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Kinetics of the Reduction of C-9 Substituted Acridinium Cations by 1,4-Dihydronicotinamides

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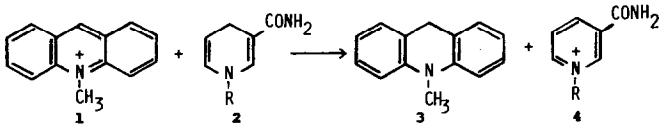
Second-order rate constants (k_2) are reported for the reduction of 9-R-10-methylacridinium cations (**5**: R = H, CH_3, CH_3CH_2, $C_6H_5CH_2$, $(CH_3)_2CH$, C_6H_5, 4-$(CH_3)_2NC_6H_4$) by 1-benzyl-1,4-dihydronicotinamide (**2**: R = $C_6H_5CH_2$) in 20% CH_3CN–80% H_2O at 25°C. All **5**: R ≠ H are reduced in the range 20- to 140-fold more slowly than **5**: R = H. However, there is no simple relationship between k_2 and the nature of R, nor between k_2 and the second-order rate constant for hydroxide ion attack at C-9 of these cations in pseudobase formation. Rates of reduction of **5** by 1-benzyl-4,4-dideuterio-1,4-dihydronicotinamide allow the calculation of the following kinetic isotope effects in this solvent medium: **5**: R, k^H/k^D: H, 1.56; $C_6H_5CH_2$, 2.7; C_6H_5, 5.4. Substituent effects upon k_2 were evaluated for the reduction of **5** by 1-(X-benzyl)-1,4-dihydronicotinamides, and lead to the following Hammett ρ parameters: **5**: R, ρ: H, −0.68; $C_6H_5CH_2$, −0.92; C_6H_5, −0.96. The latter two values require essentially complete unit positive charge generation on the nicotinamide moiety in the rate-determining transition state. It is shown that these Hammett ρ values and the above isotope effects can only be rationalized by a two-step $e^- + H^\cdot$ mechanism for hydride transfer from **2** to **5** in this solvent system. This result contrasts with our earlier conclusion of direct, one-step hydride transfer in the reduction of isoquinolinium cations by **2**, but is consistent with our observation that acridinium cations are reduced 37500-fold faster by **2** than predicted on the basis of the relative rates of nucleophilic attack (hydroxide ion) on acridinium and isoquinolinium cations. It is suggested that the availability of *both* Hammett ρ values *and* primary kinetic isotope effects will generally allow the establishment of the mechanism of hydride transfer in these systems. Application of these ideas to literature data suggests that **5**: R = H is reduced by direct hydride transfer in acetonitrile solution, in contrast to the above result in predominantly aqueous solution. The ready formation of acridanyl radicals by electron transfer to acridinium cations is demonstrated by the formation of Wurster's Blue radical cation upon mixing solutions of acridinium cations with *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

There have been many recent kinetic studies which formally involve either acridinium cations as hydride acceptors from 1,4-dihydronicotinamides, e.g. Eq. [1] (*1–11*), or, alternatively, a 9,10-dihydroacridine as hydride donor towards a variety of organic molecules (e.g., Eq. [2] where Acc is a suitable hydride acceptor species) (*12–14*).



[1]

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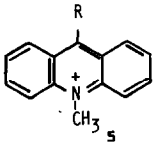
$$\mathbf{3} + \mathrm{Acc} \rightarrow \mathbf{1} + \mathrm{HAcc}^- \qquad [2]$$

The interest in these reactions revolves around the fundamental details of the mechanism of hydride transfer between the donor and acceptor species, and the relevance of such systems as models for the related transfers to and from the oxidized ($NAD(P)^+$) and reduced (NAD(P)H) forms of the nicotinamide coenzymes in enzymatic reactions. There is a long-standing discussion as to whether such reactions occur via transfer of a discrete hydride ion (a two electron process) or, alternatively, via one of several kinetically equivalent, one-electron processes ($e^- + H^\cdot$ or $2e^- + H^+$) which involve short-lived radical intermediates.

Sigman *et al.* (*1, 3*) originally interpreted kinetic studies of the reaction in Eq. [1] as requiring an intermediate species that was possibly of radical character, while Ohno *et al.* (*8*) have argued for an electron–proton–electron transfer mechanism in the reduction of **1** by 1-(substituted phenyl)-1,4-dihydronicotinamides (**2**: R = XC_6H_4). Both of these studies were subsequently shown (*10, 11*) to be complicated by unsuspected, competing reactions, and it has recently been concluded (*10, 11*) that there is no current evidence that is inconsistent with a simple, one-step hydride transfer in the reduction of acridinium cations by 1,4-dihydronicotinamides.

Colter *et al.* (*12*) have concluded that a mechanism involving direct hydride transfer is consistent with their experimental data for the reaction of Eq. [2] with a variety of acceptor species. However, more recently Lai and Colter (*15*) reported trapping the 10-methylacridanyl radical during the oxidation of **3** by 2,3-dicyano-1,4-benzoquinone, while Peters and co-workers have reported (*14*) the direct observation of this radical in picosecond spectroscopic studies of ketone reduction by **3.** This 10-methylacridanyl radical, and various derivatives of it, have been included in mechanisms of a variety of reactions of acridine derivatives (*14–20*). This tendency towards radical formation by acridinium cations indicates that exhaustive experimental data will be required before one-electron transfer mechanisms can be confidently ruled out in the dihydronicotinamide reductions of acridinium cations.

