaldehyde. Consequently, *N*-hydroxymethylation is a strong reaction as are the addition reactions of other amines to formaldehyde (36).

Thermodynamics of *N*-Hydroxymethylation of 5-Substituted Uracils—UV spectrophotometric measurements, identical to those described in the previous section, were used to calculate the *K* values for *N*-hydroxymethylation of the 5-substituted uracils (Table VI).

Although there is about a 200-fold difference in the acidity of the N–H functions of these compounds (pKa 7.7–9.9), the affinity of formaldehyde for 5-substituted uracils is essentially independent of their acidities, and substitution at the 5-position does not influence the magnitude of equilibrium constants in a systematic way. These results are consistent with reports that the equilibrium constants for the addition to formaldehyde of simple primary amines (1) are not much larger than those for addition of urea and amides (8), although the difference in the pKa of amine and amide groups is large.

Melting Point, Solubility, and Dissolution Rate of II—Comparison of uracil (I) and II indicated that II had a lower melting point (101° compared to 340°), higher water solubility (500 mg/ml compared to 2.8 mg/ml)[1], and higher water dissolution rate (82×10^{-5} *M*/min compared to 1.6 *M*/min)[1]. This behavior was predicted on the basis that *N*-hydroxymethylation changes intermolecular hydrogen-bonding possibilities in the solid phase. It supports the contention that such derivatives are potentially useful prodrugs.

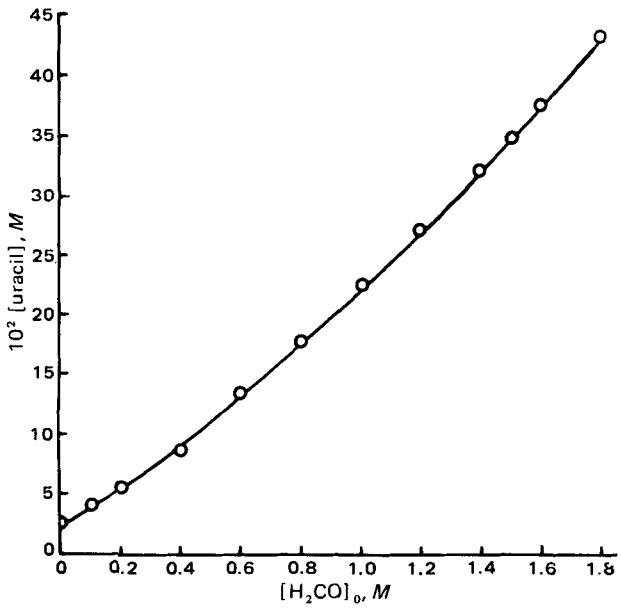


Figure 1—*Phase solubility diagram of uracil in aqueous formaldehyde at 25°, 0.1 M I.*

EXPERIMENTAL

Uracil[2], 5-fluorouracil[2], 5-bromouracil[2], 5-iodouracil[2], 5-methyluracil[2], 1-methyluracil[3], and 3-methyluracil[3] were used without further purification. 5-Chlorouracil[4] was recrystallized from aqueous ethanol. Formaldehyde (37% aqueous solution) was used without further purification, and its concentration was calculated using the USP method (38). Formaldehyde used in the NMR studies was obtained by dissolving paraformaldehyde in deuterium oxide. Buffer solutions at μ = 0.1 *M* were prepared as described previously (39).

Tetramethylsilane or sodium 4,4-dimethyl-4-silapentanesulfonate was used as the reference in NMR spectral measurements.

Preparation of *N*-Hydroxymethyl Derivatives—*1,3-Dihydroxymethyluracil (II)*—Uracil (I) (4.75 g) was dissolved in 10 ml of 37% (w/w) formaldehyde (pH adjusted to 7 with sodium hydroxide) at 25°. Refrigeration of this solution resulted in a white crystalline precipitate which, following recrystallization from acetonitrile and drying over calcium chloride, melted at 101° (melting point of uracil was 338°). The mass spectrum (*m/z* 172) and elemental analysis of the solid were consistent with the conclusion that the compound was II.

