Base Catalysis of the Reaction of Secondary Amines with *p*-Nitrophenyl Phosphate. Kinetic Evidence for an Addition Intermediate in Nucleophilic Aromatic Substitution[1]

A. J. Kirby and W. P. Jencks

Contribution No. 365 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received February 18, 1965*

The reactions of piperidine and dimethylamine with p-nitrophenyl phosphate in aqueous solution to give a substituted aniline are catalyzed by hydroxide ion. The reaction with dimethylamine is also subject to general base catalysis by a second molecule of dimethylamine. As the concentration of hydroxide ion or general base catalyst is increased, the initial rapid rate increase levels off and the second-order rate constant becomes almost independent of catalyst concentration. This is evidence for a change in rate-determining step of the reaction and for the intermediate formation of a tetrahedral addition compound. Comparison with the reaction of dimethylamine and p-nitrochlorobenzene, which is not subject to base catalysis, suggests that the catalyzed step in the reaction with p-nitrophenyl phosphate occurs after the formation of the addition intermediate. The slower reaction of methylamine with p-nitrophenyl phosphate does not show base catalysis in aqueous solution, and it is suggested that attack on the aromatic ring is rate determining with this amine.

In the course of an examination of the nucleophilic reactivity of amines toward the phosphorus atom of *p*-nitrophenyl phosphate dianion,[2] we were surprised to find that a significant fraction of the observed reaction with primary and secondary amines proceeds with attack on the 1-carbon of the aromatic ring to give a substituted aniline by nucleophilic aromatic substitution (eq. 1). The finding that the reaction with secondary amines, in aqueous solution, is subject to base catalysis stimulated a detailed examination of the reactions with dimethylamine and piperidine.

$$R_2NH + p\text{-}NO_2C_6H_4OPO_3^{2-} \rightleftharpoons p\text{-}NO_2C_6H_4NR_2 + HOPO_3^{2-} \quad (1)$$

Recently, a number of reactions have been reported which undergo a change in rate-determining step with changing catalyst concentration, so that a plot of rate against catalyst concentration is nonlinear. The existence of a change in rate-determining step requires that there be an intermediate in the reaction pathway and that the formation and decomposition of the intermediate have different sensitivities to catalysis. Many of these examples are reactions at the acyl group and at the carbonyl or substituted carbonyl group, and in a number of cases the existence of an addition intermediate in these reactions has been confirmed by independent methods.[3] There is a large amount of evidence which is consistent with the existence of a tetrahedral addition intermediate in nucleophilic aromatic substitution reactions, but there are few experiments which unequivocally demonstrate the formation of such an intermediate on the normal reaction pathway.[4-7] For example, the existence of general base catalysis[5-8] is not, in itself, evidence for the two-step nature of a reaction in view of the fact that there are reactions, such as the hydration of aldehydes, which are thought to proceed in one step by a concerted mechanism, but are subject to general acid and base catalysis.[9,10] The experiments reported here provide kinetic evidence, which we believe to be unambiguous, for a change in rate-determining step and the existence of an intermediate in the base-catalyzed reactions of dimethylamine and piperidine with *p*-nitrophenyl phosphate.

In 1958 Bunnett and Randall reported that the rate of the base-catalyzed reaction of N-methylaniline with dinitrofluorobenzene in 60% aqueous dioxane is not linear in respect to hydroxide ion concentration, and interpreted these data as evidence for an addition intermediate.[7] However, the experimental points showed considerable scatter, and the result has been criticized by Ross[5] on the ground that, within experimental error, a logarithmic plot of the rate constants against hydroxide ion concentration is linear with a slope of 1.0. Another possible interpretation of this result is that it is caused by the formation of a complex between hydroxide ion and dinitrofluorobenzene. Ross and

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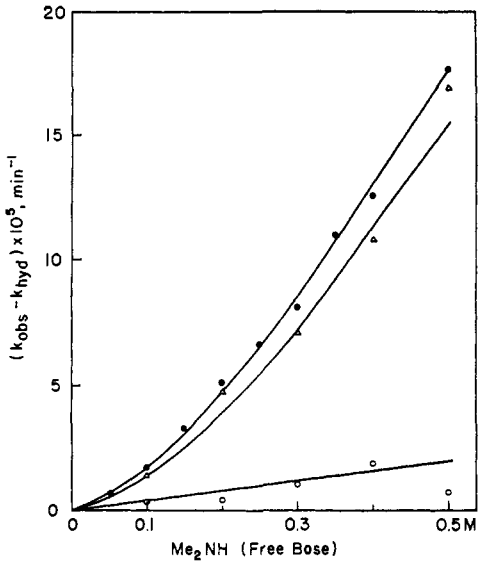

Figure 1. Observed rates of reaction of *p*-nitrophenyl phosphate with dimethylamine at pH 11.0, 39°, and ionic strength 1.0. Closed circles represent the sum of both reactions from direct measurement at 400 mμ; triangles, the rate of nucleophilic aromatic substitution, from measurements obtained by reading aliquots at 400 mμ at pH 3–4; and open circles, the rate for attack on phosphorus, by difference. The curves are calculated from eq. 7.

