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Base-Catalyzed Reactions of 1,3-Disubstituted Uracils[1]

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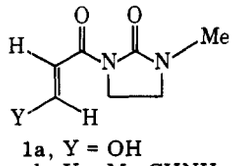
The first step in the base-catalyzed hydrolysis of 1,3-dialkyluracils (**2**) in both aqueous and Me_2SO solutions involves Michael addition to C-6 followed by ring opening between N-1 and C-6 to an enolate (**5**). From aqueous solution, with 1,3-dimethyluracil (**2a**) as substrate, formylacetic acid (**4'**) and *N,N'*-dimethylurea (**3a**) were isolated, but **5** was not observed. On the other hand, in Me_2SO evidence for the very rapid formation of the intermediate enolate was obtained. This enolate, however, was not the stable end product. In the reaction mixture it underwent a complex series of transformations leading finally to the formation of three products. Using **2a** as the disubstituted uracil, these were *N,N'*-dimethylurea (**3a**) and two *pyridine* derivatives: 1-methyl-5-(methylcarbamyl)-2-pyridone (**12a**) and 1-methyl-3-(methylaminomethylene)-2,6-pyridinedione (**13a**). 1-Ethyl-3-methyluracil (**2b**), 3-ethyl-1-methyluracil (**2c**), and 1,3-diethyluracil (**2d**) also yielded analogous, stable end products under the same conditions. A scheme is proposed to rationalize the conversion of **2** to **3**, **12**, and **13** by means of tetramethylammonium hydroxide in Me_2SO solution. The elaboration of this scheme was based on a body of data which included the isolation and characterization of an intermediate, 1,3-dimethyl-5-(methylaminomethylene)-6-(methylcarbamylmethyl)-5,6-dihydrouracil (**10a**), from the reaction of **2a** with TMAH in Me_2SO. It was found that the ethylmethyluracils, **2b** and **2c**, underwent an isomerization reaction in basic Me_2SO in which the positions of the alkyl groups were interchanged. This reaction, as well as the conversion of **2d** to **2a** by means of *N,N'*-dimethylurea in the same medium, provides additional evidence for the reversibility of the transformation of uracil, **2**, to enolate, **5**. The results obtained in this investigation provide alternative mechanisms for H-5 exchange in uracil derivatives and for a number of enzymatic C-methylation reactions. They also suggest a pathway for the biosynthesis of nudiflorine, a naturally occurring 2-pyridone, other than that previously reported.

Although the fact that 1,3-disubstituted uracils are unstable in alkaline solution has been known for more than two decades,[2] it is only recently that an aldehyde has been postulated as an intermediate in the degradation of such compounds.[3] An analogous aldehyde has been postulated as an intermediate in reactions of various substituted 6-hydroxy-5,6-dihydrouracils.[4]

During the course of a study of the reactions of 3-(β-methanesulfonyloxyethyl)-1-methyluracil with bases,[5] an aldehyde was obtained and was identified as N^1-(formylacetyl)-N^3-methylimidazolidone (**1a**). The UV absorption



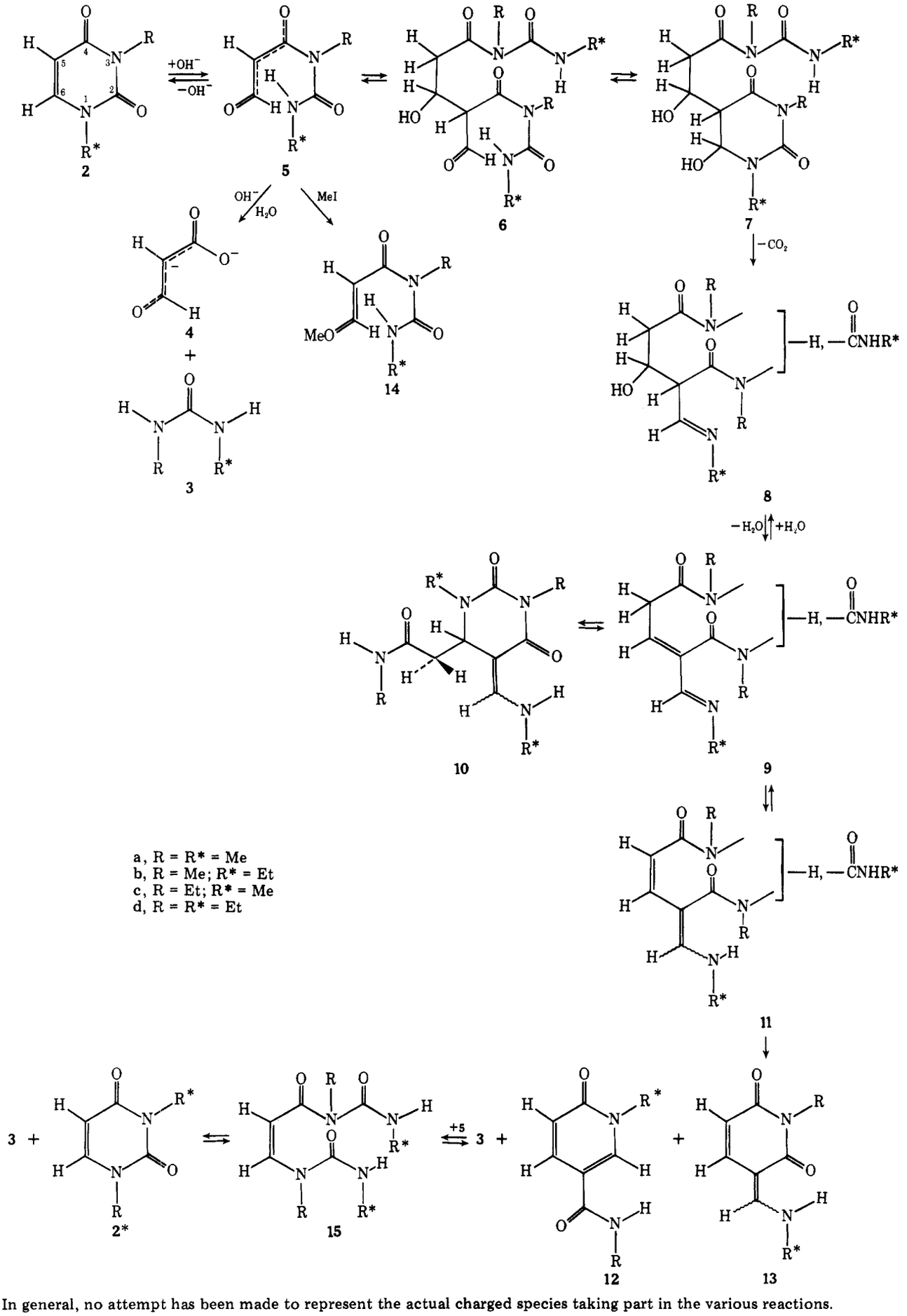
1a, Y = OH
b, Y = Me_2CHNH

characteristics of Shugar's intermediate[4b] were the same as those of **1a**. Our present experiments elucidate, in detail, the base-catalyzed reactions of 1,3-dialkyluracils in both water and dimethyl sulfoxide (Me_2SO).

