Tetrahedron. Vol. 27, pp. 953 to 960. Pergamon Press 1971. Printed in Great Britain

THE MECHANISM OF HYDROGEN-DEUTERIUM EXCHANGE OF 1-METHYL-4-PYRIMIDONE

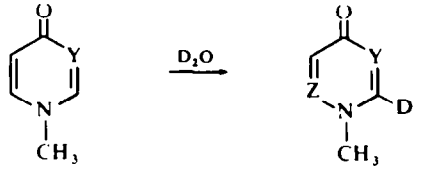
P. BEAK and R. N. WATSON

Department of Chemistry, University of Illinois, Urbana, Illinois 61801

(*Received in the USA* 9 *October* 1970; *Received in the UK for publication* 14 *October* 1970)

Abstract—H—D exchange of 1-methyl-4-pyrimidone (3) to give 1-methyl-4-pyrimidone-2*d* (4) is shown, by rate comparisons with model compounds, to involve initial deuteration at N-3 and subsequent deprotonation to give a ylide which yields 4. The change in mechanism for C-2 H—D exchange from direct proton removal for 1-methyl-4-pyridone (1) to addition-elimination for 3 is intelligible in terms of the relative kinetic acidities of the free base and the corresponding 'onium salt for each case. A suggestion is made that anionic or ylide intermediates similar to those involved in these exchanges are involved in the biosynthesis of uridylic acid.

FOLLOWING the initial observations of base-promoted H—D exchange of carbon bound hydrogen in heteroaromatic rings and proposals of ylide or anion intermediates,[1, 2] attention has focused on understanding the influence of the position and type of heteroatom on the rates of exchange.[3, 4] In the course of this work distinction between base-dependent and base-independent processes of exchange has been made.[5] A case in point is the contrast between the H—D exchange of 1-methyl-4-pyridone (1) at C-2,6, which is first-order in base, [1e] and the exchange of 1-methyl-4-pyrimidone (3) at C-2, which is independent of base.[3a, 6] In this report, a decision between two mechanisms previously advanced for the exchange of 3 is made in favor of an



1: Y = CH
3: Y = N

2: Y = CH, Z = CD
4: Y = N, Z = CH

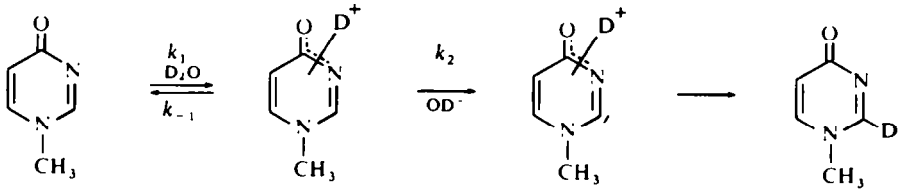
addition-elimination process. Kinetic analysis reveals that the difference in the mechanisms for exchange of 1 and 3 originates in the difference in the relative rates of exchange of the free base and the corresponding 'onium compounds of each set.

RESULTS AND DISCUSSION

Two mechanisms have been suggested to explain the base-independence of the H—D exchange of 3 at C-2.[3a] In the process outlined in Scheme I, it is presumed that 3 is deuterated to give pyrimidonium ion 5 prior to removal of the C-2 proton from 5 to yield 6 which subsequently gives 4. The rate of this process, when a steady state

concentration of **5** is assumed, is independent of base. In the alternative mechanism shown in Scheme II the donation of a deuterium ion to N-3 of **3** is considered concerted with the removal of the C-2 hydrogen.

