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KINETIC STUDY ON THE NUCLEOPHILIC SUBSTITUTION OF PURINES

A THESIS

Presented to

The Faculty of the Division of Graduate

Studies and Research

by

Alvaro Abidaud

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

in the School of Chemistry

Georgia Institute of Technology

December, 1971

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KINETIC STUDY ON THE NUCLEOPHILIC

SUBSTITUTION OF PURINES

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SUMMARY

The research described herein is an attempt to elucidate the mechanism of the nucleophilic aromatic substitution by piperidine, in particular, on 6-chloro-9-methoxymethylpurine. The work was divided in two broad areas: (1) solvent effects on the reaction rate constant and (2) the direct observation of an addition (Meisenheimer) complex by means of n.m.r. and u.v. spectroscopy.

The reaction of piperidine with 6-chloro-9-methoxymethylpurine in non-polar, aprotic solvents, such as cyclohexane and benzene, was found to be catalyzed by piperidine. A plot of the second order rate coefficient (k_{obs}), obtained from pseudo-first order kinetic data, against the concentration of piperidine was found to increase linearly with increasing piperidine concentration; however, at higher concentrations of the amine the increase was not linear and a smooth curve was obtained. This phenomenon is characteristic of the multi-step, addition-elimination mechanism originally proposed by Bunnett and co-workers for the reaction of amines with 2,4-dinitrohalobenzenes in protic media.

An isotopic study involving piperidine-N-h and -N-d was undertaken. It was demonstrated that piperidine-N-d reacted slightly faster than piperidine-N-h with the purine, in benzene and cyclohexane, at all temperatures studied. The K_H/K_D was about 0.90 ± 0.03.

In the solvent 1,4-dioxane, at 23.0°C, there is a slight catalysis of piperidine, but at 32.0°C and 44.5°C the catalytic effect of the amine is no longer observed.

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Piperidine-N-d reacted slightly faster than piperidine-N-h at 23.0°C, (K_H/K_D = 0.89 ± 0.03). At 32.0° and 44.5°C there is no observable kinetic isotope effect as indicated by the value of K_H/K_D = 1.01 ± 0.03.

At 23.0°C, in 1,4-dioxane, K_{1H}/K_{1D} (the ratio of the rate constant for the initial attack of piperidine on the purine) is equal to 0.92 ± 0.03. This difference is attributed to the slightly larger nucleophilicity of piperidine-N-d compared to piperidine-N-h. This is the first case in which this phenomenon is observed.

In polar solvents, such as water, 60 percent 1,4-dioxane - 40 percent water (v/v), dimethyl sulfoxide and methanol), there is no catalysis by piperidine, i.e., k_{obs} is independent of the concentration of the amine. These solvents help decompose the intermediate complex by assisting in the removal of HCl from it. Also, the high dielectric of these media accelerate the rate of reaction.

Finally, the direct observation of an addition (Meisenheimer complex) using 6-(2-hydroxyethoxy)-9-methoxymethylpurine and 6-methoxy-9-methoxymethylpurine was accomplished by using n.m.r. and u.v. spectroscopy. The addition complex is generally taken as the prototype of the activated complex in nucleophilic aromatic substitution.

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Table 1. Summary of the Second Order Rate Coefficients (k_{obs}) Obtained from the Reaction of Piperidine (0.4 M) with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in Different Solvents.

Solvent	k_{obs} ($sec^{-1}M^{-1}$)	t, °C
Cyclohexane	0.0117	23.0
Benzene	0.0049	23.0
1,4-Dioxane	0.0089	23.0
Water	0.1901	25.0
60 Percent 1,4-Dioxane-40 Percent Water (v/v)	0.0580	25.0
Dimethyl Sulfoxide	0.0597	25.0
Methanol	0.0186	25.0

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CHAPTER I

INTRODUCTION

Although nucleophilic substitutions at aromatic carbon atoms had been encountered as early at 1854,[1] and although many such reactions had found wide use in synthesis, questions of mechanism and reactivity received only scattered attention before the 1950's. During this decade, active research in this area was commenced independently in several laboratories. In 1951, a comprehensive review by Bunnett and the late Roland Zahler[2] and a shorter review by Miller[3] were published. At that time relatively few definite conclusions about mechanisms could be reached, but today a rather satisfactory picture can be drawn.

The great majority of aromatic nucleophilic substitutions occur by a bimolecular mechanism. In this type of reaction second-order kinetics, first-order in both substrate and reagent, are regularly observed, as are greater rates with stronger nucleophilic reagents and with substrates carrying substituents of greater electron-attracting character.

After the establishment of the $S_{N}2$ mechanism of substitution at saturated carbon atoms by Huges, Ingold and their co-workers,[4,5] it was often assumed that bimolecular aromatic nucleophilic substitution occurs by an analogous one-step mechanism of synchronous bond-formation and bond-breaking. An alternative two-step, intermediate complex mechanism was occasionally mentioned but was first forcefully advocated in 1951 by Bunnett and Zahler,[2] who showed that one-step, $S_{N}2$-like substitutions at an aromatic carbon atom was quantum mechanically improbable, while

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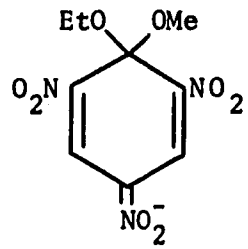
the intermediate complex mechanism was not only acceptable in this regard but was also supported by significant analogies with other phenomena. The latter included electrophilic aromatic substitutions, for which the intermediate complex mechanism was being recognized on experimental grounds,[6] and the formation of isolable addition complexes by the interaction of nucleophilic reagents with highly activated aromatic substrates. Foremost of these was complex I, formed by addition of methoxide