The reaction of 1,3-dimethyluracil (**2a**) with sodium hydroxide or tetramethylammonium hydroxide pentahydrate (TMAH) in aqueous solution was followed by means of both UV and ^1H NMR spectroscopy. The spectroscopic changes which were observed corresponded to the formation of 1,3-dimethylurea (**3a**) and the enolate of formyl acetate (**4**) (Scheme I). Unreacted **2a**, **3a**, and anion **4**, as its parent acid (**4'**),[6] were recovered from a reaction mixture by ion exchange chromatography. No intermediates were observed in this degradation even when the reaction conditions were modified. The spectroscopic properties of **4** support the structure assigned to it. The identity of **4'** was confirmed by conversion to its oxime.[7]

The behavior of **2a** in Me_2SO solution containing TMAH was much more complex than in aqueous solution. Scheme I is a representation that is in good agreement with our experimental observations. Compound **2a** was dissolved in Me_2SO containing TMAH. The changes which took place were followed by spectrophotometric observation of the reaction mixture. With a solution 0.1 M in both reactants, it was found that **2a** disappeared and a single new chromophore with λ_{max} 296 nm (**5a**) was produced. The rapidity with which this

Scheme I[a]



a, R = R* = Me
b, R = Me; R* = Et
c, R = Et; R* = Me
d, R = R* = Et

[a] In general, no attempt has been made to represent the actual charged species taking part in the various reactions.

Table I. Reactions of 1,3-Dialkyluracils with TMAH in Me_2SO

1,3-Dialkyluracil		Products[a]		
Substrate[b]	% recovered	3, %	12, %	13, %
2a	5	58	27	39
2a (excess base)	0		0	25
2b	9	45	13	25
2c	12	44	6	3
2d	19	40	2	1

[a] These yields are based on the conversion of 2 mol of **2** to 1 mol of **3** and 1 mol of **12** or **13**. [b] The reaction mixtures were ca. 0.06 M in **2** and 0.07 M in TMAH, except in the second reaction with **2a**. In this case, **2a** was ca. 0.03 M and the base 0.10 M.

chromophore reached its maximum absorbance (less than 3–4 min) was striking. As the reaction proceeded, λ_{max} 296 nm was replaced by a transitory peak at λ_{max} 320 nm (e.g., **10a**). Finally, after about 6–7 days, two stable chromophores appeared which had absorption maxima at ca. 260 (**12a**) and 365 nm (**13a**). It is to be emphasized that the precise spectrophotometric changes which are observed, and the products which are isolated from a reaction mixture, are very dependent on reaction conditions.[8] For example, if reactions are run at ten times the usual dilution, the absorption at λ_{max} 296 nm slowly disappears and there occurs a net loss of UV-absorbing compounds.

Four compounds were isolated from a 0.1 M reaction mixture in Me_2SO after it reached the stage where it was not undergoing further change. These were **2a**, **3a**, and two compounds which proved to be *pyridine* derivatives. One of these was shown to be 1-methyl-5-(methylcarbamyl)-2-pyridone (**12a**). This assignment of structure is based on a comparison of its properties with those of nudiflorine, 5-cyano-*N*-methyl-2-pyridone, and the corresponding carboxylic acid and methyl ester.[9] The other pyridine derivative was shown to be 1-methyl-3-(methylaminomethylene)-2,6-pyridinedione (**13a**) on the basis of its molecular formula, a comparison of its UV spectrum with that of **1b**,[10] and its other spectroscopic properties.

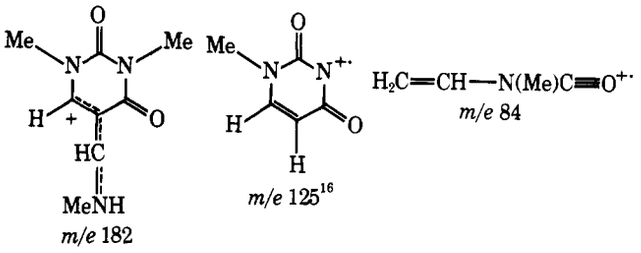
Additional significant information concerning the final reaction products was obtained in an experiment in which 1-ethyl-3-methyluracil (**2b**) was treated with TMAH in Me_2SO solution. The products were unequivocally shown to be *N*-ethyl-*N'*-methylurea (**3b**), **12b** in which the ethyl group is on the ring nitrogen, and **13b** in which the ethyl group is on the enamine nitrogen. These facts require an enamine structure to be formed prior to a ring closure step leading to **12b**.

When the reaction was run with 3-ethyl-1-methyluracil (**2c**) and 1,3-diethyluracil (**2d**), it was found by means of UV spectrophotometry that **5** was formed readily from these two compounds also. Since the stable end products, **12** and **13**, were obtained in poor yields (Table I), it is obvious that subsequent reactions of **5c** and **5d**, other than those indicated in Scheme I, predominated.

Having firmly established the end products of the reactions of **2a** and **2b** in Me_2SO containing TMAH, further evidence was sought for the nature of the intermediates. A crystalline precipitate was deposited within a few minutes after the reaction mixture containing **2a** and TMAH in Me_2SO was prepared. When attempts were made to purify the solid, the first intermediate observed, it underwent rapid chemical change. It appears to be the tetramethylammonium salt of the enolate, **5a**. Although it was not possible to purify **5a** owing to its instability, chemical and physical evidence support the assignment of structure. For example, dilution of a Me_2SO solution of **5a** with 0.1 N HCl or NaOH brings about ring closure to **2a**.[4b] With the 0.1 N NaOH, however, partial hydrolysis of **5a** to **4** also takes place. This conversion is supported by the fact that a hypsochromic shift is observed upon hydrolysis of the acyl ureido group of **5a** to a carboxyl group.[11]

Finally, **5a** was trapped as its enol ether, [(*E*)-3-methoxypropenoyl)]-*N,N'*-dimethylurea (**14a**), by addition of methyl iodide to a reaction mixture containing only **5a** (UV). The yield was 2%. In addition, 49% of **2a** and 22% of 1,3-dimethylthymine were isolated from the reaction mixture. The **2a** which was isolated after the methylation reaction was formed by ring closure.[4b] The 1,3-dimethylthymine apparently arises from methylation of **5a**[12] at C-5,[13] again followed by re-formation of the pyrimidine[4b] ring. This pathway for the formation of 1,3-dimethylthymine is reasonable. When base is added to a mixture of **2a** plus methyl iodide, the base is consumed by reaction with the latter but no 1,3-dimethylthymine is formed. Furthermore, anion formation at C-6, not C-5, of the pyrimidine ring[14] occurs under the conditions used in this experiment.

A second intermediate, which was isolated as a pure, crystalline compound, is 1,3-dimethyl-5-(methylaminomethylene)-6-(methylcarbamylmethyl)-5,6-dihydrouracil (**10a**, Scheme I). This assignment of structure is in agreement with its molecular formula and its spectroscopic[15] and chemical properties. Although the parent ion peak in the mass spectrum (*m/e* 254) is small, peaks are present which correspond to the structures



When **10a** was dissolved in a solution of TMAH in Me_2SO, 67% of **12a** and 12% of **13a** were formed after 18 h at room temperature. This ratio of **12a** to **13a** is quite different from the 2:3 ratio observed (Table I) when **12a** and **13a** are formed directly from **2a**, and may indicate that **10a** is not the compound with λ_{max} (Me_2SO) 320 nm observed spectrophotometrically in the reaction mixture, but rather an artifact of the workup. Pathways for product formation which are dependent on the position of the carbamyl group in a species such as **8** could give rise to different ratios of **12** to **13**.