Kuntz have shown that the rate of the reaction of dinitrochlorobenzene and aniline shows a leveling off with increasing amine concentration, which is caused by the formation of a complex between the two reactants.[11] While the work described here was in progress, Bunnett and Garst reported in a preliminary communication that the rate of the reaction of piperidine with 2,4-dinitrodiphenyl ether is nonlinear in respect to hydroxide ion concentration and have interpreted the results as evidence for an addition intermediate.[12]

Experimental

Materials and methods were essentially as described in the previous paper.[2] Dioxane was purified according to the method of Fieser.[13]

Reaction Products. Solutions above 0.1 *M* in *p*-nitrophenyl phosphate, containing an excess of dimethylamine as the free base, gave precipitates of N,N-dimethyl-*p*-nitroaniline within 1 hr. at 39°. From one such experiment the yellow crystalline product was identified by recrystallization from ethanol to m.p. 162.5 (reported[14] m.p. 162.5°) and by its absorption maximum at 389 mμ in ethanol (reported[15] 390 mμ). Precipitation did not occur during the course of kinetic runs, which were generally followed to less than 1% of total reaction. Similarly, strongly alkaline solutions containing piperidine gave precipitates of 1-*p*-nitrophenylpiperidine, λ_{max} 425 mμ (ε 15,700) in water, melting point and mixture melting point after recrystallization from ethanol identical with an authentic sample (102–103°, reported[16] 105°).

(11) S. D. Ross and I. Kuntz, *J. Am. Chem. Soc.*, **76**, 3000 (1954).
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Kinetic Measurements. The initial rates of *p*-nitrophenolate ion or substituted *p*-nitroaniline formation were measured by following the increase in absorption at 400 mμ, as described previously.[2] Since the extinction coefficients of *p*-nitrophenolate and of the substituted *p*-nitroanilines are not identical at 400 mμ, it was necessary to calculate the rate of the *p*-nitrophenol- and aniline-forming reactions in the following manner: The formation of the products of both reactions was followed in the thermostated cell at 400 mμ at pH values such that *p*-nitrophenolate and the substituted aniline were entirely in their colored forms. A second series of runs, identical except for a sevenfold increase in phosphate ester concentration, was followed by adding 0.5-ml. aliquots at intervals to 3.0 ml. of aqueous acetic acid at a concentration sufficient to give a final pH of approximately 3–4. Since *p*-nitrophenol is fully protonated under these conditions, the absorption at 400 mμ was due entirely to the *p*-nitroaniline and gave the proportion of the total absorbance change which resulted from attack on the aromatic ring. The difference gave the absorbance change due to the *p*-nitrophenolate which was formed by attack on phosphorus. The rate constants for the two reactions were then calculated, based on extinction coefficients at 400 mμ of 18,320 for *p*-nitrophenolate ion, 15,100 for N,N-dimethyl-4-nitroaniline, 13,500 for 1-*p*-nitrophenylpiperidine, and 16,200 for N-methylaniline. The results of a typical experiment with dimethylamine are shown in Figure 1.

The rate constants for the steady-state rate equations (6 and 7) were obtained in the following manner: The pseudo-first-order rate constants for the aniline-forming reaction were determined as described above. These were corrected for the contribution to the rate from the small rate increase with increasing hydroxide ion concentration at high hydroxide ion concentration, and were divided by the free amine concentration to give a second-order constant, k'. For the piperidine reaction, the measurable constants of eq. 6 and 7 were then obtained by plots of $1/k'$ against $1/[OH^-]$. For the dimethylamine reaction, catalysis by both hydroxide ion and dimethylamine must be considered. The ratio of the catalytic constants for these two reactions, $c = k_2/k_3$, was estimated from the initial slopes of plots of k' against the concentration of one catalyst, with the other held constant. The measurable constants of eq. 6 and 7 were then obtained from linear plots of $1/k'$ against $1/(c[OH^-] + [R_2NH])$ at constant hydroxide ion concentration and against $1/([OH^-] + [R_2NH]/c)$ at constant amine concentration. For eq. 7, the slopes of these plots give k_{-1}/k_1k_3 and k_{-1}/k_1k_2, respectively, and the intercepts of both plots give $1/k_1$. The intercepts were found to be identical, within experimental error, for both plots. The calculated line of Figure 4 is based directly on eq. 7, and that of Figure 3 is obtained from k' of eq. 7 by adding the (relatively small) corrections for the reactions giving P–O cleavage and for base catalysis at high base concentrations. The final equations used for calculation are

$$\frac{10^5(k_{obsd} - k_{hyd})}{[R_2NH]} = 4.6 + \frac{10^5[OH^-]}{67 + 2280[OH^-]} + 18.3[OH^-] \quad (2)$$

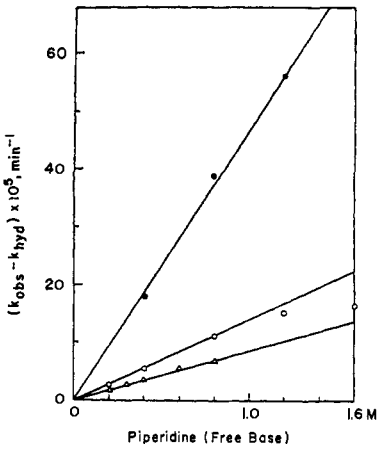

Figure 2. Total rates of reaction of *p*-nitrophenyl phosphate with piperidine as a function of piperidine concentration at different hydroxide ion concentrations at 39°, ionic strength 1.0: △ at pH 11.3, ○ at pH 11.7, and ● in 0.2 *N* NaOH. The curves are calculated from eq. 2.