(I)

ion to 2-4-6-trinitrophenetole or of ethoxide ion to 2-4-6-trinitroanisole. Evidence for the structure of (I) was adduced at the turn of the century[7,8] and has received further support recently.[9-13]

It was argued that if highly activated substrates formed isolable addition complexes as intermediates in substitution reactions, and indeed (I) can be considered to be such an intermediate, then less highly activated substrates ought also to form actual intermediates of type (II) although of stability insufficient to allow isolation. Bimolecular aromatic nucleophilic substitution was therefore represented as in equation (1). It was stressed that either the first or the second step of the mechanism

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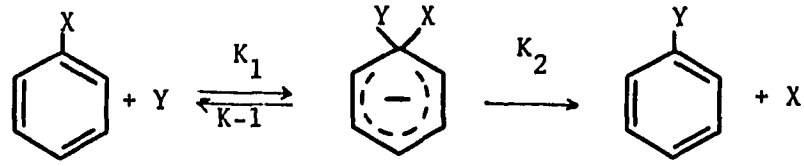
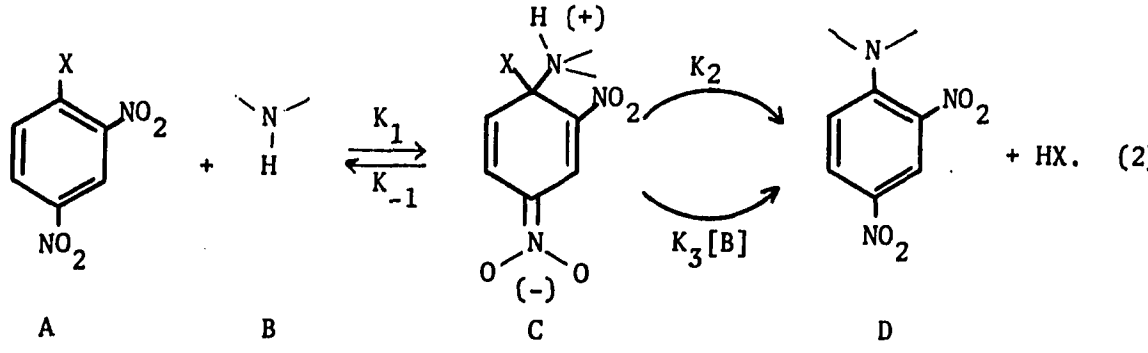


(1)

(II)

might be rate-determining, depending on the relative magnitudes of K_2 and K_{-1}. Thus if $K_2 >> K_{-1}$, the rate of the first step would in effect be the rate of the overall reaction while if $K_{-1} >> K_2$, the rate would depend on the equilibrium concentration of the intermediate complex (II) and on K_2.

Studies by Bunnett and co-workers support the theory that nucleophilic aromatic substitution on certain systems may take place in a multi-step, intermediate-complex mechanism such as the following:



A B C D

(2)

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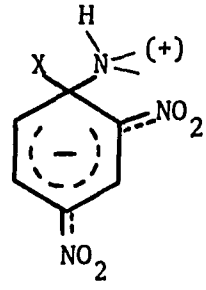
Initial attach by the nucleophile (B) on the substrate (A) produces the intermediate-complex (C), which can be represented as a Meisenheimer complex[14] such as (III):



(III)

The intermediate can decompose either through an uncatalyzed process involving spontaneous loss of a proton and ejections of the leaving group, X, or by a catalyzed route incorporating the nucleophile or another additive (B). The latter pathway has been found to be affected by various acids, bases, salts and bifunctional species, the effect being dependent on the nature of the nucleophile, the substrate and the solvent. A mathematical treatment of this theory can be developed as follows:

$$\frac{-d[A]}{dt} = \frac{d[D]}{dt} = K_1[A][B]-K_{-1}[C] \qquad (3)$$

Using the steady-state approximation on the intermediate-complex (C),

$$\frac{d[C]}{dt} = 0 = K_1[A][B]-K_{-1}[C]-K_2[C]-K_3[B][C] \qquad (4)$$

solving for C,

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$$[C] = \frac{K_1[A][B]}{K_{-1} + K_2 + K_3[B]} \tag{5}$$

Replacing [C] in equation (3),

$$\frac{d[D]}{dt} = K_1[A][B] - \frac{K_{-1}K_1[A][B]}{K_{-1} + K_2 + K_3[B]} \tag{6}$$

Rearranging terms,

$$\frac{d[D]}{dt} = \frac{K_1\{K_{-1} + K_2 + K_3[B]\}[A][B] - K_{-1}K_1[A][B]}{K_{-1} + K_2 + K_3[B]} \tag{7}$$

$$\frac{d[D]}{dt} = \frac{\{K_1K_2 + K_1K_3[B]\}[A][B]}{K_{-1} + K_2 + K_3[B]} \tag{8}$$

The observed second-order rate coefficient is given by:

$$K_{(obs)} = \frac{K_1K_2 + K_1K_3[B]}{K_{-1} + K_2 + K_3[B]} \tag{9}$$

which can be written as,

$$K_{(obs)} = \frac{K_1K_2}{K_{-1} + K_2 + K_3[B]} + \frac{K_1K_3[B]}{K_{-1} + K_2 + K_3[B]} \tag{10}$$

If $K_{-1} >> K_2 + K_3[B]$, that is, the initial formation of the intermediate-complex is fast and the decomposition of it (C) is slow then,

$$K_{(obs)} = \frac{K_1K_2}{K_{-1}} + \frac{K_1K_3[B]}{K_{-1}} \tag{11}$$

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A plot of $K_{(obs)}$ against the concentration of B is linear, with a slope = K_1K_3/K_{-1} and the intercept = K_1K_2/K_{-1}, i.e., the catalyzed and the uncatalyzed process, respectively (see Figure 1 , section "a"). At low concentrations of B this linear relationship has been observed.