Anal.—Calc. for $C_6H_8N_2O_4$: C, 41.84; H, 4.65; N, 16.28. Found: C, 41.62; H, 4.63; N, 16.38.

3-Hydroxymethyl-1-methyluracil (VI)—1-Methyluracil (V) (600 mg) was dissolved in 1 ml of 37% formaldehyde. Refrigeration of this solution resulted in a white crystalline precipitate which, on drying over calcium

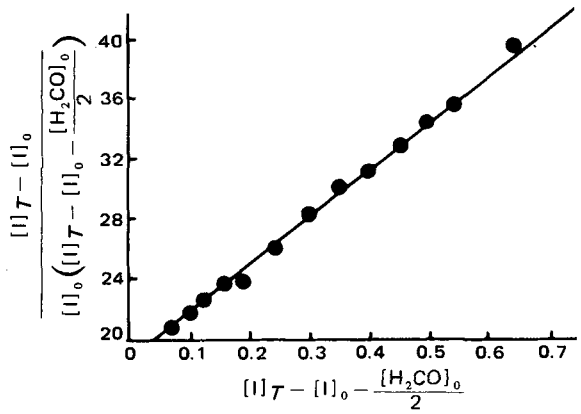


Figure 2—*Plot of* $([I]_T - [I]_0)/[I]_0([I]_T - [I]_0 - [H_2CO]_0/2)$ *versus* $[I]_T - [I]_0 - ([H_2CO]_0/2)$ *for the reaction of uracil and formaldehyde at 25°, 0.1 M I.*

[1] The methods of calculating these values were identical to those described in Ref. 37.

[2] Sigma Chemical Co.

[3] Cyclo Chemical Co.

[4] K & K Laboratories.

chloride, melted at 110° (melting point of V was 232°). The mass spectrum (*m/z* 156) and elemental analysis of the solid were consistent with the conclusion that the compound was VI.

Anal.—Calc. for $C_6H_8N_2O_3$: C, 46.15; H, 5.13; N, 17.95. Found: C, 46.28; H, 5.10; N, 18.15.

1-Hydroxymethyl-3-methyluracil (IV)—3-Methyluracil (III) (1 g) was dissolved in 1 ml of 37% formaldehyde. The crystals of IV were obtained as described for II and VI and, on drying over calcium chloride, melted at 92° (melting point of III was 178°). The mass spectrum (*m/z* 156) and elemental analysis of the solid were consistent with the conclusion that the compound was VI.

Anal.—Calc. for $C_6H_8N_2O_3$: C, 46.15; H, 5.13; N, 17.95. Found: C, 46.04; H, 5.22; N, 17.77.

Kinetic Measurements—The rates of formation of hydroxymethyl derivatives of III and V were monitored spectrophotometrically by following the change in UV absorbance that occurred when 0.1 ml of a 0.003 *M* solution of III or V was added to a 1-cm stoppered UV cell containing 3 ml of formaldehyde solution in buffer equilibrated at 25° and $\mu = 0.1$ *M*. The rates of the forward and reverse reactions and the equilibrium constants were determined as already described. All kinetic runs were conducted under pseudo-first-order conditions in which formaldehyde was used in large excess.

Determination of Equilibrium Constants by Spectrophotometry—Solutions of uracil and 5-substituted uracils (0.0001 *M*) were prepared in buffered formaldehyde solutions. The solutions were equilibrated in a thermostated bath at 25°. The UV absorbance of equilibrated solutions at a fixed wavelength was recorded at several pH values (pH 4–7), and equilibrium constants were calculated as described.

Phase Solubility Studies—Uracil (500 mg) was weighed in a series of 25-ml polyseal-lined screw-capped vials to which was added exactly 5 ml of formaldehyde of increasing concentrations (0–2 *M*) in buffer ($\mu = 0.1$ *M*). The vials were sealed, placed in a constant-temperature water bath at 25.00 ± 0.02°, and agitated with a rotating-action shaker until equilibrium was achieved (usually within 5 days). The total amount of uracil in solution in each vial was determined spectrophotometrically at λ_{max} 257 nm by diluting aliquots of filtered equilibrated solutions in the buffer.

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