The transformation of **2** to **5** in Me_2SO solution is analogous to reactions which have been described previously.[5] The first step is a Michael addition of hydroxide ion to C-6 of **2** followed by ring opening in the resulting adduct by cleavage of the N^1–C^6 bond to give **5**. The reaction of **2** with aqueous hydroxide apparently proceeds by the same pathway to give **4**, but **5** is not observed. Instead, it appears that the hydrolysis of **5** is more rapid than its formation.

The various other transformations in Scheme I involve well-known reactions, with the exception of the conversion of **7** to **8**. This mechanism, similar to that first described by Thurber and Townsend,[17] involves attack of hydroxide ion on C-2 of a dihydropyrimidine nucleus and ring opening, followed by elimination of CO_2 from the resulting carbamyl moiety. This sequence results in enamine formation at C-6 of the dihydropyrimidine ring. The conversion of **5** to **6** is an aldol condensation. The interconversion of **8** and **9** is a Michael addition and its reversal. Other transformations in this scheme involve transfer of an *N*-alkylcarbamyl group from one amido nitrogen atom to another in **8**, **9**, and **11**. The final

products are obtained by elimination of a molecule of urea only.

Further experiments with **2b** and **2c** yielded additional information of interest. When **2c** was subjected to the action of TMAH in Me_2SO solution, the 1,3-dialkyluracil which was recovered was found to be a mixture of 34% **2b** and 66% **2c**. If, instead, the experiment was carried out using **2b**, the 1,3-dialkyluracil recovered was 77% **2b** and 23% **2c**. A study of the rate of isomerization demonstrated that the reaction was slow initially and reached a plateau in approximately 24 h. Since the rate of exchange is fast by comparison[14c] with the rate of isomerization, the two reactions cannot have a common intermediate, e.g., the symmetrical species proposed by Wechter for the base-catalyzed deuterium exchange at C-6 of pyrimidines.[14b]

One explanation for the isomerization reaction is that an aldehyde intermediate, **5**, reacts with a free molecule of urea produced during the course of the final series of reactions (**11** → **3**) to give **15** (Scheme I). This compound could now undergo ring closure to an isomer of **2** (**2***). The conversion of **15** to **2*** resembles the formation of **12** and **13** from **11**. Evidence in agreement with this pathway was obtained by subjecting **2d** to the action of TMAH in Me_2SO solution in the presence of **3a**. Using approximately equimolar amounts of **2d** and **3a**, a 47% yield of **2** was recovered after a reaction time of 1 h. Its composition was 60% **2a** and 40% **2d**. The yields were independent of whether the urea was present from the beginning or whether it was added after the formation of the compound with λ_{max} 296 nm (**5d**). The result of this experiment also can be explained, however, by assuming nucleophilic attack of a molecule of urea directly on **2** either at C-4 or C-6. The synthetic applicability of this type of reaction remains to be investigated.

The results obtained in this study suggest several interesting possibilities. The fact that there can be a rapid interconversion of a cyclic pyrimidine structure, **2**, and an acyclic enolate, **5**, affords another mechanistic pathway for C-5 exchange in pyrimidines.[14] Furthermore, this rapid interconversion, and the fact that 1,3-dimethylthymine was obtained as one of the products in the experiment described above on the methylation of **5**, suggest an alternative mechanism for biological C-alkylations, e.g., those catalyzed by enzymes such as thymidylate synthetase[14a,18] and deoxycytidine 5′-phosphate and deoxyuridine 5′-phosphate hydroxymethylases.[19] It is interesting to speculate also on the possibility that the biosynthesis of nudiflorine[9] may proceed by a pathway analogous to that followed in the conversion of **2** to **12**.

Experimental Section

^1H NMR spectra were obtained on a Varian A-60A spectrometer at room temperature using tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as internal standards. A Cary 14 and a Perkin-Elmer 457 grating infrared spectrophotometer were used to obtain UV and IR spectra, respectively. UV spectra were measured in cells (Precision Cells, Inc., Hicksville, N.Y.) of 0.1 mm, 1 mm, and 1 cm path lengths. Mass spectra were obtained on a Varian M-66 mass spectrometer at an ionizing potential of 70 eV, an ionizing current of 30 μA, a resolution of ca. 2200, and with perfluorokerosene as a standard.

Thin layer chromatography was performed on Analtech silica gel G thin layer plates containing fluorescent indicator (Analtech, Inc., Newark, Del.). In all reactions involving solutions of TMAH in Me_2SO, a preliminary separation of salts from Me_2SO and organic products was achieved by pouring the reaction mixture on to silica gel Woelm (ICN, Cleveland, Ohio), 1 g for each mL, and allowing the Me_2SO to filter through the column. This was followed with a wash of approximately twice the volume of AcOEt. Evaporation gave a salt-free mixture suitable for further chromatography. Preparative chromatography (dry column) was performed on the silica gel as obtained or after activation at 100 °C for 15 h. The progress of such chromatography was monitored by TLC. When more than one eluting solvent is indicated, solvent polarity was increased by the use of a gradient after each component or group of components was eluted from the column. High-pressure liquid chromatography (HPLC) was performed using Waters ALC 202 and ALC 100 liquid chromatographs. Analytical work was done on Corasil I (4 ft × 0.125 in.) and preparative separations on Porasil A (8 ft × 0.375 in.) (Waters Associates, Inc., Framingham, Mass.).

Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Melting points are uncorrected.

1,3-Dimethyluracil (2a) and 1,3-Diethyluracil (2d). Compound **2a** was synthesized by the method of Davidson and Baudisch.[20] Compound **2d** was synthesized from either uracil[20] or 2,4-diethoxypyrimidine.[21]

3-Ethyl-1-methyluracil (2c). This was synthesized by the method of Hilbert and Johnson,[21b] mp 56–58.5 °C (lit.[21b] mp 60–61 °C).

1-Ethyl-3-methyluracil (2b). This compound was prepared from 1-ethyluracil[22] by methylation with $(CH_3)_2SO_4$.[20] An analytical sample was obtained from AcOEt/petroleum ether: mp 73.5–75.5 °C; UV (95% EtOH) λ_{max} 266 nm (ε 8800) and 207 (8050); UV (0.1 N HCl) λ_{max} 266 nm (ε 8850); UV (0.1 N NaOH) λ_{max} 266 nm (ε 9000); ^1H NMR ($CDCl_3$) δ 1.32 (t, 3, J_{Et} = 7 Hz, CH_3CH_2), 3.33 (s, 3, CH_3N), 3.81 (q, 2, J_{Et} = 7 Hz, CH_3CH_2), 5.72 (d, 1, $J_{5,6}$ = 8 Hz, H-5), and 7.11 ppm (d, 1, $J_{5,6}$ = 8 Hz, H-6); $M^+\cdot$ *m/e* 154 (100).

Anal. Calcd for $C_7H_{10}N_2O_2$: C, 54.54; H, 6.54; N, 18.17. Found: C, 54.44; H, 6.49; N, 18.03.