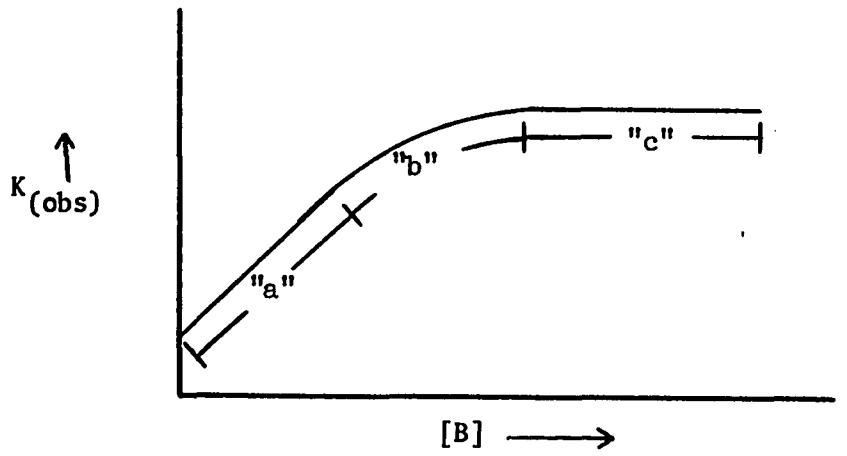

Figure 1. Relationship between $K_{(obs)}$ and [B].

Now, if $K_{-1} << K_2 + K_3[B]$, the initial formation of the intermediate-complex (C) is rate-determining and the relationship (9) reduces to (12):

$$K_{(obs)} = K_1 \qquad (12)$$

This has been found to be true at high concentrations of B, where catalysis is at a maximum and the plot of $K_{(obs)}$ versus [B] becomes parallel to the abscissa (see Figure 1 , section "c"). At intermediate concentrations, K_{-1} and $K_2 + K_3[B]$ are of comparable magnitudes and curvature of the plot occurs (see Figure 1, section "b").

Primary and secondary amines react with many 1-substituted-2,4-

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dinitrobenzenes to form 2,4-dinitrophenylamines. Some of these reactions are strongly accelerated by bases, but others are insensitive to base catalysis. The susceptibility of such a reaction to catalysis by bases has been associated with the frequency of reversion of intermediate complex "c", see page 3, to reactants. The second step of the intermediate complex mechanism of substitution is judged to be sensitive to catalysis by bases, but not the first. Consequently, only when expulsion of the leaving group X from the complex is at least partially rate limiting are reactions of this type subject to catalysis by bases. When formation of the intermediate is fully rate determining, base catalysis is not observable.

Whether or not expulsion of the leaving group is rate limiting depends upon relative rates of reversion of the intermediate to reactants and progression to products. One expects base catalysis to be observable when the leaving group is relatively slow to separate from carbon and/or when the amine moiety is easily expelled from the complex. In these terms one can understand why no reactions of amines with 2,4-dinitrochloro-or-bromobenzene have been observed to be base catalyzed, whereas reactions of piperidine with 2,4-dinitrophenyl phenyl ether[15] and 2,4-dinitroanisole[16] are strongly catalyzed by bases.

Rates of reactions of several 2,4-dinitrophenyl ethers with piperidine to form 2,4-dinitrophenylpipieridine have been measured as a function of sodium hydroxide concentration.[17] The sensitivity of these reactions to base catalysis varies with the leaving group. When the leaving group is 2,4-dinitrophenoxy or p-nitrophenoxy, catalysis by so-

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dium hydroxide is weak when it is methoxy, catalysis is strong. When catalysis is strong, the second-order rate coefficient $K_{(obs)}$ is curvilinearly related to base concentration. The reaction of 2,4-dinitrophenyl phenyl ether with piperidine in 10% dioxane-90% water (v/v) is subject to general base catalysis by piperidine and the plot of $K_{(obs)}$ versus piperidine concentration is curvilinear.

A major advance in the field of electrophilic substitution was Melander's[18] demonstration that there is no hydrogen isotope effect in typical nitration and bromination reactions, that is, tritium and protium are displaced at essentially identical rates. This showed that breaking of the carbon-hydrogen bond had made little or no progress in the rate-determining transition states of these reactions. Isotope effect experiments similar to Melander's would be desirable in the field of nucleophilic aromatic substitution but unfortunately cannot be done so conveniently. Hydrogen itself is not commonly replaced and when it is the reaction is probably more complicated than a mere one-or two-step displacement. In theory, the isotope effect in replacement of, for example, a nitro group or a chlorine atom (that is, the ratio of rates of displacement of ^{14}N vs. ^{15}N or of ^{35}Cl vs. ^{37}Cl could be used as a criterion of mechanism, but the effect would at greatest be small and measurements would have to be made with great exactitude before they could have significance.