We have commenced a detailed systematic study of substituent effects in both the hydride donor and acceptor in an attempt to further clarify the mechanism of the reduction of acridinium cations by 1,4-dihydronicotinamides. The current paper reports upon the influence of C-9 substituents upon the rates of reduction of the 10-methylacridinium cations (**5**) by 1-benzyl-1,4-dihydronicotinamide (**2**: R = $C_6H_5CH_2$) and its 4,4-dideuterio derivative. Substituent effects in the hydride donor are also probed for **5**: R = H, C_6H_5, and $C_6H_5CH_2$ using 1-(substituted benzyl)-1,4-dihydronicotinamides (**2**: R = $XC_6H_4CH_2$) and the 1-benzyl-1,4-dihydropyridine derivatives **6**: W = $CONHCH_3$, $CON(CH_3)_2$ and CN.



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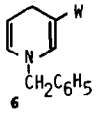

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EXPERIMENTAL DETAILS

All acridinium cations and 1,4-dihydronicotinamide derivatives were available from, or were prepared as described in, previous studies from our laboratory (*21–23*). *N,N,N′,N′*-Tetramethyl-*p*-phenylenediamine (TMPD) was recrystallized several times from hexane and had mp 49–50°C.

All kinetic studies, except where otherwise noted, were carried out in 20:80 (v/v) acetonitrile–water solution at 25°C in the presence of 0.005 *M* phosphate buffer adjusted to pH 7 at ionic strength 1.0 (1 *M* KCl). Several studies were also made at 25°C in methanol containing only the reactants. All reactions were studied under conditions in which the 1,4-dihydronicotinamide derivative was in 10- to 1000-fold excess over the acridinium cation. Acridinium cation reduction was followed by the decrease in absorbance in the vicinity of 400–440 nm, except for **5**: R = 4-$(CH_3)_2NC_6H_4$, which was followed at 500 nm, at acridinium cation concentrations in the range 0.01–0.03 m*M* as solubility of the dihydroacridine product allowed. All reactions proved to be kinetically pseudo-first-order in acridinium cation over at least 90% of the reaction. The faster reactions were investigated on a Durrum–Gibson stopped-flow spectrophotometer, while slower reactions were followed on a Varian Cary 210 spectrophotometer.

Under the current experimental conditions, the rates of hydration of the C(5)–C(6) double bond of these 1,4-dihydronicotinamides are extremely slow. For example, we have followed the decrease in absorbance at 360 nm due to the hydration of 1-benzyl-1,4-dihydronicotinamide over a period of 5 hr. From this initial reaction rate we have estimated that the first-order rate constant for hydration of this compound is approximately 3×10^{-6} sec^{-1}, which corresponds to a half-time of over 2 days. This rate constant is 6-fold smaller than the expected pseudo-first-order rate constant that we have calculated from the second-order rate constant reported by Johnson and Tuazon (*36*) for catalysis of hydration by dihydrogen phosphate monoanion in strictly aqueous solution. However, our approximate rate constant for this process in 20% CH_3CN–80% H_2O seems reasonable in the light of the known dramatic decrease in rates of hydration in the presence of organic cosolvents; e.g., in 49% ethanol in water the rate of hydration is 11-fold slower than in strictly aqueous solution (*37, 38*).

The slowest acridinium cation reduction in the current study has a half-time of less than 1 hr. However, to ensure that hydration phenomena do not influence our current study, we have always added the 1,4-dihydronicotinamide in acetonitrile solution as the last component of our reaction mixtures in studies on the Varian Cary 210 spectrophotometer. In stopped-flow studies, solutions of the 1,4-dihydronicotinamide in 40% acetonitrile–60% water containing no phosphate buffer were mixed with aqueous solutions of the acridinium cation in 0.01 *M* phosphate buffer, pH 7, containing 2 *M* KCl.

The interaction of the 10-methylacridinium cation with TMPD was investigated in acetonitrile solution containing 0.56 m*M* **1** and various concentrations of TMPD at 25°C.

RESULTS

The rates of reduction of **5**: R = H, CH_3, CH_3CH_2, $C_6H_5CH_2$, $(CH_3)_2CH$, C_6H_5, and 4-$(CH_3)_2NC_6H_4$ were studied in the presence of large excesses of 1-benzyl-1,4-dihydronicotinamides in 20% acetonitrile–80% water (v/v). In all cases, pseudo-first-order rate constants (k_{obs}) for the reduction of **5** were directly proportional to the reductant concentration up to at least [**2**] = 5 m*M*. The second-order rate constants (k_2) were evaluated from the slopes of the linear plots of k_{obs} against [**2**]. Values of k_2 for the reduction of a variety of **5** by **2**: R = $C_6H_5CH_2$ are collected in Table 1.

The value of k_2 = 400 sec^{-1} for **5**: R = H in 20% CH_3CN–80% H_2O is in good agreement with k_2 = 550 sec^{-1} in aqueous solution and k_2 = 65 sec^{-1} in neat acetonitrile reported for this reaction at 25°C by Hajdu and Sigman (*2*). The 60-fold lower rate of reduction reported for **5**: R = C_6H_5 relative to **5**: R = H in Table 1 is also in reasonable agreement with the 30-fold difference in rates reported for the reduction of these two acridinium cations in neat acetonitrile at 20° by van Laar *et al.* (*9*).

Second-order rate constants for the reduction of **5**: R = H, C_6H_5, and $C_6H_5CH_2$ by 1-benzyl-4,4-dideuterio-1,4-dihydronicotinamide are also included in Table 1, along with the (predominantly) primary kinetic isotope effects that can be calculated from these rate data.