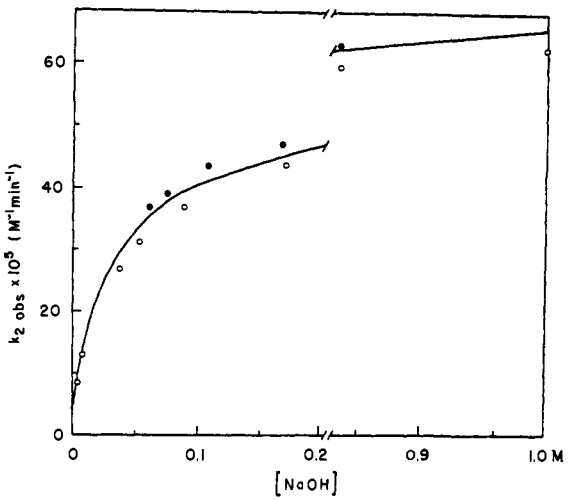

Figure 3. Second-order plot of the rates of reaction of *p*-nitrophenyl phosphate with piperidine (at constant concentrations, 1.0 *M* (●) and 0.2 *M* (○)) as a function of hydroxide ion concentration at 39°, ionic strength 1.0. The curves are calculated from eq. 2 and 7.

for Figure 3, and

$$\frac{10^5 k^*}{[R_2NH]} = \frac{10^5(k_{obsd} - k_{hyd})}{[R_2NH]} - 4 - 30.4[OH^-] = \frac{62(5100[OH^-] + 143[R_2NH])}{62 + 5100[OH^-] + 143[R_2NH]} \quad (3)$$

for Figure 4.

Reactions of *p*-nitrochlorobenzene were followed spectrophotometrically in the same way as those of the phosphate, except that this compound was added to the aqueous reaction mixture as a solution (4–5 mg./ml.) in dioxane to give a solvent that contained 16.7% dioxane by volume. The reaction with hydroxide ion was followed at 400 mμ and that with dimethylamine, to produce N,N-dimethyl-4-nitroaniline, was followed at 424 mμ (ε 19,100).

Hydroxide ion concentrations in solutions containing hydroxide ion and amine were calculated from the amount of hydroxide ion added, with a correction for the hydroxide ion produced by hydrolysis of the amine; this correction was significant only at hydroxide ion concentrations below 0.3 *M*. Observed pK_a' values of 11.01 and 11.43 for dimethylamine and piperidine, respectively, at 25° and ionic strength 1.0 were corrected to 10.61 and 11.00, respectively, at 39° using values[17,18] of 11,859 and 12,770 cal./mole for the respective heats of ionization and an interpolated value[19] of $pK_w = 13.57$ at 39°.

Determinations of the pH of reaction mixtures containing buffered solutions of amine were carried out at room temperature (25 ± 1°) with a Radiometer pHM4B pH meter equipped with a G200C glass electrode. Hydroxide ion concentrations were calculated from the observed pH and were corrected to 39°, using the heats of ionization given above. A value of 0.67 at ionic strength 1.0 was used for the activity coefficient of hydroxide ion.[20]

Results

Piperidine. The rate of the over-all reaction of *p*-nitrophenyl phosphate with piperidine is linear with respect to piperidine concentration up to 1 *M* free base, but the second-order rate constant so obtained increases with increasing pH (Figure 2). This indicates catalysis of the reaction by hydroxide ion. Plots of the second-order rate constants against hydroxide ion concentration show a marked catalysis at low hydroxide ion concentrations but a leveling off to a shallow slope above 0.2 *M* hydroxide ion (Figure 3). Spectrophotometric analysis of the products shows that at low hydroxide ion concentration the reaction proceeds primarily by P–O cleavage to give *p*-nitrophenol, regardless of piperidine concentration, but attack on the aromatic ring to give 1-*p*-nitrophenylpiperidine is the predominant reaction in alkaline solution (Table I).

Table I. *p*-Nitroaniline Formation from Piperidine and *p*-Nitrophenyl Phosphate

Piperidine, *M*	pH	Found, %	Calcd.,[a] %
0.2	11.1	35	31
1.4	11.1	35	31
0.4	0.4 *M* NaOH	92	89

[a] Calculated from eq. 7.

Above a concentration of 1 *M*, plots of rate against piperidine concentration show nonlinearity (Figure 2), such that the over-all second-order rate constants decrease with increasing piperidine concentration (Table II). This appears to be a solvent effect which results from the large amount of piperidine in the more concentrated solutions. Similar rate decreases are observed upon the addition of comparable con-

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(18) A. G. Evans and S. D. Hamann, *Trans. Faraday Soc.*, 47, 34 (1951).
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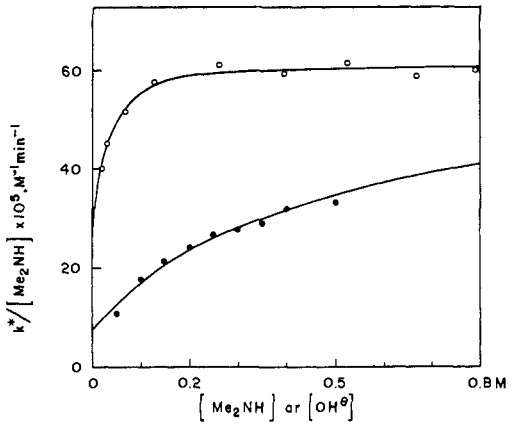


Figure 4. Second-order rate constants for the nucleophilic aromatic substitution reaction of *p*-nitrophenyl phosphate and dimethylamine at 39°, ionic strength 1.0, plotted as a function of hydroxide ion concentration in 0.2 *M* dimethylamine (○) and of dimethylamine concentration at pH 11.0 (●). The observed rates are corrected for P-O cleavage reactions and the slow hydroxide ion catalyzed reaction; *i.e.*, $k^* = k_{obsd} - k_{hyd} - 4[Me_2NH] - 30.4[Me_2NH][OH^-]$. The curves are calculated from eq. 3 and 7

centrations of dioxane or pyridine, which do not themselves react appreciably under the same experimental conditions (Table II). A similar decrease in the second-order rate constant for the reaction of piperidine with *p*-chloronitrobenzene in ethanol has been observed as the piperidine concentration is increased to 5–10 *M*.[21] The much more marked leveling off of the rate of the reaction of *p*-nitrophenyl phosphate and piperidine with increasing hydroxide ion concentration is clearly not a salt or solvent effect. All experiments were carried out at a constant ionic strength of 1.0; the addition of 0.8 *M* potassium chloride to a reaction mixture which contained 1.2 *M* piperidine and 0.2 *M* hydroxide ion was found to cause only a small increase in rate, from 5.2 to 5.7 × 10^{-4} min.$^{-1}$.