On the other hand, it is possible to study the "element effect" in nucleophilic substitutions, that is, the change in rate as the first atom of the displaceable group X is changed from one element to another.

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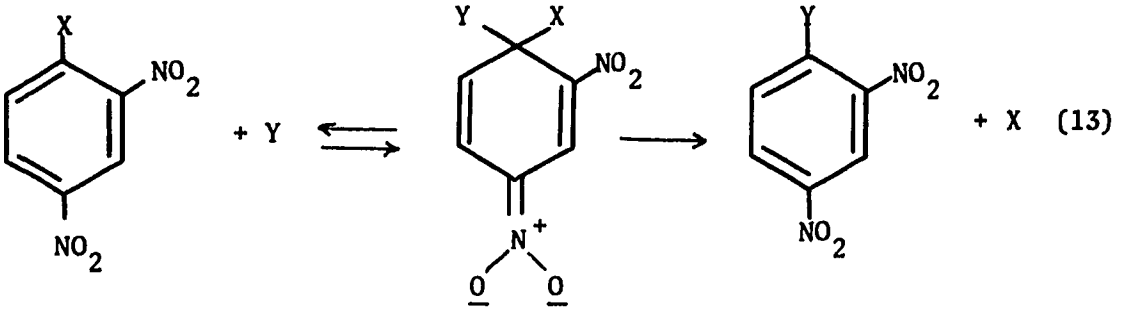

(13)

It is well known that the rates of cleavage of C-X bonds are strongly dependent on the identity of the element X.[19] The differences in rate are much greater than the differences in the rates of cleavage of C-H bonds as the hydrogen atom is changed from one isotope to another. Therefore, if C-X bond breaking has made significant progress in the transition state of the rate-determining step, the rates of displacement of groups whose first atoms represent diverse elements should differ greatly from one another.

The rates of the reaction of N-methylaniline with 1-X-2,4-dinitrobenzenes in which X is F, Cl and Br have been measured in nitrobenzene and in 99.8 percent ethanol as solvents.[20] In both media the order of reactivity, at the temperature studied, is Br > Cl > F. Estimates show that variations in the activation entropies make significant contributions to the relative reactivities. It is inferred that bond breaking must have progressed to a significant extent in the transition states.

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Table 2. Rates of Displacement on 1-X-2,4-dinitrobenzenes by N-methylaniline.

-X	Solvent	Temp., °C	K x 10^7 1. $mole^{-1}sec^{-1}$	$\Delta H^{\ddagger}$ Kcal/mole	$\Delta S,^{\ddagger}$ e.u.
F	Nitrobenzene	120	182 ± 20	10	-56
Cl	Nitrobenzene	120	2750 ± 50	12	-48
Br	Nitrobenzene	120	8450 ± 350	11	-44
F	Ethanol	50	4.53 ± 0.7	7	-68
Cl	Ethanol	50	6.22 ± 2	8	-62
Br	Ethanol	50	13.7 ± 2	12	-50

If the breaking of the C-X bond has not made significant progress in the rate-determining transition state, that is, if the activation process is entirely or almost entirely concerned with attachment of the nucleophilic reagent Y to the aromatic substrate, the effect of changing the first atom of the displaceable group is less easily predicted.

The reaction of 1-X-2,4-dinitrobenzenes, in which X is F, Cl, Br and I, with piperidine in methanol to form 2,4-dinitrophenylpipieridine have been studied.[21] The order of reactivity is F > Br > Cl > I. Heterolytic breaking of the caron-fluorine bond is characteristically slower, under comprable conditions, than heterolysis of any other carbon-halogen bond. It follows that C-F bond-breaking cannot have made significant progress in the transition state of any substitution in which fluorine is the most rapidly replaced of the halogen.

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Table 3. Reactions of 1-X-2,4-Dinitrobenzenes with Piperidine in Methanol, at 0°C.

X	Rate Coefficient 1. mole^{-1} min^{-1}	ΔE, Kcal/mole	$\Delta S^{\ddagger}$ e.u.
F	90	--	--
Br	0.118	11.8	-29.5
Cl	0.117	11.6	-30.2
I	0.0272	12.0	-31.7

The rates of reactions of six 4-substituted 2-nitrofluorobenzenes with piperidine in methanol were determined by Bunnett and co-workers.[22]

Table 4. Rates, Enthalpies and Entropies of Activation for the Reactions of 4-Substituted-2-nitrofluorobenzenes with Piperidine in Methanol.

4-Substitutent	K_{obs} at 25°C 1. mole^{-1} sec^{-1}	$\Delta H^{\ddagger}$ Kcal/mole	$\Delta S^{\ddagger}$ cal deg^{-1} mole^{-1}
H	1.29×10^{-4}	12.5	-34
Br	1.57×10^{-3}	12.0	-31
CF_3	5.86×10^{-2}	10.4	-29
CH_3CO	2.22×10^{-1}	9.9	-28
CH_3SO_2	6.93×10^{-1}	8.6	-30
NO_2	6.46	6.3	-33

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The reactions were found to be first-order in substrate and first-order in piperidine; thus they are not base catalyzed.

When linear free-energy correlations were attempted between log K_{obs} and σ values of the original Hammett type[23] rather unsatisfactory plots were obtained. On the other hand, the plot of log K_{obs} versus Jaffe's σ-values[23,24] was agreeable. For p-CF_3, σ- of +0.74 (from p-trifluoromethylanilinium ion dissociation[24] was used, and for p-Br the ordinary σ-value. Figure 2 shows the Hammell plot of log K_{obs} versus σ-for these data.