We have also investigated the kinetics of the reduction of **5**: R = H and CH_3CH_2 by **2**: R = $C_6H_5CH_2$ in methanol as solvent in view of the report of Hajdu and Sigman (*3*) of charge-transfer complex formation and a kinetic saturation effect for this reaction in methanolic solution. We have confirmed the presence of an absorption band in the vicinity of 550 nm which is produced in times less than the mixing time of the stopped-flow spectrophotometer. The decay of this absorption at 550 nm is first-order in [**5**], and pseudo-first-order rate constants for this process as a function of 1-benzyl-1,4-dihydronicotinamide concentration are shown in Fig. 1 in the form of a double-reciprocal plot. In contrast to the earlier report (*3*) of kinetic saturation for **5**: R = H, we find that k_{obs} is linear up to at least 0.3 *M* in

TABLE 1

SECOND-ORDER RATE CONSTANTS FOR THE REDUCTION OF **5** BY **2** (R = $C_6H_5CH_2$)[a]

5: R	k_2^H (M^{-1} sec^{-1})	k_2^D (M^{-1} sec^{-1})	k_2^H/k_2^D
H	400 ± 6	256 ± 3	1.56
CH_3	5.2 ± 0.5		
CH_3CH_2	12.9 ± 0.8		
$C_6H_5CH_2$	17 ± 1	6.3 ± 0.5	2.7
$(CH_3)_2CH$	8.8 ± 0.5		
C_6H_5	6.8 ± 0.2	1.25 ± 0.05	5.4
4-$(CH_3)_2NC_6H_4$	2.93 ± 0.06		

[a] At 25°C in 20% CH_3CN–80% H_2O (v/v), pH 7, ionic strength 1.0.

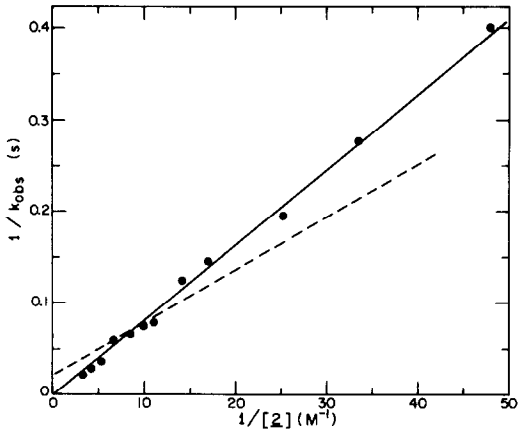


FIG. 1. Double-reciprocal plot showing the dependence of the pseudo-first-order rate constant for reduction of **5**: R = H upon the concentration of **2**: R = $C_6H_5CH_2$ in methanol at 25°C. The broken line is calculated from the kinetic parameters that are given for this reaction in Ref. (*3*).

2: R = $C_6H_5CH_2$, with the second-order rate constant in this solvent being 140 M^{-1} sec^{-1}. For **5**: R = CH_3CH_2, a slight deviation from linearity is apparent at the highest reductant concentrations (Fig. 2). This deviation presumably reflects the onset of a kinetic saturation effect, although the data up to at least 0.15 *M* is consistent with a second-order rate constant of 4.9 M^{-1} sec^{-1}.

Thus, for both of these acridinium cations we must conclude that the association constant for complex formation with 1-benzyl-1,4-dihydronicotinamide is much smaller than the value of 3 M^{-1} which was previously reported (*3*) for **5**: R = H. The broken line in Fig. 1 is calculated from the parameters reported for this reaction in Ref. (*3*). It is clear that our rate constants, while not showing any kinetic saturation effects, are of similar magnitude to those previously reported. Unfortunately, we cannot make a more direct comparison of our results with the

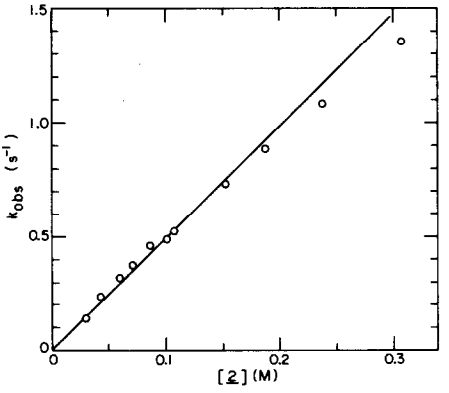


FIG. 2. Pseudo-first-order rate constants for the reduction of **5**: R = C_2H_5 by **2**: R = $C_6H_5CH_2$ in methanol at 25°C.

data of Hajdu and Sigman since these workers do not show experimental data points in their figures.

We find no evidence for the biphasic first-order plots reported by van Laar *et al.* (*9*) for the reduction of **5**: R = H by **2**: R = $C_6H_5CH_2$ in methanol. Interestingly, we have calculated $k_2 = 30\ M^{-1}\ sec^{-1}$ from the initial rate of the reaction in their Fig. 2, and this value is in excellent agreement with their data for **5**: R = H in acetonitrile, when compared with the relative rates that they report for **5**: R = C_6H_5 in methanol and acetonitrile. We suspect that the reported biphasic disappearance of absorbance at 420 nm arises from contamination of **5**: R = H by unmethylated acridine. We have shown in an unpublished study that acridine is reduced in an acid-catalyzed process (via the acridinium cation) by 1-benzyl-1,4-dihydronicotinamide. The 300-fold slower rate for the second phase of the biphasic process would then be consistent with reduction of acridine catalyzed by traces of acid in the methanolic solution.