SCHEME I



3 **5** **6** **4**

$$\frac{dP}{dt} = \frac{k_2 k_1}{k_{-1} + k_2}[D_2O][3]$$

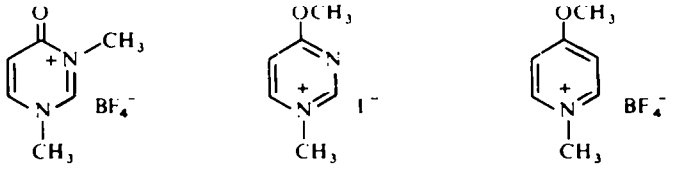
if $k_{-1} \gg k_2$,

$$\frac{dP}{dt} = \frac{k_2}{k_{-1}}[D_2O][3] = \frac{K_{D_2O}}{K_a} k_2 [3]$$

The mechanism outlined in Scheme I can be evaluated for the case in which proton removal from C-2 of **5** to give **6** is much slower than reversion of **5** to **3**, i.e., $k_{-1} \gg k_2$. In this case, the equilibrium established in the first step is measured by the acidity constant K_a. The observed overall first-order rate constant for the exchange of **3** at C-2, k_{obs}, is equal to $K_{D_2O}k_2/K_a$; each of the constants can be determined either directly or by the use of model compounds and the proposed mechanism can be judged by comparison of the observed and calculated rate constants. By measuring each constant in deuterium oxide at 70° buffered at *pD* 3·8–4·6, we avoid the comparison of values obtained at different temperatures and in different solutions which led to an inconclusive evaluation of this mechanism.[3a] Use of a common medium should also minimize ionic strength effects and eliminate differences in solvent-base composition which could invalidate the comparison of the calculated and observed rate constants.[4c, 6b]

The pK_a for 1-methyl-4-pyrimidone in deuterium oxide at 70° was determined by titration with deuterium chloride to be 2·28 ±0·03 by the method of Albert and Serjeant,[7] and the ion product for deuterium oxide at 70°, pK_{D_2O}, is 13·60.[8]

Evaluation of k_2 was achieved by use of 1,3-dimethyl-4-pyrimidonium fluoroborate (**7**)[3a] and 4-methoxy-1-methylpyrimidinium iodide (**8**) as model compounds for **5**. The pyrimidonium salt **7** is obtained by methylation of 1-methyl-4-pyrimidone and 3-methyl-4-pyrimidone and identified by virtue of its formation as the common product from these precursors. The identity of **8** follows from its preparation by methylation of 4-methoxypyrimidone and its hydrolysis to 1-methyl-4-pyrimidone.

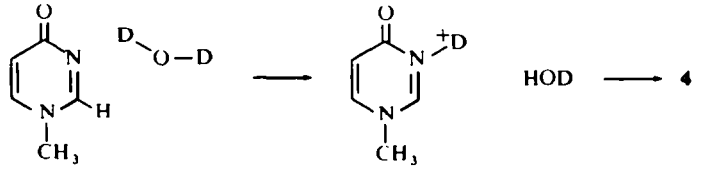


7 **8** **9**

The H—D exchange of **7** at C-2 is first-order in base[3a] with a second-order rate constant ($k_{obs}/[OD^-]$) of $9 \pm 4 \times 10^6$ l./mole-sec at 70°.* The error is due to the fact that the *pD* values of the solutions vary from 4·28 to 3·88 over the time of a kinetic run. However, good first-order kinetics are obtained for any single run. The pyrimidinium salt **8** does not undergo exchange in deuterium oxide at 70°. Instead, hydrolysis appears to occur by a reaction which is first-order in base, with a rate constant about 10^2 less than the rate of exchange of **7**. Accordingly, the rate of exchange of **8** at C-2 is at least two orders of magnitude less than that of **7**.

Using the rate of exchange of **7** as an appropriate value for k_2 in Scheme I and the above values of K_{D_2O} and K_a, we obtain a calculated first-order rate constant, $K_{D_2O}k_2/K_a$, of $4{\cdot}6 \pm 2 \times 10^{-5}$ sec^{-1} at 70° in buffered deuterium oxide. This value compares well with the observed first-order rate constant of $9{\cdot}0 \pm 0{\cdot}5 \times 10^{-5}$ sec^{-1} for the exchange of **3** under these conditions and supports the mechanism depicted in Scheme I for the exchange of **3**. The suitability of the rate of exchange of **7** as a

SCHEME II



3 **6**

model for **5**, as well as the lack of suitability of **8** as a model, provide support for the protonation of **3** on nitrogen rather than on oxygen, an assignment which was made in 1955 on the basis of UV spectroscopy[9] and has recently been supported by IR[10] and NMR[11] studies.