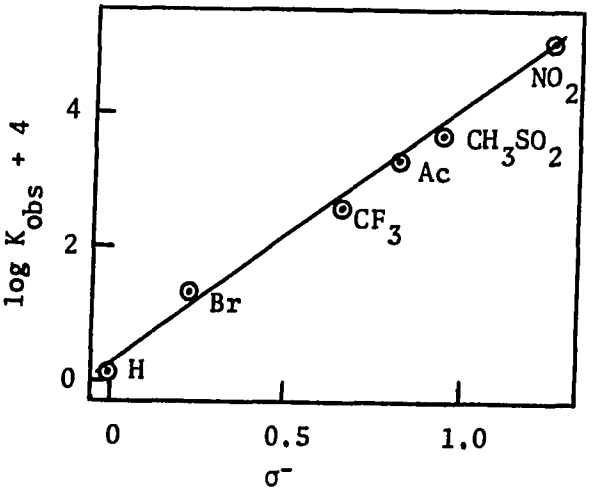

Figure 2. Hammett Plot of Log K_{obs} vs. σ -.

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The slope, ρ, of the line drawn is +3.52. This ρ is similar in magnitude to ρ observed for many other aromatic nucleophilic substitutions in nitro-activated aryl halides.[25]

Studies of nucleophilic aromatic substitution reactions in aprotic non-polar solvents indicate the effect of additives on these reactions. The reactions of 2,4-dinitrofluorobenzene and 4-nitrofluorobenzene with piperidine in benzene solution were found to be catalyzed by excess piperidine, but not by triethylamine.[26] Addition of methanol to the reaction mixture accelerated the reaction rate for the lower amine concentrations and decreased the rate at higher piperidine concentrations. These effects were explained by electrophilic catalysis in which the amine or alcohol is involved in the rate-determining abstraction of the fluoride ion through hydrogen bonding. This catalysis indicates that the breaking of the carbon-fluorine bond is important in the rate-determining transition state.

The reaction of 2,4-dinitrochlorobenzene and 4-nitrochlorobenzene with piperidine in benzene was found not to be catalyzed by the excess amine or by the addition of methanol.

Similar results on these same systems have been reported by other workers[27,28] using piperidine as the nucleophile and benzene as the solvent. In the case of the reaction of 2,4-dinitrofluorobenzene with piperidine a plot of the observed second-order rate coefficient, K_{obs}, against piperidine concentration was found to increase linearly with increasing concentration of the amine. When the reaction was run in 0.1 M methanol the second-order rate coefficient increased, but in a curvilinear manner. At higher concentrations of alcohol, 0.15 M, the rate

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constant was found to decrease with increasing piperidine concentration. The decrease in the reaction rate at higher alcohol-amine concentrations was attributed to hydrogen-bonding of piperidine with methanol, thus removing a portion of the nucleophile from the reaction. This theory was confirmed by adding increasing amounts of methanol to the 2,4-dinitrochlorobenzene-piperidine reaction which does not appear to be catalyzed by additives. The rate constant in this case was found to decrease with increasing concentrations of the alcohol.

Experiments conducted by Pietra and co-workers[29] show that α-pyridone substantially increases the rate of the reaction of piperidine with 2,4-dinitrofluorobenzene in benzene solution while N-methyl-α-pyridone does not affect the rate at all.

Table 5. Reaction of 2,4-Dinitrofluorobenzene with Piperidine in Benzene at 25°C. Catalytic Coefficient of Various Substances.

Added Substance	Catalytic Coefficient $mole^{-2}\ l^{2}\ sec^{-1}$
α-pyridone	3200
Piperidine	600
Phenol	220
Triethylenediamine	32
Methanol	21
Pyridine	2
Triethylamine	0
N-methyl-α-pyridone	0

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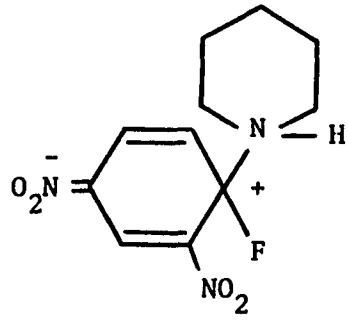
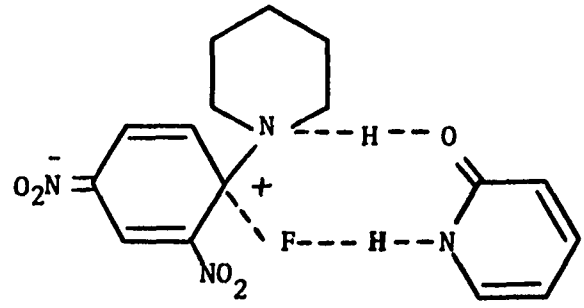
Examinations of Table 5 shows that the reaction of 2,4-dinitrofluorobenzene is catalyzed by substances having either the predominant character of bases or that of acids. However, the most efficient catalyst, α-pyridone, is very much weaker as a base (in water) than pyridine[30] and much weaker as an acid (in water) than phenol.[30] The only likely explanation is that α-pyridone acts as a "bifunctional" catalyst assisting the concerted separation of both ammonium proton and fluoride from the intermediate IV. The rate limiting transition state would then be approximated by V.