Rates of reduction of **5**: R = H, C_6H_5 and $C_6H_5CH_2$ by a series of 1-(X-benzyl)-1,4-dihydronicotinamides (**2**: R = $XC_6H_4CH_2$) were measured in aqueous acetonitrile in the presence of large excesses of **2.** In all cases these reactions were pseudo-first-order in the acridinium cation concentration, with pseudo-first-order rate constants that were directly proportional to [**2**]. The second-order rate constants that were obtained for each of these reactions are given in Table 2. These second-order rate constants (k_2) are correlated with the Hammett σ constants for the substituents X by Eqs. [3], [4], and [5] for **5**: R = H, C_6H_5, and $C_6H_5CH_2$, respectively:

$$\log k_2 = -0.68(\pm 0.05)\sigma + 2.56(\pm 0.02)\ (r = 0.991) \quad [3]$$

$$\log k_2 = -0.92(\pm 0.07)\sigma + 1.28(\pm 0.03)\ (r = 0.991) \quad [4]$$

$$\log k_2 = -0.96(\pm 0.07)\sigma + 0.81(\pm 0.03)\ (r = 0.988) \quad [5]$$

Second-order rate constants were also determined as described above for the reduction of **5**: R = H, C_6H_5, and $C_6H_5CH_2$ by the 1,4-dihydronicotinamide

TABLE 2

SECOND-ORDER RATE CONSTANTS FOR THE REDUCTION OF **5** BY **2**(R = $XC_6H_4CH_2$)[a]

	$k_2(M^{-1}\ sec^{-1})$		
2: X	**5**: R = H	**5**: R = $C_6H_5CH_2$	**5**: R = C_6H_5
H	400 ± 6	17 ± 1	6.8 ± 0.2
4-F	306 ± 6	15.5 ± 0.7	5.5 ± 0.2
4-Br	242 ± 4	12 ± 1	3.5 ± 0.2
3-F	221 ± 4		2.7 ± 0.1
3-CN	150 ± 2	5.6 ± 0.7	1.91 ± 0.06
4-CN	128 ± 1	4.3 ± 0.7	1.39 ± 0.08

[a] At 25°C, in 20% CH_3CN–80% H_2O, pH 7, ionic strength 1.0.

TABLE 3

SECOND-ORDER RATE CONSTANTS FOR THE REDUCTION OF **5** BY **6**[a]

	$k_2(M^{-1}\ sec^{-1})$			
5:R	**6**:W = CN	$CONH_2$	$CONHCH_3$	$CON(CH_3)_2$
H	10.9 ± 0.6	400 ± 6		
$C_6H_5CH_2$		17 ± 1	31 ± 3	59 ± 4
C_6H_5	0.072 ± 0.006	6.8 ± 0.2	9.6 ± 0.2	12.0 ± 0.8
IQ[b]	0.52	29	58	106

[a] At 25°C, in 20% CH_3CN–80% H_2O, pH 7, ionic strength 1.0.
[b] Isoquinolinium cation **12**: W = CN. Data from Ref. (*22*).

derivatives **6**: W = CN, $CONHCH_3$, and $CON(CH_3)_2$ in aqueous acetonitrile solutions. Rate constants for these reactions are collected in Table 3.

The absorption maximum in the vicinity of 550 nm reported by Hajdu and Sigman (*3*) and confirmed in the present work for the species present immediately upon mixing the 10-methylacridinium cation and 1-benzyl-1,4-dihydronicotinamide is similar to the spectra of charge–transfer complexes which other workers have reported to be formed in solutions containing acridinium cations and various aniline derivatives (*20, 24*). We have commenced a systematic study of such species for a variety of substituted anilines, and while this study is still incomplete, we feel that the observations in Fig. 3 are relevant to the current study. Figure 3 shows the absorption spectra of solutions containing the 10-methylacridinium cation and various concentrations of TMPD. These solutions, immediately

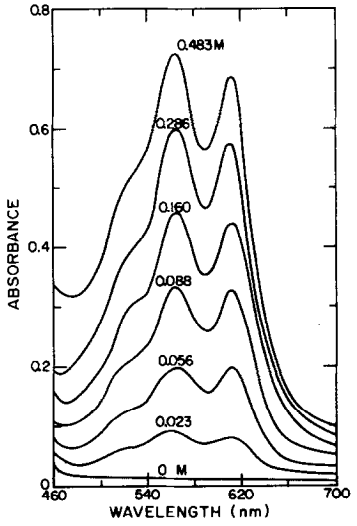
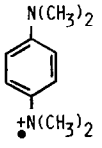


FIG. 3. Absorption spectra of solutions of **5**: R = H (0.56 m*M*) in the presence of various concentrations (as indicated) of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine in acetonitrile solution.

upon mixing, display spectra having maxima at 563 and 614 nm. This spectrum is very characteristic of the well-known radical cation **7** which is commonly referred to as Wurster's blue (*25–27*). The ready formation of this species from TMPD in the presence of **1** is suggestive of a facile single-electron transfer from TMPD to **1** to generate **7** and the 10-methylacridanyl radical **8**: R = H.



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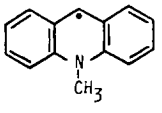

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Similar spectral observations of the formation of the Wurster's Blue radical cation were made for solutions containing TMPD and various examples of the 9-substituted acridinium cations **5** in Table 1. Some of these spectra proved to be unstable over periods of several hours, presumably due to subsequent radical combination reactions of the type described by Ballard *et al.* (*20*). We also note that the charge–transfer spectra of mixtures of quinolinium or isoquinolinium cations and TMPD do *not* show this characteristic Wurster's Blue radical cation spectrum.

DISCUSSION

From the data in Table 1 it is clear that the presence of any alkyl or aryl substituent at C-9 significantly decreases the rate of reduction of the 10-methylacridinium cation by 1-benzyl-1,4-dihydronicotinamide. There seems to be no simple relationship between k_2 and whether R is a primary alkyl, secondary alkyl, or aryl substituent. In fact, there is more variation of k_2 within the series of primary alkyl R groups (methyl, ethyl, and benzyl) than there is between the methyl, isopropyl, and phenyl substituents. Thus, it is clear that steric differences between different C-9 alkyl and aryl substituents are relatively unimportant in determining k_2, although there is presumably a predominant steric component that is responsible for the decrease in k_2 for any substituent relative to the C-9 unsubstituted 10-methylacridinium cation.