Table II. Second-Order Rate Constants for the Reaction of 1.2 *M* Piperidine with *p*-Nitrophenyl Phosphate[a]

Piperidine		1.2 *M* piperidine + solvent		
	k, M^{-1} min.$^{-1}$	Added solvent,	Dioxane k, M^{-1} min.$^{-1}$	Pyridine[b] k, M^{-1} min.$^{-1}$
M	× 10^4	*M*	× 10^4	× 10^4
0.8	4.8			
1.2	4.7	...	4.7	4.7
1.6	3.7	0.5	4.0	3.2
2.0	3.0	1.0	3.4	2.6

[a] In the presence of organic solvents in 0.2 *M* NaOH at 39°, ionic strength 1.0. [b] Pyridine itself reacts with *p*-nitrophenyl phosphate at some 2% of the observed rate under these experimental conditions.

Dimethylamine. N,N-Dimethyl-*p*-nitroaniline is the predominant product of the reaction of *p*-nitrophenyl phosphate with dimethylamine even at low hydroxide ion concentrations (Figure 1). This reaction is similar to the piperidine reaction in that it is catalyzed by hydroxide ion but, in contrast to the piperidine reaction, the rate of the dimethylamine reaction increases more rapidly than the first power of the amine concentration (Figure 1). This is evidence for a term in the rate law for this reaction which is second-order in respect to amine and, therefore, for general base catalysis of the reaction by a second mole of amine. The relative amounts of P-O and C-O splitting were determined spectrophotometrically in the same way as for the piperidine reaction, and the rate constants for attack at the aromatic ring, corrected for the shallow increase in rate at high hydroxide ion concentration (see below), are plotted in Figure 4. Plots of rate against hydroxide ion concentration at constant amine concentration (upper curve) show a steep initial rise, followed by a leveling off similar to that observed with piperidine. The second-order rate constants increase with increasing dimethylamine concentration (lower curve), reflecting general base catalysis, but these plots also show a leveling off with increasing amine concentration and, as will be shown below, approach the same limiting rate as the hydroxide ion catalyzed reaction at high catalyst concentrations.

Reactions with Other Amines. Of a number of amines examined,[2] only morpholine, another secondary amine, shows a significant base-catalyzed reaction with *p*-nitrophenyl phosphate (Table III). Since the extinction coefficient of the *p*-nitroaniline produced in this reaction is not known, the rate constants are based upon the extinction coefficient of the *p*-nitrophenolate ion and are, therefore, only approximate; however, the increase in rate in alkaline solution as well as a qualitative demonstration that a substituted aniline is formed under alkaline conditions indicate that a significant base-catalyzed aromatic substitution reaction occurs with this amine.

Table III. Second-Order Rate Constants for the Reaction of Morpholine (1.2 *M*) with *p*-Nitrophenyl Phosphate[a]

Hydroxide concn., *M*	k_2, M^{-1} min.$^{-1}$ × 10^5
10^{-4}	2.0
0.2	5.5
0.6	7.0

[a] In the presence and absence of hydroxide ion at 39° and ionic strength 1.0.

Several primary amines give an appreciable amount of aromatic substitution.[2] The reaction with methylamine, however, does not show significant catalysis by the amine itself nor by added hydroxide ion (Table IV). The mean value of the over-all second-order rate constant, for the reaction of methylamine at both

Table IV. Reaction of Methylamine with *p*-Nitrophenyl Phosphate at 39°, Ionic Strength 1.0

Conditions	k_2, M^{-1} min.$^{-1}$ × 10^5	% nitroaniline produced
0.27 *M* $MeNH_2$, pH 11.2	7.2	31
0.4 *M* $MeNH_2$, pH 11.2	7.2	31
0.67 *M* $MeNH_2$, pH 11.2	7.6	30
0.4 *M* $MeNH_2$, in 0.4 *M* NaOH	7.7	34

(21) N. E. Sbarbati, T. H. Suárez, and J. A. Brieux, *Chem. Ind.* (London), 1754 (1964).

phosphorus and carbon, is 7.4×10^{-5} M^{-1} min.$^{-1}$, and that for the aniline-forming reaction is 2.3×10^{-5} M^{-1} min.$^{-1}$.

p-Chloronitrobenzene. The reactions of *p*-chloronitrobenzene with hydroxide ion and dimethylamine were followed in 16.7% dioxane–water under conditions otherwise identical with those for the phosphate. The observed first-order rate constants are directly proportional to the concentrations of hydroxide ion and of methylamine, respectively, and there is no catalysis of the amine reaction by hydroxide ion (Table V).