Isolation of Formylacetic Acid (4′). Two hundred milligrams (1.43 mmol) of **2a** was dissolved in 3 mL (3.33 mmol) of 1.1 N aqueous TMAH solution. Samples were removed for ^1H NMR and UV spectra. The resonances for **2a** slowly decreased and a singlet at 2.59 ppm due to **3a** and a doublet at 8.09 ppm (J = 11 Hz) due to **4** appeared. After 22 h, the ^1H NMR spectrum showed 22% **2a**, 78% **3a**, 54% of formylacetate (**4**), and 7% of an unidentified component with a resonance at 8.30 ppm. The reaction mixture was diluted to 10 mL with water. Aliquots in 0.1 N NaOH and 0.1 N HCl showed ca. 20% unreacted **2a** and two pH-dependent λ_{max} at 260 and at 325 nm. An estimate of ε for **4**, derived from combined UV and ^1H NMR data, is 18 000. Ion exchange chromatography [Rexyn RG 50 (H^+), Fischer Scientific Co.], using H_2O as eluent, afforded 50 mg of a yellow oil, **4′** [λ_{max} (0.1 N NaOH) 259 nm; ^1H NMR (CD_3CN) δ 3.40 (d, 2, J = 2.5 Hz, CH_2), 7.55 (broad, carboxyl proton[23]), and 9.67 ppm (t, 1, J = 2.5 Hz, CH=O), plus resonances due to some **2a**]; 16 mg of pure **2a** (8%); and 79 mg of **3a** (63%).

Repetition of the reaction with NaOH gave similar results.

Preparation of the Oxime of Formylacetic Acid. One gram (7.15 mmol) of **2a** was dissolved in 16 mL (17.6 mmol) of 1.1 N aqueous TMAH solution. After 13 h at room temperature, a sample was removed for ^1H NMR. The reaction mixture contained 27% **2a**, 73% **3a**, and 55% of formylacetate, **4**. The reaction mixture was cooled in ice and 940 mg (13.5 mmol) of hydroxylamine hydrochloride was added. After 90 min the reaction mixture was extracted with $CHCl_3$. The aqueous layer was acidified to pH 3 with 3 N H_2SO_4 (4.4 mL) and extracted with Et_2O. The Et_2O extracts were dried ($CaCl_2$) and evaporated in vacuo. The crude weight of oxime was 255 mg (63%). A small portion of Et_2O was added to the crude material and 120 mg of crystalline material was collected: mp 111–112 °C;[7] ^1H NMR (Me_2SO-d_6)[24] δ 3.15 (25%) and 3.27 (75%) (d, 2, J = 6.5 and 5.0 Hz, CH_2), 6.87 (75%) and 7.37 (25%) (t, 1, J = 6.5 and 5.0 Hz, CHNOH), and 10.37 ppm (broad, 2, NOH and COOH). All protons exchange in D_2O.

Anal. Calcd for $C_3H_5NO_3$: C, 34.96; H, 4.89; N, 13.59. Found: C, 35.10; H, 4.92; N, 13.53.

UV Spectral Studies of Reaction of 2a with TMAH in Me_2SO. Standard Reaction Conditions. Compound **2a** (116.6 mg, 0.833 mmol) was placed in a 10-mL volumetric flask and diluted to the mark with a 0.1 N solution of TMAH in Me_2SO.[25] The solid dissolved within 3 min and the solution was transferred to a 0.1-mm cell. A crystalline precipitate separated after ca. 5 min. The UV spectrum was obtained using calibrated screens in the reference path to compensate for the high concentration. The initial spectrum had a sharp λ_{max} at 296 nm. This λ_{max} disappeared and was replaced (1 h) by two maxima at 320 and 276 nm. These maxima did not persist. During the course of the next 18 h, other transient maxima were observed, but by 68 h three maxima were present at 265, 315, and 365 nm. These were still present after 7 days. An estimate of the extinction coefficient for **5a** from this experiment gives 6300.[26]

UV Spectral Studies of Reaction of 2a with TMAH in Me_2SO. Dilute Solutions. Compound **2a** (9.90 mg, 7.07×10^{-2} mmol) was dissolved in 2 mL of Me_2SO and 9 mL of a 9.05×10^{-2} N solution of TMAH (0.815 mmol) in Me_2SO was added. The UV spectrum was obtained within 4 min of mixing. A sharp maximum was observed at

296 nm (A = 1.47, corresponding to ϵ 23 000).[26] The absorbance slowly decreased, with ~50% loss in 3 h. After 6 h, a new maximum was evident at 266 nm whose absorbance increased slightly after another day and then remained constant for 7 days at an absorbance of 0.31. It is presumed to be due to 42% of **2a** which was regenerated by ring closure of **5a** as the concentration of base decreased.[4b]

The value of ϵ 23 000 for **5a**, compared to that of 6300 found in the previous experiment, suggests that the crystalline precipitate noted in that experiment possesses the chromophore corresponding to λ_{max} 296 nm, i.e., the precipitate is the tetramethylammonium salt of **5a**. To obtain confirmatory evidence for this explanation, **2a** (22.25 mg, 0.159 mmol) was treated with 10 mL of 0.1 N TMAH in Me_2SO in a centrifuge tube. After the initial solid dissolved, a new solid separated (5 min). The reaction mixture was centrifuged and the resulting precipitate was dissolved in absolute ethanol. The UV spectrum of this solution had maxima at 296 and 270 nm. Within 8 min, the λ_{max} at 296 nm decreased by 30%.

UV Spectral Studies of Reaction of 2a with TMAH in Me_2SO. Dilution of Aliquots with Aqueous Acid and Base. One hundred milligrams (0.715 mmol) of **2a** was placed in a 10-mL volumetric flask and diluted to the mark with a solution of 0.1 N TMAH in Me_2SO. Aliquots of 20 μL were removed periodically and diluted with 0.1 N NaOH and 0.1 N HCl for the measurement of UV spectra. The spectrum in NaOH solution of an aliquot taken after 5 min had λ_{max} 267 nm and a shoulder at 295 nm which rapidly decreased as the former increased. The initial spectrum in acid of an aliquot taken at the same time had λ_{max} 267 nm and an absorbance that was half that in base. Within 1 h the spectrum of an aliquot in basic solution had a maximum at 310 nm, in addition to that at 267 nm. The 310-nm absorption was not present in the corresponding aliquot diluted with acid. After 7 days the spectrum of an aliquot in base had maxima at 265 and 345 nm, while the spectrum in acid had maxima at 260 and 348 nm.

Reaction of 2a with TMAH in Me_2SO. Preparation of 1-Methyl-5-(methylcarbamyl)-2-pyridone (12a) and 1-Methyl-3-(methylaminomethylene)-2,6-pyridinedione (13a). TMAH (3.6 g, 20 mmol) was dissolved in 225 mL of Me_2SO in an atmosphere of dry, oxygen-free nitrogen by warming to 60–70 °C. The solution was then cooled to room temperature. Compound **2a** (2.3 g, 16 mmol) in 50 mL of Me_2SO was added. Aliquots were removed periodically and diluted with 0.1 N NaOH and 0.1 N HCl for measurement of UV spectra. The reaction mixture turned yellow immediately and a crystalline precipitate appeared. These crystals redissolved and within several hours fine white needles separated. This latter precipitate may be tetramethylammonium carbonate, since gas evolution takes place immediately upon dissolving it in dilute acid. In 24 h, the reaction mixture had turned bright red. After 7 days no further change in the UV spectrum was taking place. The reaction mixture was desalted and chromatographed on silica using $CHCl_3$, $CHCl_3$/AcOEt, AcOEt, and AcOEt/EtOH as the developing solvents. Mixtures of **2a**, Me_2SO_2,[27] and **13a** (658 mg), and **3a** and **12a** (940 mg), were obtained. The latter mixture afforded **12a** on crystallization from MeCN: mp 186.5–188 °C; IR (KBr) 3370 and 3320 (NH), 1665 (NC=O), and 1620 cm^{-1} (C=C); UV (95% EtOH) λ_{max} 304 nm (ϵ 4100) and 257 (14 700); UV (0.1 N HCl) λ_{max} 298 nm (ϵ 4800) and 257 (14 400); UV (0.1 N NaOH) λ_{max} 298 nm (ϵ 4800) and 257 (14 700); ^1H NMR (Me_2SO-d_6) δ 2.77 (d, 3, $J_{Me,NH}$ = 4.5 Hz, CH_3NH), 3.51 (s, 3, CH_3N), 6.41 (d, 1, $J_{3,4}$ = 9.5 Hz, H-3), 7.87 (d of d, 1, $J_{3,4}$ = 9.5, $J_{4,6}$ = 3 Hz, H-4), 8.16 (broad, 1, NH), and 8.32 ppm (d, 1, $J_{4,6}$ = 3 Hz, H-6); M^+· m/e 166 (100).