The lack of a simple steric influence from C-9 substituents upon k_2 contrasts with substituent effects upon the second-order rate constants (k_{OH}) for hydroxide ion attack at C-9 of **5** to form the pseudobases (**9**). Variation of k_{OH} with R in **5** does follow the order (H > primary alkyl > secondary alkyl > aryl) expected on the basis of steric effects (*23*). Relative substituent effects upon k_2 for the reduction of **5** and upon k_{OH} for hydroxide ion attack are compared in Table 4. At least a qualitative relationship between steric effects for hydroxide ion attack at C-9 and an assumed hydride ion attack at C-9 would be expected, since in both cases the partial rehybridization that occurs at C-9 in the transition state leads to some relief of the peri interactions between the C-9 substituent and the hydrogen atoms on C-1 and C-8. Such peri interactions are expected to increasingly destabilize the cation **5** with increasing size of R. The lack of any simple relationship between k_2

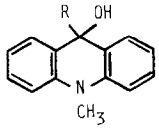
TABLE 4

RELATIVE RATES OF REDUCTION AND HYDROXIDE ION ATTACK ON 10-METHYLACRIDINIUM CATIONS (5)

5 : R	Relative k_2[a]	Relative k_{OH}[b]
H	1	1
CH_3	0.013	0.089
CH_3CH_2	0.032	0.105
$C_6H_5CH_2$	0.043	0.089
$(CH_3)_2CH$	0.022	0.014
C_6H_5	0.017	0.0024
4-$(CH_3)_2NC_6H_4$	0.0074	0.00125

[a] From data in Table 1.
[b] From data in Ref. (*23*).

and k_{OH} then suggests that the rate constant k_2 does not simply reflect rate-determining hydride attack at C-9.



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The two C-9 aryl substituents in particular seem to impart a far greater reactivity towards reduction (k_2) than expected upon the basis of relative rates of hydroxide ion attack (k_{OH}). It should be noted, however, that for a constant steric interaction such as is expected for **5** : R = C_6H_5 and 4-$(CH_3)_2NC_6H_4$, the substituent effect of the dimethylamino group is similar for both k_2 and k_{OH}.

The Hammett $\rho = -0.68$ (Eq. [3]) for the reduction of **5** : R = H by the 1,4-dihydronicotinamides **2** : R = $XC_6H_4CH_2$ may be compared with $\rho = -0.95$ in the same solvent for the equilibrium constants for cyanide ion dissociation from 1-(X-benzyl)-4-cyano-1,4-dihydronicotinamides to give the 1-(X-benzyl)nicotinamide cations (*28*). This comparison indicates that, in the transition state for 10-methylacridinium cation reduction, the nicotinamide moiety bears approximately 70% of the positive charge of the nicotinamide cation. This estimate is slightly smaller than the estimates of approximately 80% of the nicotinamide cation charge being borne in the transition state by the nicotinamide moiety during the reduction of isoquinolinium and 3,4-dihydroisoquinolinium cations by **2** : R = $XC_6H_4CH_2$ (*21*, *29*).

The above $\rho = -0.68$ for the reduction of **5** : R = H is significantly smaller than $\rho = -0.92$ and -0.96 (Eqs. [4] and [5]) for the reduction of **5** : R = $C_6H_5CH_2$ and C_6H_5, respectively. In fact, the Hammett ρ values for these two C-9 substituted cations are identical within experimental error of the above-quoted equilibrium $\rho = -0.95$ for nicotinamide cation formation from a 1,4-dihydronicotinamide derivative. These values require essentially a full positive charge to be borne by the

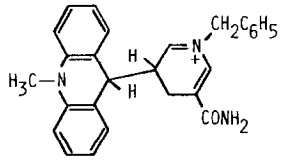
nicotinamide moiety in the transition states for the reduction of **5**: R = $C_6H_5CH_2$ and **5**: R = C_6H_5. Thus the presence of a C-9 substituent in the 10-methylacridinium cation reduces the rate of reduction, and also appears to cause the nicotinamide species to adopt an essentially product-like electron density in the rate-determining transition state. These C-9 substituent effects upon k_2 and Hammett ρ are formally consistent with reactivity–selectivity considerations, but, in view of the current controversy in this area, and the complexity of the current reaction mechanisms, we will not pursue these considerations further.

The magnitudes of the primary kinetic isotope effects for these reductions (Table 1) also show a pronounced dependence upon the presence (and apparently the nature (compare $C_6H_5CH_2$ and C_6H_5)) of a C-9 substituent in the 10-methylacridinium cation. Our value of $k^H/k^D = 1.56$ in 20% CH_3CN–80% H_2O for the reduction of **5**: R = H by **2**: R = $C_6H_5CH_2$ is very similar to $k^H/k^D = 1.38$ reported for this reaction in methanol (*3*) and also to $k^H/k^D = 1.46$ for the reduction of this acridinium cation by **2**: R = $CH_3(CH_2)_2$ in aqueous solution (*1*). These values contrast with primary isotope effects of 4.6 (*9*) and 4.11 (*10*) recently reported for the reduction of **5**: R = H by **2**: R = $C_6H_5CH_2$ in acetonitrile solution. Thus there would seem to be a pronounced solvent effect upon k^H/k^D for the reduction of the 10-methylacridinium cation. Such a dramatic solvent dependence of the primary kinetic isotope effect is also remarkable in view of the less than 10-fold decrease in rate that is observed for this reaction in acetonitrile relative to aqueous solution (*2*). This same solvent dependence does not seem to be present in the reduction of the 9-phenyl-10-methylacridinium cation. Thus, our value of $k^H/k^D = 5.4$ (Table 1) in 20% acetonitrile in water is actually somewhat larger than $k^H/k^D = 4.47$ which van Laar *et al.* report (*9*) for the reduction of this cation in neat acetonitrile.