Table V. Reaction of *p*-Chloronitrobenzene with Dimethylamine and Hydroxide Ion[a]

Dimethylamine		Hydroxide ion	
M	$k_1 \times 10^5$, min.$^{-1}$	M	$k_1 \times 10^7$, min.$^{-1}$
0.1, pH 11.0	2.67	0.33	4.16
0.3, pH 11.0	8.6	0.67	8.7
0.5, pH 11.0	14.0	1.0	13.6
0.33 (free base)	9.3		
0.33 + 0.33 M OH^-	9.3		
0.33 + 0.67 M OH^-	9.1		
Mean $k_2 = 2.8 \times 10^{-4}$ M^{-1} min.$^{-1}$		Mean $k_2 = 1.34 \times 10^{-6}$ M^{-1} min.$^{-1}$	

[a] In 16.7% dioxane–water at 39° and ionic strength 1.0.

Discussion

Reactions with Piperidine and Dimethylamine. Catalysis by hydroxide ion of the reaction of *p*-nitrophenyl phosphate with piperidine in aqueous solution is an example of the base catalysis which has been observed for a number of other aromatic substitution reactions[5,7,8] and shows that proton transfer to or from one of the reactants occurs in the rate-determining step of the reaction. The fact that the rate of the hydroxide-catalyzed reaction levels off to a shallow slope as the hydroxide ion concentration is increased requires either (a) that nearly all of one of the starting materials be converted to a complex with hydroxide ion or (b) that the reaction undergoes a change in rate-determining step with increasing hydroxide ion concentration. The leveling off of the rate is not the result of complexing of piperidine by hydroxide ion because the leveling off occurs at an hydroxide ion concentration lower than the piperidine concentration, and it cannot be due to removal of hydroxide ion by complexing with piperidine because the leveling off is also observed at concentrations of hydroxide ion which are larger than the concentration of piperidine and the leveling off occurs at the same hydroxide ion concentration at two different piperidine concentrations (Figure 3). Furthermore, hydroxide ion has no effect on the reaction of dimethylamine with *p*-nitrochlorobenzene (Table V), although a leveling off with increasing hydroxide ion concentration occurs in the corresponding reaction with *p*-nitrophenyl phosphate (Figure 4). The leveling off is not due to complexing of *p*-nitrophenyl phosphate because other reactions of *p*-nitrophenyl phosphate show no such leveling off in the presence of hydroxide ion (*e.g.*, Table IV). The leveling off is, therefore, evidence for a change from a rate-determining step at low basicity, which is strongly catalyzed by hydroxide ion, to a rate-determining step at high hydroxide ion concentrations, which shows little or no such catalysis. A change in rate-determining step demands the existence of an intermediate in the reaction. We reject a charge-transfer complex as the intermediate on the grounds that its rate of formation would not be expected to be as slow as the observed reaction at either high or low basicity, and conclude that the intermediate is a tetrahedral addition compound.

Either of the mechanisms shown in eq. 4 and 5 is consistent with the results. In mechanism I, the base-

Mechanism I

$$R_2NH + {}^{2-}O_3POArNO_2 \underset{k_{-1}[OH^-],\ k_2[B]}{\overset{k_1[OH^-],\ k_2[B]}{\rightleftharpoons}} IH^{2-} \xrightarrow{k_3} \text{products} \quad (4)$$

Mechanism II

$$R_2NH + {}^{2-}O_3POArNO_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} IH^{2-} \xrightarrow{k_2[OH^-],\ k_3[B]} \text{products} \quad (5)$$

catalyzed formation of the intermediate, IH^{2-}, is rate determining at low basicity, and the breakdown of the intermediate is rate determining at high basicity. In mechanism II the base-catalyzed breakdown of the intermediate is rate determining at low basicity, and the uncatalyzed formation of the intermediate is rate determining at high basicity. The steady-state treatment gives the rate eq. 6 and 7 for mechanisms I and II, respectively.

$$\frac{\text{rate}}{[R_2NH][Ar]} = k' = \frac{k_3(k_1[OH^-] + k_2[B])}{k_3 + k_{-1}[OH] + k_{-2}[B]} \quad (6)$$

$$\frac{\text{rate}}{[R_2NH][Ar]} = k' = \frac{k_1(k_2[OH^-] + k_3[B])}{k_{-1} + k_2[OH^-] + k_3[B]} \quad (7)$$

where Ar = *p*-nitrophenyl phosphate and k' is the observed second-order rate constant for nucleophilic aromatic substitution. Equations 6 and 7 are mathematically of the same form and mechanisms I and II are, therefore, kinetically indistinguishable in the absence of further information. For the piperidine reaction, the terms containing B, corresponding to general base catalysis by piperidine, are negligible, presumably because of the large size of the piperidine molecule. It is assumed that the hydroxide term in the piperidine reaction represents general base catalysis by analogy with the dimethylamine reaction and other nucleophilic aromatic substitution reactions,[5,7,8] but the argument would be the same if this term represented specific base catalysis. There presumably exists a water reaction in which water is the base, B, but no evidence for a significant contribution of such a reaction was obtained in our experiments, which were carried out in alkaline solution.