The alternative mechanism, shown in Scheme II, could be supported by the observation of a rate acceleration in the presence of prospective tautomeric catalysts.[12] In fact, the rate of reaction of 0·1 M **3** at 100° in deuterium oxide is slightly retarded by the presence of 0·1 M 2-pyridone as well as by 0·2 and 1·0 M pyrazole in a potassium deuterium phosphate-sodium deuteroxide buffer or by 1·0 M potassium deuterium phosphate. However, this result is not unequivocal, since the efficiency of the potential bifunctional catalyst would have to be considerably greater than that of the bifunctional solvent, deuterium oxide, for its effect on the rate to be observable. Nonetheless, in view of the above favorable evaluation of Scheme I, the process of ylide formation suggested in Scheme II appears to be unnecessary.

H—D exchange by processes involving initial formation of an 'onium species has been reported for a number of heteroaromatics,[1h, 5] and it appears to be a general, but sometimes unrecognized, reaction. The difference in mechanisms of C-2 exchange for 1-methyl-4-pyrimidone (**3**) and 1-methyl-4-pyridone (**1**) shows that neither formal structural similarity nor relative basicity† can be used to predict mechanism.

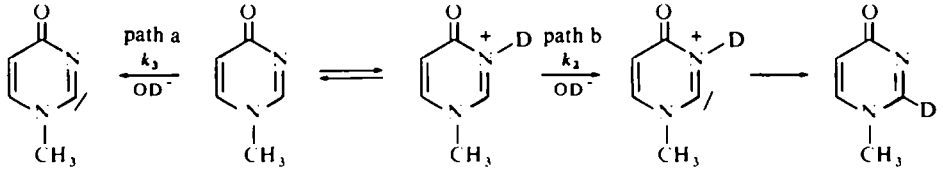
Analysis of the mechanisms of exchange of **1** and **3** reveals that the divergence is

* Deuteroxide is the active base for proton removal since the rate is unaffected by changing buffer concentration at constant pD.

† **1** is $10^{1{\cdot}3}$ more basic than **3**.[13]

due to the differences in the relative kinetic acidities of the 'onium species and the free bases at C-2. If it is assumed that the kinetic acidity of 1-methyl-4-pyrimidone (**3**) for removal of the C-2 proton by direct reaction with base is modeled by the kinetic acidity of 1-methyl-4-pyridone* (**1**) and that the kinetic acidity of the 1-methyl-4-pyrimidonium ion (**5**) is modeled by that of 1,3-dimethylpyrimidonium fluoroborate (**7**), the ratio of kinetic acidities of **5** and **3** at C-2 is *ca* 10^{12}.[3a] † This difference is used in Scheme III to estimate the relative rate ratio for exchange by addition-elimination (path a) and direct proton removal (path b) as $k_a/k_b = 5/[OD^-]$. Although this result is only a semi-quantitative indication of the real situation, it establishes that the concentration of deuteroxide is too low for exchange by path b to be observed under

SCHEME III



$$\frac{k_2}{k_3} \sim 10^{12} \qquad \frac{k_a(\text{first-order})}{k_b(\text{first-order})} = \frac{k_2 K_{D_2O} K_a^{-1}[3]}{k_3[3][OD^-]} = \frac{5}{[OD^-]}$$

the conditions investigated and quantitatively illustrates the dependence of the pathway for exchange on the relative acidities of the free base and the 'onium salt.

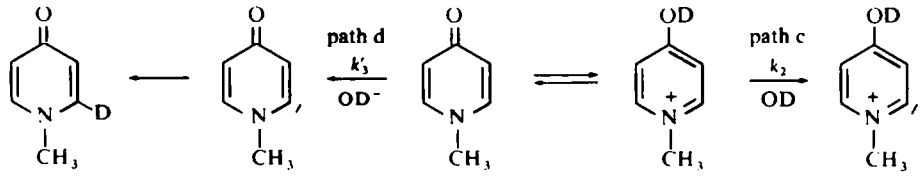
The relative kinetic acidities of 1-methyl-4-pyridone (**1**) and its corresponding pyridinium salt **10** at C-2 is estimated as 5×10^3, with **9** being used as a model. Substituting the known acidity constant of **1** for K_a[13] and using 5×10^3 for k'_2/k'_3, we obtain (Scheme IV) a ratio of first-order rate constants for exchange by addition-elimination (path c) and direct proton removal (path d) of $2 \times 10^{-7}/[OD^-]$. Accordingly, exchange of **1** at C-2 by addition-elimination is too slow to compete with direct exchange under the conditions investigated.[1d]