Another curious feature of the primary kinetic isotope effects in Table 1 lies in their complete contradiction to what one would have predicted in view of the nature of the transition states deduced above from the Hammett ρ parameters in these reactions. The Westheimer theory of isotope effect dependence on transition state structure predicts (*30*) a primary kinetic isotope effect close to unity for reactions which display very product-like transition states. Although a quite product-like transition state seems to be indicated from the Hammett ρ values for the reductions of the 9-benzyl- and 9-phenyl-10-methylacridinium cations, these reactions display primary kinetic isotope effects (2.7 and 5.4, respectively) that are more typical of more "symmetrical" transition states. In contrast, the 10-methylacridinium cation apparently has a less product-like transition state when judged by the Hammett correlations, and yet has only a relatively small primary kinetic isotope effect in predominantly aqueous or methanolic solutions.

The data for the reactions of the 10-methylacridinium cation with the 1,4-dihydronicotinamides might be dismissed on the grounds that these reactions are complicated by the alternative reaction of the electrophilic C-9 of **5** with the nucleophilic C-5 of **2** to give an adduct such as **10**. There has been speculation on this possibility in the literature (*9, 31*) following the observation of analogous reactions between **2** and trifluoroacetophenone (*32*) or pyridine-2-carboxaldehyde (*31*). However, there does not seem to have been any specific experimental evidence reported in support of the formation of **10** in a reaction between **5** and **2**,

whereas essentially quantitative isolation of 9,10-dihydro-10-methylacridine (**3**) has been reported (*1, 4*) from these reactions. In view of this, we prefer to accept the rate constants for the reduction of the 10-methylacridinium cation at face value, and this approach seems to be justified by the satisfying mechanistic rationalization that is presented below for all of the current experimental data. For 9-alkyl and especially 9-aryl acridine derivatives, steric hindrance to the formation of species such as **10** at any significant rate would be expected to be prohibitive, and consequently resort to such species cannot be made in attempting to rationalize the current data.



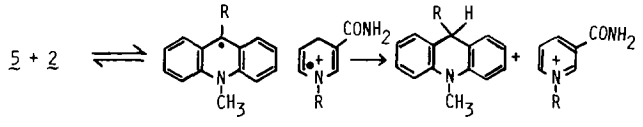
10

The mechanisms of the reduction of isoquinolinium (*21, 22*) and 3,4-dihydroisoquinolinium (*29*) cations are most readily interpreted in terms of rate-determining, one-step hydride transfer from the 1,4-dihydronicotinamide to these cationic hydride acceptors. Detailed substituent effect correlations in such reactions indicate that C—H bond breaking at C-4 of the nicotinamide moiety is considerably more advanced in the transition state than is C—H bond formation in the hydride acceptor (*21, 29*). This discrepancy between C—H bond fission and formation results in the accumulation of a considerable fractional negative charge on the migrating hydride species in the transition state. In the reduction of both 5-nitroisoquinolinium and 3,4-dihydroisoquinolinium cations it is estimated that transition state charge development on the nicotinamide moiety is 80% of that present on the nicotinamide cation product. Thus the nicotinamide species is quite product-like in these transition states.

As noted above, the Hammett ρ values for the reduction of the 9-benzyl- and 9-phenyl-10-methylacridinium cations by **2**: R = $XC_6H_4CH_2$ require essentially full development of unit positive charge on the nicotinamide moiety in the transition states for these reactions. This might be looked upon as an even more extreme product-like transition state nicotinamide moiety in these cases than was found in the cases of the isoquinolinium cation reactions, and would imply essentially complete C—H bond-breaking in the transition state. In view of the steric inhibitions to attack at C-9 in these acridinium cations, it would seem unlikely that this complete C—H bond fission from the hydride donor is accompanied by essentially complete transition state C—H bond formation at C-9 of the hydride acceptor. Consequently, a significant fractional negative charge would be expected to be borne by the migrating hydride species in these acridinium cation reductions. This interpretation might also explain the magnitudes of the primary kinetic isotope effects for **5**: R = $C_6H_5CH_2$ and C_6H_5. These isotope effects are clearly inconsistent with a transition state that is product-like both in terms of the hydride donor and the hydride acceptor.

An alternative mechanistic interpretation of the Hammett ρ values and the

kinetic isotope effects for the reduction of **5**:R = $C_6H_5CH_2$ and C_6H_5 is also consistent with all the current experimental information. This is outlined in Scheme 1, and involves the $e^- + H^\cdot$ mechanistic formalism that is often considered for hydride transfer. A rapid electron transfer, followed by a rate-determining hydrogen atom migration, would be quite consistent with the current experimental data. Thus a one-electron transfer from the dihydronicotinamide to the acridinium cation would generate a nicotinamide radical cation bearing a full positive charge, as suggested by the ρ values. Subsequent rate-determining hydrogen atom migration would not result in any further net charge change upon the nicotinamide moiety. A relatively symmetrical transition state for hydrogen atom migration would then be consistent with the observed kinetic isotope effects.