IV



V

This interpretation is substantiated by the finding that N-methyl-α-pyridone which, compared to α-pyridone lacks the acidic hydrogen, is devoid of any catalytic activity.

The kinetics of the nucleophilic displacement of halogen atoms from aromatic carbon atoms in appropriately substituted halogeno benzenes have received considerable attention. When, however,the aromatic carbon atom forms part of a heterocyclic system, our knowledge of the kinetics of these displacement reactions is extremely scanty.

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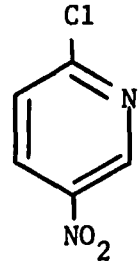
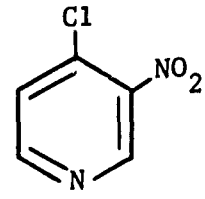
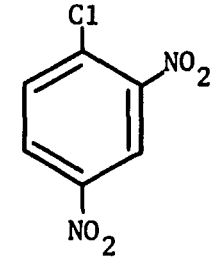
It is commonly asserted that in aromatic systems the cyclic nitrogen atom and the substituted nitro-group cause similar disturbances of the aromatic electron cloud. Bishop and co-workers[31] have studied the reactions of 2-chloro-5-nitropyridine(VI) and 4-chloro-3-nitropyridine(VII) with pyridine in ethanol solution.



VI



VII



VIII

The results were compared with those of the reaction of 2,4-dinitrochlorobenzene with pyridine in ethanol.

Table 6. Reactions of Pyridine with 2-Chloro-5-nitropyridine, 4-Chloro-3-nitropyridine and 2,4-Dinitrochlorobenzene in Ethanol, at 55°C.

Chloro-Compound	$K_{obs} \times 10^6$ 1. mole^{-1} sec^{-1}	ΔE, Kcal/mole
2,4-dinitrochlorobenzene	11.1 ± 0.2	16.7
2-chloro-5-nitropyridine	1.97 ± 0.04	18.1
4-chloro-3-nitro-pyridine	32.1 ± 0.6	16.9

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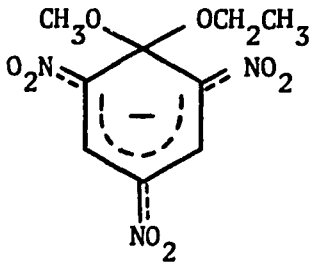
As can be seen on Table 6, the rate constants are of comparable magnitude. It is thought that 2-chloro-5-nitropyridine reacts slightly slower due to lack of steric compression by an o-nitro-group in the transition state.

Conventional kinetic studies have provided us with considerable insight into the general mechanism of activated nucleophilic aromatic substitution.[2] The main feature is that the nucleophile attacks the aromatic substrate (generally activated by one or several nitro or other electron-withdrawing groups) to form a high-enery intermediate, often referred to as a Meisenheimer complex, which can either proceed to products in a second-step or revert to reactants as illustrated previously in equation (2), page 3.

In 1902 Meisenheimer[8] succeeded in the isolation and structure determination of a compound, IX, which is today commonly taken as the



IX

prototype of the activated complex in nucleophilic aromatic substitution. Since that time, the structure of the anions formed from other trinitro aromatic compounds has been the subject of numerous investigations and

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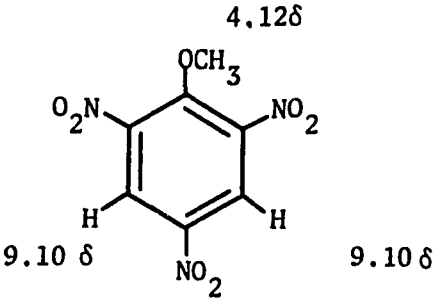
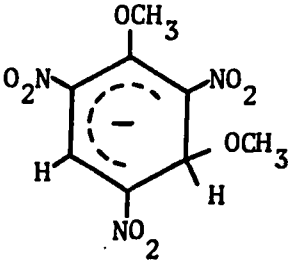
much speculation.[32-40]

The stability of Meisenheimer complexes depends significantly on the ability of the ring substitutents to accept a negative charge. Servis showed[41] that the reaction of methyl picrate (X) with methoxide

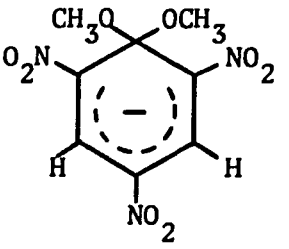


X

ion in dimethylsulfoxide initially yields the 1,3-dimethylcyclohexadienylide (XI). This undergoes a rapid conversion to the thermodynamically more stable complex (XII).



XI



XII

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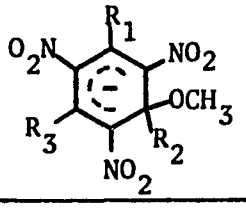
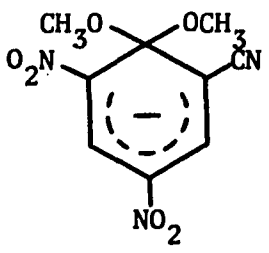
Table 7. Chemical Shift of Anions from Methyl Picrate Reacting with Methoxide Ion in Dimethyl Sulfoxide.

	Compound				Chemical Shift, ppm[a]			
		R_1	R_2	R_3	R_1	R_2	R_3	OCH_3
	XI	OCH_3	H	H	3.45	6.17	8.42	3.20
	XII	H	OCH_3	H	8.67	3.06	8.67	3.06

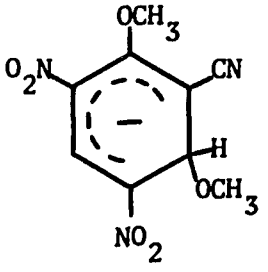
[a]Relative to tetramethylsilane internal standard = 0.0 ppm.