Comparison of the mechanistically determining factor in the exchanges of **1** and **3** suggests that the difference may be attributed to the high C-2-hydrogen kinetic acidity of **5** relative to that of **3** or **10**. The inductive effect of a positive nitrogen[1c, e, f, h, 2a, d, 5h] is expected to have a large effect on the acidity of an adjacent C—H. Moreover, the difference in exchange rates of **7** and **8** is consistent with the retardation of exchange at positions adjacent to an atom bearing an sp^2 electron pair

* The kinetic acidity of **3** at C-2 is probably greater than that of **1**. Zoltewicz, *et al.* have shown that pyrimidine is *ca* 10^2 more acidic than pyridine at C-2.[3c] On the other hand, the estimate of the relative kinetic acidities of **5** and **3** is probably *ca* a factor of 10 low because the exchange of **7** is measured at 70° and that of **1** at 100°. In order for the subsequent analysis (Scheme III) to be affected, the estimated relative kinetic acidities of **3** and **5** would have to be in error by at least a factor of 10^5.

† An attempt was made to evaluate the exchange of 1-methyl-4-pyrimidone by direct proton removal by heating **3** in a deuterium oxide solution buffered at pD 11·8. Under these conditions exchange at C-6 as well as C-2 was observed. Considerable decomposition[6] and a change in the pD to 7·5 precluded determination of the order in base. A pseudo first-order rate constant for the exchange of **3** at C-6, with the initial base concentration being used, was found to be $1{\cdot}7 \pm 0{\cdot}2 \times 10^{-6}$ sec^{-1}, in remarkable agreement with the value of $3{\cdot}1 \pm{\cdot}3 \times 10^{-6}$ sec^{-1} found for the exchange of **1** at C-2 under the same conditions.

SCHEME IV

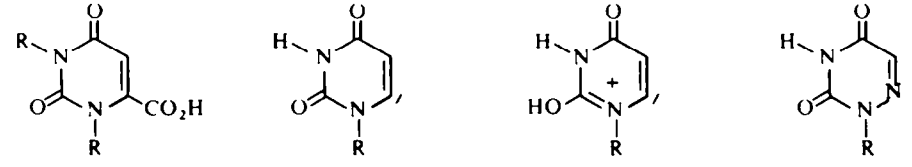


2 **1** **10**

$\frac{k'_2}{k'_3} \sim 5 \times 10^3$ $\qquad \frac{k_c\,(\text{first-order})}{k_d\,(\text{first-order})} = \frac{k'_2 K_{D_2O} K_a\,{}^1[1]}{k'_3[1][OD^-]} = \frac{2 \times 10^{-7}}{[OD^-]}$

previously reported,[3c, d, j, 14] and is attributable to electron pair repulsions, but the operation of other effects cannot be ruled out.

The anionic or ylide intermediates postulated in the hydrogen-deuterium exchange of **1** and **3** may provide an appropriate model for an intermediate in the biosynthesis of the pyrimidine nucleotides. The conversion of orotidine-5'-phosphate to uridylic acid by orotidylic decarboxylase[15] could be represented by the conversion of **11** to carbon dioxide and an anion **12a** or a ylide **12b** intermediate. Analogy for this speculation is found in the decarboxylations of a number of heteroaromatic 'onium salts.[16] It is interesting that 6-azauracil (**13**), which structurally resembles the suggested intermediate, is an effective inhibitor of biosynthetic conversion or orotic acid to the pyrimidines.[17]