At high hydroxide concentrations the rate does not level off completely, but shows a continued increase with a shallow slope which is much smaller than the initial slope, but is greater than the experimental error of the measurements. This continued small increase could represent a specific salt effect or could represent a small amount of catalysis by hydroxide ion of the step (k_3 in eq. 4, k_1 in eq. 5) which is relatively insensitive to base catalysis. If the latter suggestion is correct, the argument would be the same as given above, but the steady-state rate equations would contain an extra

term for such catalysis. For mechanism II, for example, the steady-state equation would be

$$\frac{\text{rate}}{[R_2NH][Ar]} = k' = \frac{(k_1 + k_4[OH^-])(k_2[OH^-] + k_3[B])}{k_{-1} + k_{-4}[OH^-] + k_2[OH^-] + k_3[B]} \quad (7a)$$

where k_4 and k_{-4} are the added rate constants for hydroxide ion catalysis of the first step and $k_{-4} << k_2$. At high hydroxide ion concentrations this reduces to $k' = k_1 + k_4[OH^-]$, which would account for the small continued increase in the observed rate at high base concentrations. In the absence of more definite information about the nature of this effect, we have chosen to treat the data with eq. 6 and 7 and to make a correction to the calculated rates for this small rate increase which, whatever its nature, is proportional to the concentration of hydroxide ion.

For the reasons discussed below, the rate constants for the reactions with piperidine and dimethylamine were evaluated for mechanism II and eq. 7, as described in the Experimental section, and are summarized in Table VI. The calculated rates, based on

Table VI. Rate Constants for the Formation of Substituted *p*-Nitroanilines[a] from the Reactions of *p*-Nitrophenyl Phosphate with Piperidine and Dimethylamine (according to eq. 7, at 39°, ionic strength 1.0)

	k_1, M^{-1} min.$^{-1}$ $\times 10^5$	k_2/k_{-1}, M^{-1}	k_3/k_{-1}, M^{-1}
Dimethylamine	62	82	2.3
Piperidine	44	34	...

these rate constants, are shown as the solid lines in Figures 3 and 4. In Figure 3 the rates of the P–O splitting reaction and the small rate increase proportional to hydroxide ion concentration have been added to the rate constants calculated from eq. 7, while in Figure 4 these corrections have been subtracted from the observed rates in order to facilitate comparison of hydroxide and amine catalysis. The calculated rates show satisfactory agreement with the experimental results. At low concentrations of base, eq. 7 reduces to $k' = (k_1/k_{-1})(k_2[OH^-] + k_3[B])$, which corresponds to a pre-equilibrium formation and base-catalyzed breakdown of the addition intermediate and accounts for the observed base catalysis under these conditions. At high base concentrations, eq. 7 reduces to $k' = k_1$, the formation of the intermediate is rate determining, and little or no base catalysis is observed. Although, because of the greater catalytic effectiveness of hydroxide ion than of dimethylamine, it is not immediately apparent from the figure, the same maximal rates are approached with both hydroxide and amine catalysis; *i.e.*, k_1 is the same for both types of catalysis. Entirely equivalent results are obtained if the calculations are based on mechanism I and eq. 6, but the rate constants of Table VI would be assigned differently in this case.

Kinetic data obtained with a single compound do not permit a choice between mechanisms I and II, but data obtained with another compound may permit such a choice. To this end, the reaction of dimethylamine with *p*-chloronitrobenzene was examined and was found to proceed with a second-order rate constant of 28×10^{-5} M^{-1} min.$^{-1}$ and to show no evidence for base catalysis by hydroxide ion or dimethylamine (Table V). Granted that the reaction proceeds through an addition intermediate (eq. 8), it would be expected that

$$R_2NH + ClArNO_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \begin{matrix} R_2HN^+ \\ \diagdown \\ ArNO_2 \\ \diagup \\ Cl \end{matrix}^- \xrightarrow{k_2} R_2NArNO_2 + Cl^- + H^+ \quad (8)$$

the attack of dimethylamine on the aromatic ring, rather than the leaving of chloride ion, would be rate determining for this reaction in aqueous solution, because chloride ($pK_a < 0$) is a much better leaving group than dimethylamine ($pK = 10.6$), so that $k_2 >> k_{-1}$, and nearly every molecule of intermediate that is formed would go on to products. This assumption is supported by the finding of Bunnett, *et al.*, that in the reaction of piperidine with a series of 1-substituted 2,4-dinitrobenzenes, including 2,4-dinitrochlorobenzene, the rate differs by less than fivefold, whereas a large difference in rate would be expected if expulsion of the anion made a significant contribution to the rate-determining step.[6]

The results suggest, therefore, that in the reaction of dimethylamine with *p*-nitrophenyl phosphate mechanism II (eq. 5) is correct, and that the formation of the addition intermediate, k_1, is the rate-determining step at high concentrations of base because (a) this is the step that does not show significant base catalysis in the reaction of dimethylamine with either chlorobenzene or *p*-nitrophenyl phosphate, and (b) the rate constant for the reaction with chlorobenzene (28×10^{-5} M^{-1} min.$^{-1}$ in 16.7% dioxane) is very similar to the limiting rate constant, k_1, for the reaction with *p*-nitrophenyl phosphate (62×10^{-5} M^{-1} min.$^{-1}$ in water), as would be expected if both of these rate constants referred to rate-determining attack on the aromatic ring. The alternative assignment (mechanism I) would require that attack on the aromatic ring be base catalyzed for the *p*-nitrophenyl phosphate reaction but not for the *p*-chloronitrobenzene reaction, and that the similarity of rate constants be coincidental. Mechanism II is essentially the same as that assigned by Bunnett and Randall to the general base catalyzed reaction of N-methylaniline with 2,4-dinitrofluorobenzene.[7] The conclusion receives some further support from the fact that the rate constant for the reaction of hydroxide ion with *p*-chloronitrobenzene ($k = 1.34 \times 10^{-6}$ M^{-1} min.$^{-1}$) is similar to that for the reaction with *p*-nitrophenyl phosphate[2] ($k = 4.9 \times 10^{-7}$ M^{-1} min.$^{-1}$). It is assumed that the latter reaction involves attack at the aromatic ring in view of the well-known small sensitivity of phosphate ester dianions to attack by hydroxide ion; the small rate difference is presumably an electrostatic effect. Attack on the aromatic ring would be expected to be rate determining for both of these reactions.