Anal. Calcd for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07; N, 16.86. Found: C, 58.16; H, 6.18; N, 17.05.

The residue from the above crystallization was sublimed in vacuo at 72 °C (bath temperature) and 1 × 10^{-4} mm to give 315 mg of **3a**: mp 102.5–105.5 °C (lit.[28] mp 105 °C); ^1H NMR ($CDCl_3$) δ 2.75 (d, 3, $J_{Me,NH}$ = 4.5 Hz, CH_3N) and 5.22 ppm (broad, 1, NH); M^+· m/e 88 (100).

The first mixture was rechromatographed on silica gel ($CHCl_3$) to give 35 mg of **2a**; 219 mg of a mixture of **2a**, Me_2SO_2,[27] and **13a** (141 mg by UV); and **13a** (370 mg). The latter was recrystallized twice from n-hexane–$CHCl_3$ to give **13a**, which was pale yellow in color: mp 124.5–126 °C; IR (KBr) 3450 (NH), 1680 (NC=O) and 1600 cm^{-1} (C=C); UV (95% EtOH) λ_{max} 345 nm (ϵ 30 500), 280 (1850), and 240 (8400); UV (0.1 N HCl) λ_{max} 348 nm (ϵ 30 800), 280 (2200), and 237 (9100); UV (0.1 N NaOH) λ_{max} 345 nm (ϵ 20 300) and 275 (6500); ^1H NMR ($CDCl_3$) δ 3.19 (d, 3, $J_{Me,NH}$ = 5 Hz, CH_3NH), 3.30 (s, 3, CH_3N), 5.75 (d, 1, $J_{4,5}$ = 9.5 Hz, H-5), 6.92 (d, 1, $J_{4,5}$ = 9.5 Hz, H-4), 7.25 (d, 1, $J_{CH,NH}$ = 13.5 Hz, NHC**H**=), and 10.30 ppm (broad, 1, NH); M^+· m/e 166 (100).

Anal. Calcd for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07; H, 16.86. Found: C, 58.04; H, 6.18; N, 16.95.

The overall yields of products follow: **2a**, 5%; **3a**, 58%; **12a**, 27%; and **13a**, 39%.

Repetition of the previous experiment with 26.7 mg (0.176 mmol) of **2a** in 5 mL of 0.1 N TMAH (0.5 mmol) in Me_2SO afforded no **12a**, a 25% yield of **13a**, and another compound with an absorption at λ_{max} 290 nm (pH dependent).

Reaction of 1,3-Diethyluracil (2d) with TMAH in Me_2SO. A solution of 3.05 g of TMAH (16.7 mmol) in 167 mL of Me_2SO was prepared as described above. To this was added 2.3 g (13.7 mmol) of **2d** in 25 mL of Me_2SO plus another 17 mL of Me_2SO. The reaction was followed by UV. After 7 days, the reaction mixture was desalted. Chromatography on silica gel (CH_2Cl_2) afforded a mixture of **2d** and Me_2SO_2[27] (450 mg); several minor components; **3d** (290 mg) [^1H NMR ($CDCl_3$) δ 1.12 (t, 3, J_{Et} = 7.5 Hz, CH_3CH_2), 3.22 (d of q, 2, J_{Et} = 7.5 and $J_{CH_2,NH}$ = 5.5 Hz, CH_3CH_2NH), and 5.75 ppm (broad, 1, NH)]; and a mixture containing mostly **12d** [UV (0.1 N NaOH) λ 290 nm (shoulder) and λ_{max} 257 nm; ^1H NMR ($CDCl_3$) δ 1.22 (t, 3, J_{Et} = 7 Hz, CH_3CH_2NH), 1.36 (t, 3, J_{Et} = 7 Hz, CH_3CH_2N), 3.40 (m, 2, J_{Et} = 7 Hz, CH_3CH_2NH), 4.09 (q, 2, J_{Et} = 7 Hz, CH_3CH_2N), 6.59 (d, 1, $J_{3,4}$ = 9.5 Hz, H-3), 7.67 (broad, 1, NH), 7.97 (d of d, 1, $J_{3,4}$ = 9.5, $J_{4,6}$ = 3 Hz, H-4), and 8.38 ppm (d, 1, $J_{4,6}$ = 3 Hz, H-6); M^+· m/e 194 (100)]. The latter mixture contained 0.256 mmol (2%) of **12d** (UV), assuming ϵ to be the same as for **12a**. The UV assay of the total reaction mixture indicated that 0.8% of 1-ethyl-3-(ethylaminomethylene)-2,6-pyridinedione (**13d**) was present. The yield of **3d** was 40% and 19% of **2d** was recovered.

Reaction of 1-Ethyl-3-methyluracil (2b) with TMAH in Me_2SO. Compound **2b** (2.11 g, 13.5 mmol) in 25 mL of Me_2SO was added to a solution, prepared as described previously, of TMAH (3.05 g, 16.8 mmol) in 184 mL of Me_2SO. The reaction was followed by UV. After 7 days, the reaction mixture was desalted. Chromatography on silica gel (CH_2Cl_2) afforded the following fractions: a mixture (275 mg) of Me_2SO_2,[27] **2b** and **2c** (HPLC, see below), and **13b**; **13b** and an unidentified component (350 mg); and a mixture of **3b** and **12b**. Rechromatography ($CHCl_3$) of the second mixture gave 70 mg of the dialkyluracils, **2b** and **2c** (HPLC), and pure **13b** (166 mg). The latter was sublimed in vacuo at ~100 °C (bath temperature) and 1 × 10^{-4} mm to give 100 mg of pale yellow 1-methyl-3-(ethylaminomethylene)-2,6-pyridinedione (**13b**), which turned pink after standing for several days at room temperature: mp 50.5–55.5 °C; IR (KBr) 3450 (NH), 1675 (NC=O), and 1600 cm^{-1} (C=C); UV (95% EtOH) λ_{max} 347 nm (ϵ 30 800), λ_{sh} 280 nm (ϵ 2250), and λ_{max} 241 nm (ϵ 9000); UV (0.1 N HCl) λ_{max} 349 nm (ϵ 32 000), 280 (2350), and 237 (9500); UV (0.1 N NaOH) λ_{max} 345 nm (ϵ 21 200) and 272 (7300); ^1H NMR ($CDCl_3$) δ 1.34 (t, 3, J_{Et} = 7.5 Hz, CH_3CH_2), 3.32 (s, 3, CH_3N), 3.54 (d of q, 2, J_{Et} = 7.5 Hz, $J_{CH_2,NH}$ = 13.5 Hz, CH_2NH), 5.77 (d, 1, $J_{4,5}$ = 9.5 Hz, H-5), 6.99 (d, 1, $J_{4,5}$ = 9.5 Hz, H-4), 7.35 (d, 1, $J_{CH,NH}$ = 13.5 Hz, NHC**H**=), and 10.21 ppm (broad, 1, NH); M^+· m/e 180 (100).