11 **12a** **12b** **13**

EXPERIMENTAL*

4-Methoxy-1-methylpyrimidinium iodide (**8**). Methylation of 4-methoxypyridinium[18] (0·965 g; $8{\cdot}76 \times 10^{-3}$ mol) with equimolar amounts of MeI in ethylene chloride for 2 days at 0° gave yellow crystals (1·45 g; 66%). Recrystallization from acetone gave **8** as white crystals, m.p. 102–104° (dec); NMR (D_2O *vs* DSS) δ 4·20 (s, 3, CH_3), 4·24 (s, 3, CH_3), 7·43 (d, 1, J = 7·5 Hz, H-5), 8·72 (d–q, 1, J = 7·5, 2 Hz, H-6), and 9·18 ppm (s–d, 1, H-2). (Found: C, 28·48; H, 3·66; N, 10·96. Calcd. for $C_6H_9IN_2O$: C, 28·57; H, 3·57; N, 11·11%).

The structure of **8** was established by its hydrolysis in D_2O to a product which has NMR signals superimposable on those of added **3**. The signals observed are not identical to those of added 3-methyl-4-pyrimidone. Attempts to prepare the fluoroborate salt of this pyrimidinium iodide were unsuccessful.

Methanol-O-d was prepared by the method of Pasto and Meyer[19] from 37·8 g; 2·48 $\times 10^{-1}$ mol (K and K Laboratories) $Si(OMe)_4$; and D_2O (10 g; 0·50 mol). The wet MeOD (~100%) was dried with magnesium methoxide and distilled before use. No OH absorption was seen in the NMR spectrum of the MeOD.

* M.ps were determined in open capillaries in a Thomas-Hoover m.p. apparatus and are corrected. All NMR were determined on Varian Associates Model A-60, A-60A, A-56/60, or HA/100 spectrometers. IR and UV spectra were determined on a Perkin–Elmer Infracord Model 137 or 521 and Carey Model 14 spectrophotometers, respectively; solvents are noted. Microanalyses were carried out by Mr. J. Nemeth and associates. All pH and pD measurements were made on a Corning Model 12 pH meter with a Sargent 30070–10 miniature combination electrode. Reagents obtained commercially were not purified unless specific drying or purification procedures are noted.

Kinetic experiments. In a typical kinetic run, an NMR tube containing the appropriate weight of substrate, buffer, or base, and solvent was heated in an insulated bath for an appropriate length of time. The samples were quenched in ice water or Dry Ice and kept at 0° or lower until NMR integrations could be made. All proton exchanges were monitored by NMR spectroscopy; three to 5 integrations were done for each point and the average of these was used to compute rates. Integrations were made relative to a non-exchanging proton absorption in the same substrate or to an internal standard. In exchanges not run at NMR probe temps (41–42°), negligible exchange occurred during the time of the integral determinations. The rate constants were calculated either by a least squares program or by the graphical method, with first-order kinetics being assumed in each case. Specific rate constants were calculated by division of the observed rate constants by the base concentration.

Exchange of 1-*methyl*-4-*pyrimidone* (3). All pyrimidone exchanges were carried out in D_2O with pyrimidone concentrations of 1·0 M at 70 or 100°, with phosphate or acetate buffers being used. Unless otherwise specified, exchange was observed only at H-2.

Exchanges of 1,3-*dimethyl*-4-*pyrimidonium fluoroborate* (7), 1-*methyl*-4-*pyridone* (1), *and* 4-*methoxy*-1-*methylpyridinium fluoroborate* (9). In these exchanges the disappearance or appearance of the low field absorbance was observed (H-2 or H-2,6). Table 1 gives the conditions used.

TABLE 1. EXCHANGES OF 1,3-DIMETHYL-4-PYRIMIDONIUM FLUOROBORATE (7), 1-METHYL-4-PYRIDONE (1), AND 4-METHOXY-1-METHYLPYRIDINIUM FLUOROBORATE (9) IN D_2O

Components	Molarity	Half-lives followed	Temperature (°C)	*pD*	k_{obs} (sec^{-1})
7	0·9	4	70	4·28–3·88 (70°)	$2·61 \pm 0·19 \times 10^{-3}$
NaOAc	0·1				
HOAc	1·1				
1	1·55	5	100	11·7–11·6 (25°)	$3·9 \times 10^{-6}$
KH_2PO_4	0·17				
K_3PO_4	0·37				
9	1·0	1[a]	100	7·3–6·8 (25°)	$5·3 \times 10^{-7}$
H_3BO_3	0·13				
KH_2PO_4	1·3				
K_3PO_4	0·43				

[a] Hydrolysis occurred to the extent of about 50% during the runs. Linear plots of log H2,6/H3,5 *vs* time were obtained.