These conclusions are summarized diagrammatically in the transition-state diagram of Figure 5 for the reactions of dimethylamine with *p*-nitrophenyl phosphate

and *p*-chloronitrobenzene. This diagram is based on the free energies of activation obtained from the rate constants for the two steps of the reaction at different concentrations of catalyst; *i.e.*, the barrier for the first step is based on k_1 and that for the second on $(k_2k_1/k_{-1})[OH^-] = k_2K_{eq}[OH^-]$, where K_{eq} is the equilibrium constant for formation of the addition intermediate. This procedure makes it possible to show how the free energy barrier for each step varies with catalyst concentration and with the structure of the reactants. At low base concentration the second step of the reaction has the largest free energy barrier and is rate determining; as the base concentration is increased this energy barrier drops and the barrier to the first step becomes dominant. For the chloro compound, the energy barrier to the first step is similar to that for the phosphate, but that for the second step is smaller so that base catalysis would not be observed, even if the second step of this reaction is subject to catalysis.

It should not be concluded that base catalysis never occurs in the first step of the attack of strongly basic amines on aromatic systems. Catalysis of the over-all reaction has been observed in solvents which are less effective proton-transferring reagents than water under conditions in which the first step of the attack of amines on aromatic nitrohalides is probably rate determining,[5,8] and the small rate increase in concentrated base with *p*-nitrophenyl phosphate, discussed above, may represent such catalysis.

The findings (a) that the reactions of piperidine with dinitrophenyl halides and the *p*-nitrophenyl ether of dinitrophenol (pK_a of *p*-nitrophenol = 7.0) proceed at similar rates[6] and (b) that for *p*-chloronitrobenzene $k_2 >> k_{-1}$, while for *p*-nitrophenyl phosphate (pK_a of HPO_4^{2-} = 12.3) $k_{-1} \sim k_2$, demonstrate that k_{-1} and k_2 undergo the expected change in relative magnitude with changing leaving group and suggest the prediction, if the assignment of mechanism II is correct, that a *p*-nitrophenyl compound with a less acidic oxygen atom in the leaving group than phosphate would react with piperidine more slowly than *p*-nitrophenyl phosphate and would show base catalysis over the whole range of reaction. In terms of the transition-state diagram of Figure 5 this would correspond to an increase in the free energy barrier of the base-catalyzed second step, which would, therefore, become rate determining over a wide range of base concentration.

Possible detailed mechanisms for eq. 5 (mechanism II) are shown in eq. 9–12. Similar mechanisms have been proposed by Bunnett and co-workers for the reaction of N-methylaniline with 2,4-dinitrofluorobenzene.[7,22] A rate-determining simple proton transfer from the intermediate to the catalyzing base (eq. 10) does not appear probable because the rates of such proton transfer reactions are generally diffusion controlled.[23] It is particularly difficult to account for the much greater catalytic efficiency of hydroxide ion than of dimethylamine, if this is the rate-determining step, because diffusion-controlled proton transfer from the intermediate to such strong bases should show little dependence on the strength of the base. A mechanism which involves concerted proton removal

(22) J. F. Bunnett and G. T. Davis, *J. Am. Chem. Soc.*, 82, 665 (1960), footnote 27.
(23) M. Eigen, *Pure Appl. Chem.*, 6, 97 (1963).

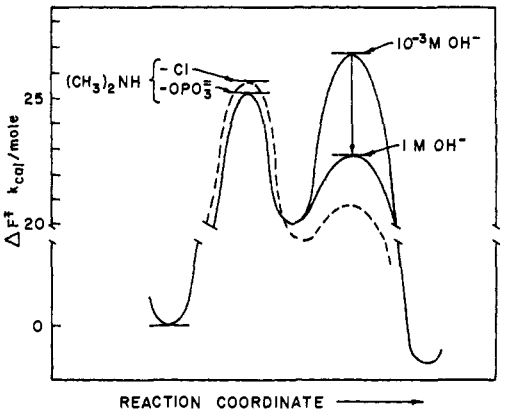
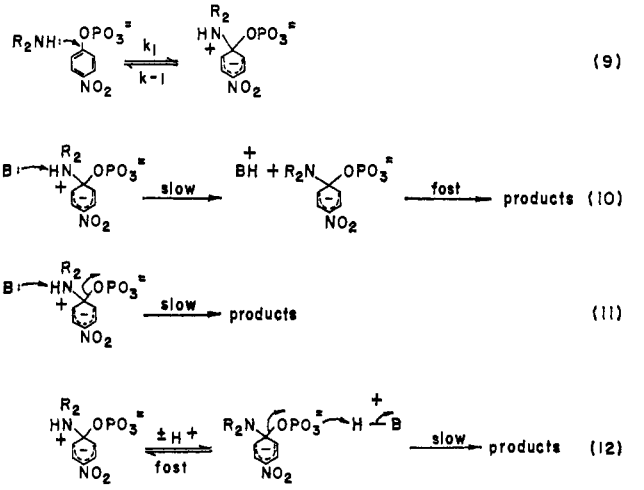


Figure 5. Transition state diagram based on the observed second-order rate constants for the reactions of dimethylamine with monosubstituted nitrobenzenes. Solid line: the reaction of dimethylamine with *p*-nitrophenyl phosphate in the presence of 10^{-3} and 1.0 *M* hydroxide ion. Dashed line: the reaction of dimethylamine with *p*-chloronitrobenzene. The free energies of activation which are based on experiment are shown by horizontal lines; other values are arbitrary.