Anal. Calcd for $C_9H_{12}N_2O_2$: C, 59.99; H, 6.71; N, 15.54. Found: C, 59.79; H, 6.59; N, 15.43.

The mixture of **3b** and **12b** was sublimed below 100 °C (bath temperature) at 1 × 10^{-4} nm to give 110 mg of **3b**: ^1H NMR ($CDCl_3$) δ 1.10 (t, 3, J_{Et} = 7 Hz, CH_3CH_2), 2.72 (s, 3, CH_3), 3.16 (q, 2, J_{Et} = 7 Hz, CH_3CH_2), and 5.22 (broad, 2, NH). Sublimation at a temperature above 100 °C at 1 × 10^{-4} mm gave 1-ethyl-5-(methylcarbamyl)-2-pyridone (**12b**). A sample for analysis was recrystallized from n-hexane–$CHCl_3$: mp 157.5–158.5 °C; IR (KBr) 3320 (NH), 1680 (NC=O), and 1635 cm^{-1} (C=C); UV (95% EtOH) λ_{max} 305 nm (ϵ 4500) and 259 (15 700); UV (0.1 N HCl) λ_{max} 300 (ϵ 4800) and 257 (14 900); UV (0.1 N NaOH) λ_{max} 299 nm (ϵ 5080) and 257 (15 600); ^1H NMR ($CDCl_3$) δ 1.35 (t, 3, J_{Et} = 7 Hz, CH_3CH_2), 2.91 (d, 3, $J_{Me,NH}$ = 4.5 Hz, CH_3NH), 3.99 (q, 2, J_{Et} = 7 Hz, CH_3CH_2), 6.42 (d, 1, $J_{3,4}$ = 9.5 Hz, H-3), 7.30 (broad, 1, NH), 7.78 (d of d, 1, $J_{3,4}$ = 9.5, $J_{4,6}$ = 3 Hz, H-4), and 8.13 ppm (d, 1, $J_{4,6}$ = 3 Hz, H-6); M^+· m/e 180 (100).

Anal. Calcd for $C_9H_{12}N_2O_2$: C, 59.99; H, 6.71; N, 15.54. Found: C, 60.17; H, 6.75; N, 15.71.

The overall yield of products was 45% of **3b**, 13% of **12b**, and 25% of **13b**. Nine percent of **2** was recovered.

The mixture of **2b** and **2c** was separated by preparative HPLC using 7.5% EtOH in n-hexane as solvent. Compound **2b** had κ' = 4.06 and **2c** had κ' = 6.25. The two compounds, 77 and 23%, respectively, were identified by comparison of ^1H NMR spectra with those of authentic samples.

Reaction of 3-Ethyl-1-methyluracil (2c) with TMAH in Me_2SO. Compound **2c** (2.11 g, 13.7 mmol) in 25 mL of Me_2SO was added to a solution, prepared as described above, of TMAH (3.05 g, 16.8 mmol) in 184 mL of Me_2SO. The progress of the reaction was followed as usual. After 7 days the reaction mixture was desalted. Chromatography on silica gel (CH_2Cl_2) yielded several fractions. These were a mixture (265 mg) of Me_2SO_2,[27] **2b** and **2c** (HPLC; see

below); an unidentified component and 1-ethyl-3-(methylaminomethylene)-2,6-pyridinedione (**13c**) (2.7% by UV) [^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3, J_{Et} = 7 Hz, CH_3CH_2), 3.26 (d, 3, $J_{Me,NH}$ = 5 Hz, CH_3NH), 4.07 (q, 2, J_{Et} = 7 Hz, CH_3CH_2N), 5.82 (d, 1, $J_{4,5}$ = 9.5 Hz, H-5), 7.11 (d, 1, $J_{4,5}$ = 9.5 Hz, H-4), 7.52 (d, 1, $J_{CH,NH}$ = 13.5 Hz, NHCH=), and 10.50 ppm (broad, 1, NH)]; a 50:50 mixture (85 mg) of **13c** and **3b**; and **3b** plus 1-methyl(5-ethylcarbamyl)-2-pyridone (**12c**) (5.5% by UV) [^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3, J_{Et} = 7 Hz, CH_3CH_2), 3.64 (s, 3, CH_3N), 6.56 (d, 1, $J_{3,4}$ = 9.5 Hz, H-3), 8.09 (d of d, 1, $J_{3,4}$ = 9.5, $J_{4,5}$ = 3 Hz, H-4), 8.27 (broad, 1, NH), and 9.29 ppm (d, 1, $J_{4,6}$ = 3 Hz, H-6)]. In this last spectrum, the resonance corresponding to the methylene moiety of the ethyl group was presumed to be under the resonance due to the methylene protons of the **3b** present in the sample.

The mixture of **2b** and **2c** was identified by means of analytical HPLC using *n*-hexane/5% EtOH as the developing solvent: κ' for **2b** (34%) = 1.62, κ' for **2c** (66%) = 2.28.

Reaction of 5a with Methyl Iodide. [(*E*)-3-Methoxypropenoyl]-*N,N'*-dimethylurea (14a) and 1,3-Dimethylthymine. A mixture of **2a** (1.29 g, 9.2 mmol) and TMAH (1.77 g, 9.7 mmol) in 100 mL of Me_2SO was shaken for 17 min. Methyl iodide (1 mL, 16 mmol) was added. The solution became neutral within 6 min. It was evaporated in vacuo at 45 °C and the residue, chromatographed on activated silica gel (AcOEt), afforded a mixture of a new compound of molecular weight 172 and 1,3-dimethylthymine (130 mg); a 1:1 mixture of **2a** and 1,3-dimethylthymine (438 mg); and **2a** (420 mg). The new compound (30 mg), κ' = 2.15, and 1,3-dimethylthymine[29] (67 mg), κ' = 4.55, were separated by means of preparative HPLC (7.5% EtOH/*n*-hexane). The new component was recrystallized from *n*-hexane to give 10 mg of analytically pure [(*E*)-3-methoxypropenoyl]-*N,N'*-dimethylurea (**14a**): mp 74.5–77.5 °C; IR (Nujol) 3300 (NH), 1700, 1630, and 1600 cm^{-1} (C=C and C=O); UV (95% EtOH) λ_{max} 247 nm (ϵ 12 800); UV (0.1 N HCl) λ_{max} 250 nm (ϵ 12 000); UV (0.1 N NaOH)[30] λ_{max} 265 nm (ϵ 5300); ^{1}H NMR ($CDCl_3$) δ 2.85 (d, 3, $J_{Me,NH}$ = 4.5 Hz, CH_3NH), 3.30 (s, 3, CH_3N), 3.75 (s, 3, CH_3O), 5.63 (d, 1, $J_{2,3}$ = 12 Hz, H-2), 7.61 (d, 1, $J_{2,3}$ = 12 Hz, H-3), and 9.15 ppm (broad, 1, NH); $M^+\cdot$ *m/e* 172 (72).