Attempted exchange of 4-*methoxy*-1-*methylpyridinium iodide.* Solns composed of 1·0 M **8** and borate buffer (*pD* 3·9–2·7) in D_2O or 0·89 M **8** in MeOD with added NaOMe at 70, 100, and 42°, respectively, showed only hydrolysis, and no exchange at H-2 was detected in the time that more than 95% of the salt hydrolyzed.

pD *measurements.* The assembly was calibrated prior to each determination with a buffer in H_2O. Buffer standards with a pH of 4·00, 7·00, or 9·00 were used for calibration, the one closest to the *pD* being determined being chosen. The correction from *pH* (measured) to *pD* was made according to the equation *pD* = *pH* (measured) + 0·34. This correction factor was determined at 70° by comparing the *pH* (measured) on a Corning Model 12 with a Sargent combination electrode with the reported *pD* of a soln of 0·05 M HOAc and 0·05 M NaOAc in D_2O.[20] A two-point straight line plot was made between the 0·34 correction factor at 70° and the 0·40 correction factor reported for 25°. Correction factors used at other temps were taken from this plot.

Measurements of *pD* were made directly on the NMR samples (~0·5 ml) before and after a run. All *pD* measurements except those on the 70° kinetic run of 1-methyl-4-pyrimidone and 1,3-dimethyl-4-pyrimidonium fluoroborate were determined at room temp. When *pD* was used to determine D_2O concentrations for specific rate constants, calculations were not corrected for temp. Temp corrections were made for pK_{H_2O} and pK_{D_2O}.

The solns containing fluoroborate salts gave unstable *pD* measurements which fluctuated by a maximum of 0·05–0·01 *pD* units. In these cases, an average value in the range of the fluctuations was chosen as the *pD*.

For the exchange of 1-methyl-4-pyrimidone at high *pD*, the *pD* values before and after a kinetic run show a large variation, 11·84–7·55. In this case, the initial value was used in the calculations. Repeat runs gave the same results, and the pseudo first-order plots are reasonably straight lines.

pD *measurements at* 70°. The *pD* of solns of 1,3-dimethyl-4-pyrimidonium salt and 1-methyl-4-pyrimidone used for kinetic runs was determined at 70° by immersing the sample cup in an insulated, heated bath. The electrode was equilibrated for 10–20 min at 70°. Potassium acid tartrate (saturated at 25°) and potassium acid phthalate (0·05 M at 25°), which have pH values of 3·58 and 4·13, respectively, at 70°, were used as calibration standards.[21]

Samples of 1,3-dimethyl-4-pyrimidonium salt and buffer were made with the same molar concentrations in D_2O at 70° as the kinetic samples, and the *pD* was taken immediately. The *pD* was observed during the course of the exchange and was found to decrease from 3·8 to 3·5 over a 20-min period.

Determination of the pKa *of* 1-*ethyl-4-pyrimidone at* 70° *in* D_2O was accomplished by titration in D_2O of a pyrimidone-D_2O solution with DCl in the insulated bath.[7] Potassium acid phthalate and potassium tartrate were used as calibration standards for the *pH* electrodes. The pK_a was determined from the Eq (1), where $(f_\pm^{1:1})$ is calculated from $-\log(f_\pm^{1:1}) = 0{\cdot}505\sqrt{I}/1{\cdot}0 + 1{\cdot}6\sqrt{I}$, with $I = [BH^+]_{stoi} - \{D^+\}$. The quantity $\{D^+\}$ is obtained from the measured *pD*.[7]

$$pK_a = pD + \log\left(\frac{[BH^+]_{stoi}(f_\pm^{1:1}) - \{D^+\}}{[B]_{stoi} + \{D^+\}}\right) \quad (1)$$

Acknowledgement—We are grateful to the Alfred P. Sloan Foundation and the Public Health Service (GM-12595) for support, to the Eli Lilly Company for a fellowship to Richard N. Watson, and to Professor John Zoltewicz for criticism of this manuscript.