and phosphate elimination (eq. 11) avoids these difficulties. However, it is probable that the labile proton of the intermediate of eq. 11 is lost rapidly in a diffusion-controlled reaction in the alkaline solutions in which these experiments were carried out, so that the concentration of this protonated intermediate would be small and catalysis of its decomposition would not be expected to cause an appreciable rate increase, compared to the rate of decomposition of the basic form of the intermediate, in which this proton is completely removed. For these reasons, the most probable mechanism appears to be that of eq. 12, in which the conjugate acid of the general base catalyst donates a proton to the conjugate base of the intermediate, to make the phosphate a better leaving group. The transition state for this reaction has the same stoichiometric composition as those for the mechanisms of eq. 10 and 11; these three mechanisms are, therefore, kinetically indistinguishable.

Reactions with Primary Amines. The reaction of *p*-nitrophenyl phosphate with primary amines, in contrast to secondary amines, is not subject to general base catalysis. The over-all rate constant for the reaction of methylamine with *p*-nitrophenyl phosphate is

7.4×10^{-5} M^{-1} min.$^{-1}$, while that for reaction at the aromatic ring is 2.3×10^{-5} M^{-1} min.$^{-1}$ (Table IV). Thus, the rate of reaction of methylamine at the aromatic ring is 27 times slower than the rate constant of 62 M^{-1} min.$^{-1}$, which was assigned to the attack of dimethylamine on the aromatic ring (Table VI). The absence of base catalysis of the methylamine reaction would reasonably be accounted for if the relatively slow rate of this reaction reflects a large free energy barrier for attack at the aromatic ring. It was concluded above that the attack of amine on the ring is not subject to appreciable base catalysis, and if this step is rate determining in the reaction with primary amines, base catalysis would not be significant for these reactions. The mechanism and rate constants for the second step of the methylamine reaction would be expected to be similar to those for the secondary amine reactions. (It is probable, by analogy with other base-catalyzed reactions, that water may act as a proton transfer catalyst for the second step. A mechanism which involves proton transfer from the amine to the leaving phosphate, either directly or through a water molecule, is possible and would be especially attractive for reactions of primary amines. The result of reaction paths of this kind would be to lower the energy barrier for the second step of the reaction at low base concentrations.) This analysis is consistent with the previous observation of Brady and Cropper that dimethylamine reacts 11 times faster than methylamine with 2,4-dinitrochlorobenzene in alcohol, in spite of the steric effect of the *o*-nitro group in this compound and the generally higher nucleophilic reactivity of secondary than of primary amines, if steric effects are not dominant.[24]

Thus, the disappearance of detectable general base catalysis in the reactions of amines with monosubstituted nitrobenzenes could occur through either an increase in the energy barrier of the uncatalyzed first step of the reaction or through a decrease in the energy barrier of the second step, as in the reaction of dimethylamine with *p*-chloronitrobenzene or with *p*-nitrophenyl phosphate at high concentrations of base catalyst. Analogous changes in sensitivity to base catalysis are seen in reactions of acyl compounds as the leaving group is varied.[25]

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The Migration and Elimination of Hydrogen during Biosynthesis of Cholesterol from Squalene[1]

J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popják, Y. Shimizu,[2a] S. Ichii,[2b] E. Forchielli, and E. Caspi[2c]

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Lanosterol and cholesterol biosynthesized from 4R-4-H^3-mevalonic acid retain, respectively, five and three of the six labeled atoms present in the intermediate squalene. The cholesterol was degraded and, of the three H^3 atoms retained, one is at the 17α-position. The remaining two tritium atoms are most probably located at C-20 and C-24. This pattern of loss, retention, and distribution of H^3 is in complete harmony with the theoretical mechanism of squalene cyclization. The absence of tritium in the steroidal nucleus confirms the intermediate oxidation of the C-3 hydroxyl during biosynthesis and shows that when the double bond of cholesterol is formed the 5α-hydrogen atom is eliminated, not rearranged.

Eschenmoser, *et al.*,[3] reasoning from what was then known of the cyclization of squalene (II) to lanosterol (IV) and assuming that the stereochemistry of this enzymic process would be that observed for analogous chemical reactions, put forward a detailed mechanism for this cyclization. Briefly, the attack of an electron-deficient species, equivalent to an hydroxyl cation, initiates the cyclization of squalene to a cationic intermediate (III). Four rearrangements then follow: hydrogen from C-17 to C-20 and from C-13 to C-17, methyl from C-14 to C-13 and from C-8 to C-14. Finally a proton is lost from C-9 to give lanosterol. This mechanism has been strongly supported by the demonstration[4,5] that the rearrangements of methyl groups do occur as postulated. There has been no experimental support until now for the hydrogen migrations except the fact that enzymic cyclizations of squalene run in D_2O gave lanosterol free from stably bound deuterium.[6]

(1) (a) This work was initiated while one of us (E. C.) was a visiting scientist at the Shell Research, Ltd., Milstead Laboratory in Sittingbourne, Kent, England, during July–Aug. 1963, and was continued at the Worcester Foundation, Shrewsbury, Mass. (b) The work at the Worcester Foundation was supported by Grants CA-07137, A-5326, and CA-04663 from the U. S. Public Health Service.
(2) (a) Postdoctoral Fellow 1963–1964 on leave of absence from Hokkaido University, Sapporo, Japan; (b) Postdoctoral Fellow 1960–1964; (c) recipient of Research Career Program Award CA-K3-16614 from the National Cancer Institute.
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