Anal. Calcd for $C_7H_{12}N_2O_3$: C, 48.83; H, 7.02; N, 16.07. Found: C, 48.88; H, 6.98; N, 16.17.

The total yields of the various products were 22% 1,3-dimethylthymine, 49% **2a**, and 2% **14a**.

Isolation of 1,3-Dimethyl-6-(*N*-methylcarbamylmethyl)-5-(methylaminomethylene)-5,6-dihydrouracil (10a). Compound **2a** (2.03 g, 14.5 mmol) was added to 190 mL of 0.09 N TMAH (17.1 mmol) in Me_2SO. Three minutes after the initial solid dissolved, another solid separated. The reaction was followed by UV. After 4.5 h, when the absorption at 320 nm had reached a maximum, glacial acetic acid (1 mL, 17.6 mmol) was added to stop further reaction. The mixture was chromatographed to remove salts. The residue obtained was dissolved in 25 mL of absolute EtOH and an aliquot was analyzed by HPLC (5% EtOH in *n*-hexane). Four components were present: **2a**; a compound with λ_{max} 310 nm (κ' = 6.15); and two others. A 29% yield (UV) of the component with λ_{max} 310 nm was present. Chromatography (AcOEt and 3:1 AcOEt/EtOH) of the main portion of the residue, after evaporation of the EtOH, afforded 390 mg (19%) of **2a**; a mixture (140 mg) of **2a** (5%, UV) and **13a** (2.5%, UV); **13a** (300 mg; 2%, UV); and two other components (1.103 g), one of which was **10a**. The latter mixture afforded a white solid, mp 158–164 °C, on addition of 1,2-dichloroethane. Recrystallization from $CHCl_3$/1,2-dichloroethane afforded 80 mg of pure **10a**: mp 171–173 °C; IR ($CHCl_3$) 3460 and 3310 (NH and OH), 1660, 1651 (NC=O), and 1600 cm^{-1} (C=C); UV (95% EtOH) λ_{max} 310 nm (ϵ 18 200); UV (0.1 N HCl) shoulders on end absorption at 260 nm (ϵ 3800) and 225 (6340); UV (0.1 N NaOH) λ_{max} 309 nm (ϵ 18 900); ^{1}H NMR ($CDCl_3$) δ 2.20 (d of d, 1, $J_{CH_2,6}$ = 9, J_{gem} = 13 Hz, CH_2CH), 2.42 (d of d, $J_{CH_2,6}$ = 5, J_{gem} = 13 Hz, CH_2CH), 2.68 (d, 3, $J_{Me,NH}$ = 4.5 Hz, CH_3NH),[31] 2.88 (d, 3, $J_{Me,NH}$ = 4 Hz, CH_3NH),[31] 2.94 (s, 3, CH_3N),[31] 3.02 (s, 3, CH_3N),[31] 4.12 (d of d, 1, $J_{CH_2,6}$ = 5 and 9 Hz, CH_2CH), 5.83 (broad, 1, NH), 6.55 (d, 1, $J_{CH,NH}$ = 13 Hz, CHNH), and 8.01 ppm (broad, 1, NH); mass spectrum *m/e* (rel intensity) $M^+\cdot$ 254 (1), 182 (100), 125 (12), and 84 (17).

Anal. Calcd for $C_{11}H_{18}N_4O_3$: C, 51.95; H, 7.13; N, 22.03. Found: C, 51.79; H, 7.10; N, 21.66.

Reaction of 10a with TMAH in Me_2SO. Compound **10a** (40 mg, 0.158 mmol) was dissolved in 300 μL of Me_2SO-h_6 and the ^{1}H NMR spectrum was obtained: NH hydrogens at 7.81 and 7.61, vinyl hydrogen at 6.59 (J = 13.5 Hz), CH_2 group at 4.01 (J = 5 and 8 Hz), and methyl groups at ca. 2.82, 2.90, and 2.94 ppm. The remaining proton resonances were obscured by the absorption of the solvent. The solution was removed from the ^{1}H NMR tube and added to 20 mg (0.11 mmol) of TMAH, which did not dissolve completely. The solution turned yellow. It was returned to the ^{1}H NMR tube without transferring solid. The spectrum had a single broad resonance centered at 7.40 ppm and methyl group resonances at 2.92, 2.85, and 2.77 ppm, in addition to solvent, water, and tetramethylammonium resonances. After 18 h, resonances corresponding to **12a** were present. UV analysis in 0.1 N NaOH and 0.1 N HCl indicated the presence of 67% of **12a** and 12% of **13a**.

Degree of Isomerization of 2c as a Function of Time. A sample of **2c** (151 mg, 0.98 mmol) was placed in a 10-mL volumetric flask. A 0.1 N solution of TMAH (1 mmol) in Me_2SO was added to the mark. Aliquots (2 mL) were removed after 2 min, 0.5, 2.5, 6.5, and 24 h. Each aliquot was treated with 50 μL (0.88 mmol) of glacial acetic acid and desalted. The residues were dissolved in 10 mL of absolute EtOH for UV determinations in 0.1 N NaOH and 0.1 N HCl, and HPLC analysis using 5% EtOH in *n*-hexane as developing solvent. The extent of isomerization was 0, 1, 9, 17, and 23%, respectively.

Reaction of 1,3-Diethyluracil (2d) with 1,3-Dimethylurea (3a) in the Presence of TMAH. Compound **2d** (150 mg, 0.89 mmol) and **3a** (80 mg, 0.91 mmol) were placed in a 10-mL volumetric flask and 0.11 N TMAH (1.1 mmol) in Me_2SO was added to the mark. Aliquots (3 mL) of this solution were removed after 1 and 2 h. Each was treated with 200 μL of glacial acetic acid and desalted. The residues obtained were dissolved separately in absolute EtOH. The solutions were subjected to UV analysis and HPLC. By UV it was found that both solutions contained **10** and the chromophore present in **2**. The sample taken at 1 h contained 56% (UV, 0.1 N HCl) of the latter chromophore, while the 2-h sample contained 47%. HPLC analysis (5% EtOH in *n*-hexane) showed that the content of **2** of both samples was made up of 63% of **2a** and 37% of **2d**.

In a similar reaction, after a chromatographic separation on silica gel, **3a** and **3d** were found (^{1}H NMR) to be present in addition to the **2a** and **2d**.

A solution of **2d** (150 mg, 0.89 mmol) and 10 mL of 0.1 N TMAH (1.0 mmol) in Me_2SO was allowed to react for 1.75 h. Then **3a** (80 mg, 0.91 mmol) was added and the solution was allowed to stand for another 2.75 h. At the end of this time, the reaction mixture was worked up as described above. The total yield of **2** by UV was 50%, consisting of 61% **2a** and 39% **2d** (HPLC).