REFERENCES

[1] 6-Membered rings:
[a] H. E. Dubb, M. Saunders and J. H. Wang, *J. Am. Chem. Soc.* **80**, 1767 (1958);
[b] I. F. Tupitsyn and N. K. Semenova, *Tr. Gos. Inst. Prikl. Khim.* **49**, 120 (1962), *Chem. Abstr.* **60**, 6721[c] (1964);
[c] N. N. Zatsepina, I. F. Tupitsyn and L. S. Efros, *J. Gen. Chem. USSR* **34**, 4124 (1964);
[d] P. Beak and J. Bonham, *Tetrahedron Letters* 3083 (1964);
[e] P. Beak and J. Bonham, *J. Am. Chem. Soc.* **87**, 3365 (1965);
[f] T. J. Curphey, *Ibid.* **87**, 2063 (1965);
[g] J. A. Zoltewicz and C. L. Smith, *Ibid.* **88**, 4766 (1966);
[h] J. A. Zoltewicz and C. L. Smith, *Ibid.* **89**, 3358 (1967);
[i] R. K. Howe and K. W. Ratts, *Tetrahedron Letters* 4743 (1967)
[2] 5-Membered rings:
[a] R. Breslow, *J. Am. Chem. Soc.* **80**, 3719 (1958);
[b] R. J. Gillespie, A. Grisom, J. H. Ridd and R. F. M. White, *J. Chem. Soc.* 3228 (1958);
[c] P. Haake and W. B. Miller, *J. Am. Chem. Soc.* **91**, 4044 (1963);
[d] R. A. Olofson, W. R. Thompson and J. S. Michaelman, *Ibid.* **86**, 1865 (1964);
[e] H. Prinzbach, E. Futterer and A. Luttringhaus, *Angew. Chem.* Intern. Ed., **5**, 513 (1965);
[f] H. A. Staab, H. Irngartinger, A. Mannschreck and M. T. Wu, *Liebigs Ann.* **695**, 55 (1966)
[3] 6-Membered rings:
[a] P. Beak and E. M. Monroe, *J. Org. Chem.* **34**, 589 (1969);
[b] J. A. Zoltewicz, G. M. Kauffman and C. L. Smith, *J. Am. Chem. Soc.* **90**, 5939 (1968);
[c] J. A. Zoltewicz, G. Grahe and C. L. Smith, *Ibid.* **91**, 5501 (1969);
[d] I. F. Tupitsyn, N. N. Zatsepina, A. V. Kirova and Y. M. Kapustin, *Reakts. Sposobnost. Org. Soedin* **5**, 601 (1968); *Chem. Abstr.* **70**, 76940[x] (1969);
[e] Y. Kawazoe, Y. Koshioka, M. Yamada and H. Igeta, *Chem. Pharm. Bull. Tokyo* **15**, 2000 (1967);
[f] R. A. Abramovitch, G. M. Singer and A. R. Vinutha, *Chem. Commun.* 55 (1967);
[g] H. V. Blank, I. Wempen and J. J. Fox, *J. Org. Chem.* **35**, 1131 (1970);
[h] W. W. Paudler and L. S. Helmik, *Ibid.* **33**, 1087 (1968) and refs cited