8 **11**

This mechanism predicts essentially complete charge neutralization on the acridine in the transition state, since a neutral acridanyl radical is formed in the initial electron transfer step. This contrasts with the one-step hydride transfer mechanism considered above, which predicts considerable cationic character on the acridine moiety in the transition state. The nature of the acridine moiety in the transition state may be probed using substituent electronic effects in these acridinium cations as probes of charge changes occurring between the reactants and transition state. Such studies are potentially capable of distinguishing between the direct one step hydride transfer and indirect $e^- + H^\cdot$ reduction mechanisms that are considered above. These studies will require considerable synthetic effort and have not yet been attempted.

We feel that the $e^- + H^\cdot$ mechanism for acridinium cation reduction by 1,4-dihydronicotinamides is consistent with all the experimental evidence presented in the current work. The initial electron transfer generates the acridanyl radical (**8**). The usual order of radical stabilities suggests that the 9-alkyl radicals **8** should display enhanced stabilities relative to the corresponding cations **1** in comparison with the 9-unsubstituted species **8**:R = H. For **8**:R = aryl, additional benzylic radical stabilization is potentially possible, although this additional resonance stabilization is probably quite small because of the inability of the 9-aryl substituent to achieve coplanarity with the acridine ring system (*23, 33*).

Furthermore, these relative radical stabilities combined with steric inhibition to attack at C-9 should cause **8**:R = H to be more reactive as a hydrogen atom abstractor than **8**:R = alkyl or aryl. This situation is summarized in Fig. 4, where the energy changes for R = H and R = alkyl (or aryl) are compared relative to the corresponding cations, for the simple two-step process of Eq. [6]. In Eq. [6], the radical pair intermediate is assumed to exist in a cage, which is treated as the single species *C*.

$$\mathbf{5} + \mathbf{2} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} C \xrightarrow{k_b} \mathbf{3} + \mathbf{4} \qquad [6]$$

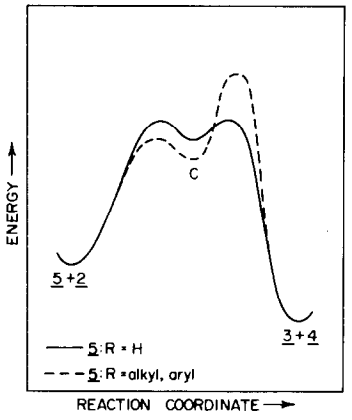


FIG. 4. Substituent effects upon the energy diagram for reduction of **5** by **2** via the $e^- + H^\cdot$ mechanism.

If C is treated as a steady-state intermediate, then Eq. [6] predicts that the observed second-order rate constant for this reaction is given by

$$k_2 = k_a k_b/(k_{-a} + k_b). \qquad [7]$$

Kinetic isotope effects upon k_a and k_{-a} should be negligible, and thus

$$\frac{k_2^H}{k_2^D} = \frac{k_b^H(k_{-a} + k_b^D)}{k_b^D(k_{-a} + k_b^H)}.$$

According to Fig. 4, $k_b \ll k_{-a}$ for R = alkyl or aryl, and thus $k_2^H/k_2^D = k_b^H/k_b^D$, which is the true primary kinetic isotope effect for the hydrogen atom transfer step. On the other hand, if k_b approaches k_{-a} in magnitude, then $k_2^H/k_2^D < k_b^H/k_b^D$. This is the situation portrayed for R = H in Fig. 4. These predictions are consistent with the current experimental observations of kinetic isotope effects of 1.56 for R = H, 2.7 for R = benzyl, and 5.4 for R = phenyl. In fact, these isotope effects exactly reflect the expected orders of k_b for R = H, alkyl, and aryl on the basis of both radical stability and steric inhibition to attack at C-9.

These same considerations in terms of Eq. [6] and Fig. 4 are also consistent with the observed Hammett ρ values for R = H, $C_6H_5CH_2$, and C_6H_5. Thus, for R = benzyl and phenyl, electron transfer is complete prior to the rate-determining transition state, and, consequently, ρ in these cases reflects a unit positive charge generation on the nicotinamide moiety. For R = H, there is no clear-cut rate-determining transition state, and consequently the observed k_2 and derived ρ value are complex functions of all rate constants (Eq. [7]).

We commented above upon the apparent solvent dependence of k_2^H/k_2^D for the reduction of the 10-methylacridinium cation. In terms of Fig. 4, k_2^H/k_2^D in the range 4–4.5, as observed for reduction of this cation in acetonitrile, should correspond to rate-determining hydrogen atom migration after initial rapid electron transfer, and a ρ value appropriate for full charge development on the nicotinamide moiety should be observed in this solvent. Hajdu and Sigman (*2*) give

second-order rate constants for reduction of **1**: R = H in acetonitrile by five **2**: R = 4-$XC_6H_4CH_2$ (X = OCH_3, CH_3, H, CO_2CH_3, CN). We have calculated $\rho = -0.56 \pm 0.05$ ($r = 0.990$) from these data. This value is less than we find for this reaction in 20% CH_3CN–80% H_2O, and must be scaled of course to allow for the expected more negative ρ value for unit charge development in the less polar acetonitrile as compared with aqueous solution. Although we are unable to make a quantitative comparison, it is clear that there must be significantly less positive charge development on the nicotinamide in the transition state in acetonitrile than in predominantly aqueous medium. Thus, in acetonitrile solution, the reduction of **1**: R = H is not consistent with the $e^- + H^\cdot$ mechanism proposed above for predominantly aqueous medium, but rather seems to be quite consistent with a one-step hydride transfer mechanism as concluded by Powell and Bruice (*10, 11*).