Crampton and Gold[42] had earlier suggested the reason for this isomerization is that while (XII) is a less strained structure than methyl picrate (X), the transition state leading to its formation is more strained than for (XI).

Further examples of 1,3-complex formation have been reported. Fendler, et al.,[43] followed the course of the reaction of 2-cyano-4,6-dinitroanisole with methoxide ion by ^1H nmr spectroscopy. The spectrum of the known complex (XII) was observed together with that of the 1,3-complex (XIV) which had a half-life of approximately 1 hour.



XIII



XIV

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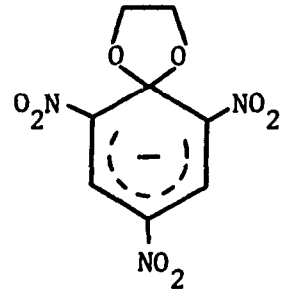
Similar observations were made in studies of the reactions of methoxide ion with 4-cyano-2,6-dinitroanisole and 2,4-dicyano-6-nitroanisole. In both cases the 1,3-complexes are again formed initially and then undergo conversion to the isomeric 1,1-complexes. The isolation of the 1,1-complexes of the former compound has also been reported by other workers.[44]

When sodium 2-hydroxyethoxide in ethylene glycol is added to glycol 2,4,6-trinitrophenyl ether [1-(2-hydroxyethoxy)-2,4,6-trinitrobenzene] a bright orange-red solution is obtained.[45] By removal of the solvent an orange amorphous solid is obtained which is soluble in water but insoluble in aprotic solvents of low dielectric constant.



XV

the nmr spectrum of the compound in acetone-d_6 shows only two proton resonances, one at 8.7δ and a second at 4.3δ. This observation is in accord with structure (XV). The absorption at 8.7δ is due to the ring protons of the ion. The second line at 4.3δ results from the two cyclic methylene groups, which being equivalent, show no spin-spin splitting. The intensity of this line, double that of the absorption at 8.7δ, is consistent with structure (XV).

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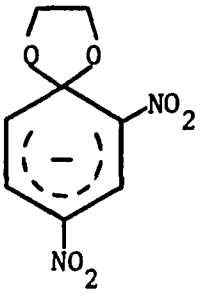
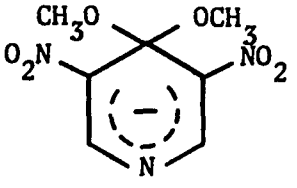
The bright red color formed on the addition of sodium hydroxide to an acetone solution of 1-(2-hydroxyethoxy)-2,4-dinitrobenzene[46] was thought to be due to the formation of a spiro-Meisenheimer complex (XVI).



XVI

The nmr spectrum substantiated the postulated structure.

Several investigators have reported evidence for the formation of Meisenheimer complexes in nucleophilic displacement reactions of N-heteroaromatic substrates. Illuminati and Stegel[47] reported the formation of a Meisenheimer complex upon the action of methoxide ion on 4-methoxy-3,5-dinitropyridine in methanol solution. Structure XVII was proved by elemental analysis and nmr spectral evidence.



XVII

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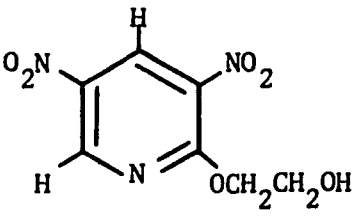
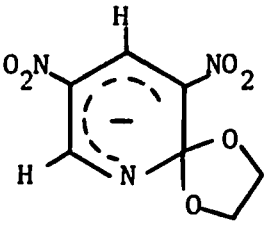
In dimethylsulfoxide-d_6 solution only two singlets were found, their δ values being 8.78 and 2.92 with relative intensities 1 and 3 respectively.

Addition of sodium methoxide to a solution of 2-(2-hydroxyethoxy)-3,5-dinitropyridine (XVIII) in dimethylsulfoxide gives a red coloration (λmax = 462 mμ) and causes the disappearance of the nmr spectrum of the



XVIII



XIX

aromatic compound at 9.31 δ and 9.10δ and the appearance of a tightly coupled AB system centered at 8.35δ (relative intensity 1) and a singlet (relative intensity 2) at 4.12δ corresponding to the ring and methylene protons of the spiro-compound (XIX).[48] 2-Hydroxy-3,5-dinitropyridine absorbs at 8.8 δ in basic dimethyl sulfoxide, so it would appear that no hydrolysis has taken place. Acidification of a solution of (XIX) regenerates the spectrum of (XVIII).