[4] 5-Membered rings:
[a] R. A. Olofson and J. M. Landesberg, *J. Am. Chem. Soc.* **88**, 4263 (1966);
[b] R. A. Olofson, J. M. Landesberg, K. N. Houk and J. S. Michaelman, *Ibid.* **88**, 4265 (1966);
[c] P. Haake, L. B. Bausher and W. B. Miller, *Ibid.* **91**, 1113 (1969);
[d] K. T. Potts, H. R. Burton and S. K. Roy, *J. Org. Chem.* **31**, 265 (1966);
[e] A. Rochat and R. A. Olofson, *Tetrahedron Letters* 3377 (1969) and refs cited
[5] [a] J. A. Zoltewicz and J. D. Meyer, *Ibid.* 421 (1968);
[b] R. A. Coburn, J. M. Landesberg, D. S. Kemp and R. A. Olofson, *Tetrahedron* **26**, 685 (1970);
[c] T. M. Harris and J. C. Randall, *Chem. & Ind.* 1728 (1965);
[d] J. L. Wong and J. H. Keck, *Abstracts of the 158th National ACS Meeting*, Or51, New York, N. Y., Sept. (1969);
[e] N. N. Zatsepina, Y. L. Kaminskii and I. F. Tupitsyn, *Reakts. Sposobnost. Org. Soedin.* **4**, 433 (1967); *Chem. Abstr.* **69**, 85848[e] (1968);
[f] H. Igeta, M. Yamada, Y. Koshicka and Y. Kawazoe, *Chem. Pharm. Bull. Tokyo* **15**, 1411 (1967);
[g] F. Bergmann, D. Lichtenberg and Z. Neiman, *Chem. Commun.* 992 (1969);
[h] J. D. Vaughan, Z. Mughrabi and E. C. Wu, *J. Org. Chem.* **35**, 1141 (1970);
[i] E. C. Wu and J. D. Vaughan, *Ibid.* **35**, 1146 (1970) and refs cited
[6] G. E. Wright, L. Bauer and C. L. Bell, *J. Heterocycl. Chem.* **3**, 440 (1966)
[7] A. Albert and E. P. Serjeant, *Ionization Constants of Acids and Bases*, pp. 27–63. Wiley, New York, N. Y. (1962)
[8] A. K. Covington, R. A. Robinson and R. G. Bates, *J. Chem. Phys.* **69**, 2750 (1965)
[9] D. J. Brown, E. Hoerger and S. F. Mason, *J. Chem. Soc.* 211 (1955)
[10] J. P. Schoffner, L. Bauer and C. L. Bell, *J. Heterocycl. Chem.* **7**, 487 (1970)
[11] R. Wagner and W. von Phillipsborn, *Helv. Chim. Acta* **53**, 299 (1970)
[12] P. E. Rony, *J. Am. Chem. Soc.* **91**, 6090 (1969) and refs cited
[13] A. Albert, *Physical Methods in Heterocyclic Chemistry* (Edited by A. R. Katritzky), pp. 79–81. Academic Press, New York, N. Y. (1963)
[14] I. F. Tupitsyn, N. N. Zatsepina and A. V. Kirova, Isotopenpraxis, **3**, 136 (1967); *Chem. Abstr.* **71**, 21351w (1969); R. A. Olofson, R. V. Kendall, A. C. Rochat, J. M. Landesberg, W. R. Thompson and J. S. Michelman, *Abstracts of the 153rd National Meeting of the American Chemical Society*, Q34, Miami, Fla., April (1967)
[15] G. W. Crosbie, *The Nucleic Acids, III* (Edited by E. Charaguff and J. N. Davidson), p. 327. Academic Press, New York, N. Y. (1960)
[16] B. R. Brown and D. L. Hammick, *J. Chem. Soc.* 659 (1949); P. Haake and J. Mantecon, *J. Am. Chem. Soc.* **86**, 5230 (1964); K. W. Ratts, R. K. Howe and W. G. Phillips, *Ibid.* **91**, 6115 (1969); H. Quast and E. Schmitt, *Liebigs Ann.* **732**, 43 (1970) and refs cited
[17] J. Sköda, *Progress in Nucleic Acid Research* **2**, 205 (1963)
[18] D. J. Brown and L. N. Short, *J. Chem. Soc.* 331 (1953); D. J. Brown, *J. Soc. Chem. Ind.* **69**, 353 (1950); L. Bauer, G. E. Wright, B. A. Mikrut and C. L. Bell, *J. Heterocycl. Chem.* **2**, 447 (1965)
[19] D. J. Pasto and G. R. Meyer, *J. Org. Chem.* **33**, 1257 (1968)
[20] R. Gary, R. G. Bates and R. A. Robinson, *J. Chem. Phys.* **69**, 2750 (1965)
[21] R. G. Bates, *Determination of pH, Theory and Practice*, p. 76. Wiley, New York, N. Y. (1964)