Registry No.—**2a**, 874-14-6; **2b**, 62415-62-7; **2c**, 59495-24-8; **2d**, 22390-04-1; **3a**, 96-31-1; **3b**, 28145-10-0; **3d**, 623-76-7; **4'**, 926-61-4; **4'** oxime, 62415-63-8; **5a**, 62415-64-9; **10a**, 62415-65-0; **12a**, 62415-66-1; **12b**, 62415-67-2; **12c**, 62415-68-3; **12d**, 62415-69-4; **13a**, 62415-70-7; **13b**, 62415-71-8; **13c**, 62415-72-9; **14a**, 62415-73-0; 1-ethyluracil, 6490-42-2; $(CH_3)_2SO_4$, 77-78-1; hydroxylamine HCl, 5470-11-1; methyl iodide, 74-88-4.

References and Notes

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(13) The numbering system used for designating the atoms in 2 is retained for purposes of simplification and ease of comparison.
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(15) The UV spectrum of 10a, which varies with pH, is in good agreement with that of 1b, which has a related chromophore. The only significant difference between the spectra of 10a and the imidazolidone, 1b, is in the extinction coefficients. This difference may well be due to a difference in the stereochemistry of the two chromophores. See ref 5.
(16) The species m/e 125 may be a radical cation or a cation.
(17) T. C. Thurber and L. B. Townsend, J. Org. Chem., 41, 1041 (1976).
(18) D. V. Santi and C. F. Brewer, Biochemistry, 12, 2416 (1973); D. V. Santi, C. S. McHenry, and H. Sommer, ibid., 13, 471 (1974).
(19) See, for example, R. B. Dunlap, N. G. L. Harding, and F. M. Huennekens, Ann. N.Y. Acad. Sci., 186, 153 (1971).
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(21) (a) G. E. Hilbert and T. B. Johnson, J. Am. Chem. Soc., 52, 1152 (1930); (b) ibid., 52, 2001 (1930).
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(23) This area integrates for ca. 3 protons due to the fact that not all water was removed from the original sample by evaporation.
(24) The assignment of the resonances to the syn and anti isomers is arbitrary. These were present in a ratio of 25:75.
(25) Me_2SO was purified by azeotropic distillation with benzene to remove the bulk of the water followed by vacuum distillation from CaH_2.
(26) The value of ε given for 5a is based on 100% conversion of 2a.
(27) Dimethyl sulfone was a ubiquitous minor component of all of these reactions. A control reaction in which the dialkyluracil was omitted did not produce any sulfone.
(28) Fr. Fichter and B. Becker, Chem. Ber., 44, 3481 (1911).
(29) This material was identical with a sample of 1,3-dimethylthymine prepared by the methylation of thymine.[20]
(30) The UV spectra in both 95% EtOH and 0.1 N HCl are reasonable for the structure assigned to 14a. The initial spectrum of this compound in 0.1 N NaOH showed a shoulder at ~290 nm which disappeared in a short time. The spectrum was invariant after 1 h. The value of λ_{max} 265 nm reported is probably due to 2a which is formed by a Michael addition of hydroxide ion to the enol ether carbon atom of 14a, followed by the expulsion of methoxide ion in a reverse Michael addition. The resulting product is 5a (λ_{max} 296 nm in Me_2SO), which then undergoes ring closure to 2a (59% yield).
(31) The assignment of these methyl groups cannot be made with certainty.

Nucleophile and Borate Reactivity with Nicotinamide Adenine Dinucleotide and Its Analogues[1]

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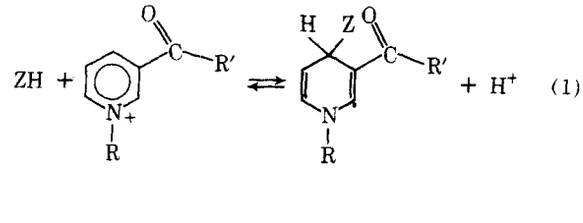
Nucleophilic reactivity for a variety of nucleophiles to the ring addition reaction of pyridine nucleotides has been measured. There is no relationship between rate of reaction and equilibrium affinity for the nucleophile. This situation is similar to the reaction of nucleophiles with the carbonyl carbon or with carbonium ions. The position of equilibrium correlates with γ, the position of equilibrium for nucleophilic reaction with the carbonyl group. However, a better correlation between pyridinium ion addition reactions has been obtained: A new affinity scale for the pyridinium ion, P^+, is defined. Boration of the ribose adjacent to the pyridinium ring reduces the equilibrium constant for nucleophile addition three- to sevenfold, regardless of the structure of the pyridinium moiety.

Nicotinamide adenine dinucleotide (NAD^+)[2] is transformed into ring addition complexes by a variety of agents. Sulfite,[3] hydroxide,[4] cyanide,[5] and enols[6] form favorable complexes. Weaker interactions take place with mercaptans, hydroxylamine, and imidazole.[6,7] A number of stable inhibitory ternary NAD^+–nucleophile–dehydrogenase complexes are formed.[7-11] Though NAD^+ ring addition complexes are formed in the absence of enzyme, complex formation is favored on the enzyme surface when the nucleophile bears a structural resemblance to the natural substrate. Kaplan and Everse[12] have proposed that the ternary complex formed by NAD^+, pyruvate, and lactate dehydrogenase plays a role in metabolite regulation in the living cell.

Borate complexes with the ribose adjacent to the nicotinamide cation in NAD^+ to form NADB, which forms less favorable nucleophilic ring addition complexes at a lower rate, in comparison with NAD^+.[13] The borate complexation with NAD^+ is the cause of the competitive inhibition of a number of dehydrogenases by borate with respect to NAD^+.[14]

In order to understand what chemical properties of the nucleophile favor a stable addition complex, a study of both the rate and equilibrium constants was initiated. This reaction as given in eq 1 is easily followed because the complex is



(1)

chromophoric, absorbing maximally at 310–360 nm (depending upon the nucleophile and the pyridinium ion). Rate and equilibrium constants were obtained as a function of pH, in order to elucidate the overall stoichiometry of the reaction and of the transition state. NAD^+, 3-acetylpyridine adenine dinucleotide ($APAD^+$), and their nucleotide and alkyl analogues were studied. In addition we have measured the boration and phenylboration equilibrium of $APAD^+$, nicotinamide adenine dinucleotide phosphate (NADP), and other analogues of NAD^+, and we have measured the effect of boration on nucleophilic reactivity and affinity. We have compared the scale of reactivity and affinity of nucleophiles to NAD^+ with the reactivity and affinity scales of carbonium ions and carbonyl compounds. A new pyridinium ion affinity scale, P_+, is defined.

Experimental Section

Materials. The pyridine nucleotides were products of Sigma Chemical Co. The concentration of pyridine nucleotide was assayed using horse or yeast alcohol dehydrogenase from Sigma. 1-Benzyl-3-acetylpyridinium chloride ($BzAP^+Cl^-$) was prepared by mixing benzyl chloride with 3-acetylpyridine in benzene at room temperature. Recrystallization from ethanol yielded a white product, mp 189–190 °C (uncorrected). 1-Methyl-3-acetylpyridinium iodide ($MeAP^+I^-$) was prepared by mixing methyl iodide with 3-acetylpyridine at room temperature. Recrystallization from 1-propanol yielded yellow crystals, mp 163–165 °C (uncorrected). 1-Methyl-3-carboxymethylpyridinium iodide ($McCP^+I^-$), *N*-methylnicotinamide iodide ($MeNic^+I^-$), and *N*-methylisonicotinamide iodide ($MeIsonic^+I^-$) were prepared in the same way as $MeAP^+$, using methyl iodide and the corresponding pyridine derivative, mp 258–260, 207–211, and 129–133