Primary kinetic isotope effects and Hammett ρ values are now available for reduction of a variety of cationic hydride acceptors by **2**: R = $XC_6H_4CH_2$. We suggest that, when primary kinetic isotope effects are considered in conjunction with Hammett ρ values, these combined criteria are capable of allowing the mechanistic distinctions indicated above. These combined criteria also allow the elimination of the $e^- + H^+ + e^-$ stepwise mechanism as the predominant pathway for reduction in these systems. The kinetic isotope effect (k_2^H/k_2^D) for the reduction of **5**: R = C_6H_5 would require that proton transfer be rate determining in such a mechanism. However, this would, in turn, require that the nicotinamide should bear considerably less than a full unit positive charge in the transition state, and this would be inconsistent with the observed ρ value for this reduction.

Our conclusion of an $e^- + H^\cdot$ mechanism for the reduction of acridinium cations in predominantly aqueous medium requires that this two-step mechanism be faster than direct one-step hydride transfer in these solutions. In Table 5 we compare rates of reduction and rates of hydroxide ion attack for the 10-methylacridinium cation and the 4-benzoyl-2-methylisoquinolinium cation (**12**: W = COC_6H_5). It has been established that isoquinolinium cations in general are re-

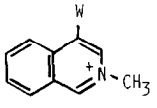
TABLE 5

COMPARISON OF RATE CONSTANTS FOR REDUCTION AND HYDROXIDE ION ATTACK ON ACRIDINIUM AND ISOQUINOLINIUM CATIONS

	5: R = H	**12**: W = COC_6H_5
pK_{R+}	10.01[a]	9.34[b]
k_{OH} (M^{-1} sec^{-1})	550[a]	4.1×10^4[b]
Rel. k_{OH}	1	75
k_2 (M^{-1} sec^{-1})	400	0.79[c]
Rel. k_2	500	1

[a] Data from Ref. (*34*).
[b] Data from Ref. (*35*).
[c] Data from Ref. (*22*).

duced by 1,4-dihydronicotinamides by direct one-step hydride transfer (*22*), and the second-order rate constants for this reduction are correlated with the second-order rate constants for hydroxide ion attack on these cations. This particular isoquinolinium cation is chosen for Table 5 since it most closely resembles the 10-methylacridinium cation in pK_{R+} for pseudobase formation. Thus, these two cations can be directly compared in kinetic terms without any significant thermodynamic influences complicating the comparison. Isoquinolinium cation **12**: W = COC_6H_5 reacts with hydroxide ion 75-fold faster than does the 10-methylacridinium cation. However, this latter cation is reduced by **2**: R = $C_6H_5CH_2$ at a 500-fold greater rate than **12**: W = COC_6H_5 is reduced. Thus the 10-methylacridinium cation appears to be reduced 75 × 500 = 37500-fold faster than expected for a simple nucleophilic process. Such a rate enhancement suggests that a mechanism that is faster than direct hydride transfer may be available for the reduction of the acridinium cation. We have argued above in favor of a two-step e^- + $H^\cdot$ mechanism.



12

The influence of the substituents W in **6** on k_2 for the reduction of **5** (Table 3) closely follows these same substituent effects in the reduction of isoquinolinium cations. It should be noted that, to a first approximation, electronic effects from W substituents will be similar for both direct hydride transfer and the two-step e^- + $H^\cdot$ mechanism, since in each case the pyridine ring of the hydride donor is significantly electron deficient in the transition state. Consequently, the substituent effects seen in Table 3 are consistent with either of these two mechanistic possibilities.

The ready formation of the Wurster's Blue radical cation from the interaction of TMPD with acridinium cations (Fig. 3) indicates the ready formation of acridanyl radicals from acridinium cations in the presence of a suitable electron donor. There are also a number of other observations in the literature of the ready formation of radicals by electron transfer to acridinium cations (*16–20*). Thus, a reaction mechanism for reduction of acridinium cations that involves an initial electron transfer to these cations has suitable pecedent in the known chemistry of these species.

The suggestion of a difference in mechanism for the reduction of the 10-methylacridinium cation in aqueous solution and acetonitrile solution is consistent with the expected relative stabilities of the nicotinamide radical cation and the appropriate transition states for the two different mechanisms in these two solvents. The nicotinamide radical cation required as an intermediate in the e^- + $H^\cdot$ mechanism would be expected to be less stable in acetonitrile than in a predominantly aqueous environment. In acetonitrile solution, the one-step hydride transfer involving only fractional positive charge development on the nicotinamide moiety in the transition state is apparently the lowest energy pathway. It seems that the

energetics of the one-step hydride transfer and two-step $e^- + H^\cdot$ mechanisms are similar for these reductions of acridinium cations, with the preferred pathway being susceptible to influence by solvent and substituent effects. Further detailed studies of solvent and substituent effects upon these reductions will provide important tests of the mechanistic conclusions that we have drawn in the current study.

Previous workers have also concluded that multistep mechanisms are operative for hydride transfer from 1,4-dihydronicotinamides to acridinium cations. These conclusions have been heavily based upon the observation of discrepancies between kinetic and product isotope effects when the reductions are performed using 1,4-dihydronicotinamides bearing a single deuterium label at C-4. The recent work of Powell and Bruice (*10*) has clearly established that such discrepancies are actually due to scrambling of the isotopic label in the 9,10-dihydroacridine product due to hydrogen exchange of this species with unreacted acridinium cation at a rate that is significant relative to the overall rate of reduction. The present work makes no use of such "discrepancies," and, furthermore, the two-step $e^- + H^\cdot$ mechanism does not predict any such difference in kinetic and product isotope effects since the hydrogen atom transfer step occurs in an essentially irreversible step in a reaction that thermodynamically lies heavily in favour of the 9,10-dihydroacridine and nicotinamide cation products.

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