The 2,4,6-trinitroanilines do not react with either secondary or tertiary amines in dimethyl sulfoxide.[41] However, 2,4,6-trinitroanisole reacts with both types of amines. Addition occurs at the 1 position to generate a Meisenheimer type complex as follows:

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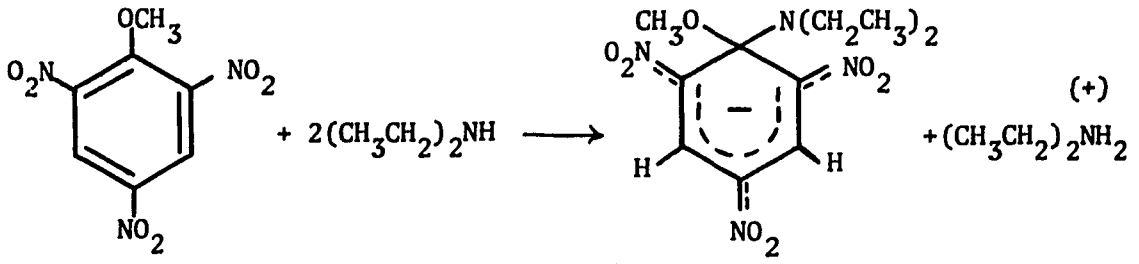
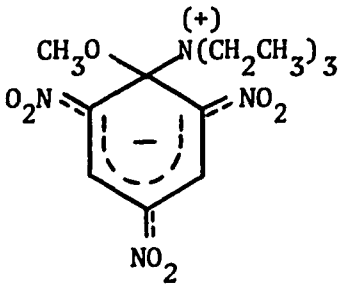


XX XXI

Somewhat surprisingly, triethylamine in dimethyl sulfoxide also reacts with 2,4,6-trinitroanisole to generate the zwitterionic complex (XXII).[41]



XXII

Compounds (XXI) and (XXII) were identified by their nmr spectra.

The spiro Meisenheimer complexes possess a number of unique features which are relevant to the chemistry, particularly the structures and stabilities of Meisenheimer complexes, and to their role in nucleophilic aromatic substitution. The greater stabilities of the spiro complexes relative to the acyclic complexes should be reflected in the mag-

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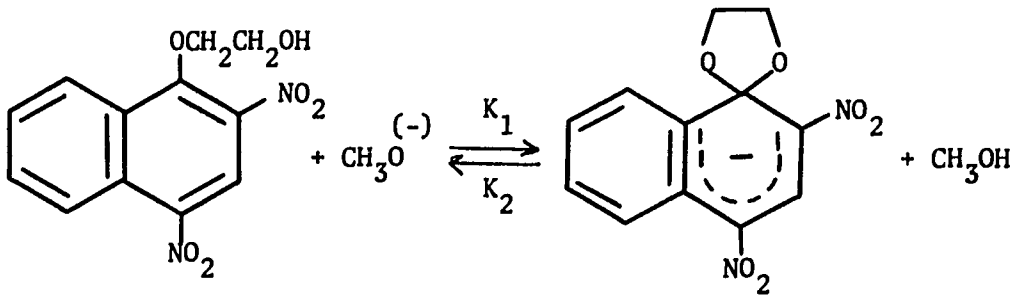
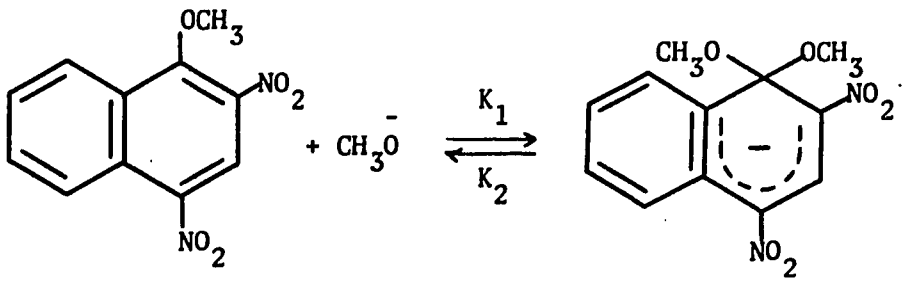
nitudes of the appropriate kinetic and thermodynamic parameters; entropy effects might be expected to be particularly important for the spiro complexes. A convincing demonstration of the structures of the spiro complexes should provide particularly compelling evidence for the sp^3 hybridization at C-1 of the cyclohexadienyl systems of Meisenheimer complexes.

Fendler and co-workers[49] reported the rate constants for the formation and decomposition of the spiro Meisenheimer complexes (XXV) and (XXVI) formed by the reaction of methoxide ion with 1-(2-hydroxy-



XXIII XXV



XXIV XXVI

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ethoxy)-2,4-dinitronaphthalene (XXIII) and 1-methoxy-2,4-dinitronaphthalene (XXIV), respectively, in methanolic solution at 6.65°, 15.00° and 25.00°C, allowing a determination of Keq., K_1 and K_2, and the energies and entropies of activation for the reactions.

Table 8. Kinetic and Thermodynamic Parameters for the Formation and Decomposition of Meisenheimer Complexes XXV and XXVI in Methanol at 25.00°C.

	XXV	XXVI
K_1, 1. mole^{-1} sec^{-1}	1.28	0.92
$K_2 \times 10^3$, sec^{-1}	3.60	4.0
Keq., 1. mole^{-1}	356	230
E_1, Kcal mole^{-1}	11.8 $\pm$ 0.8	13.8 $\pm$ 0.8
ΔS_1^*, e.u.	-20 $\pm$ 2[a,b]	-17 $\pm$ 2[a,b]
E_2, Kcal mole^{-1}	20.0 $\pm$ 0.8	16.5 $\pm$ 0.8
ΔS_2^*, e.u.	-5 $\pm$ 2[a,c]	-18 $\pm$ 2[a,c]

[a]Calculated at 25.00°C.
[b]Calculated by using the second-order rate constants, K_1.
[c]Calculated by using the first-order rate constants, K_2.

Data in Table 8 indicate tha the spiro Meisenheimer complex (XXV) is approximately 50 percent more stable than the analogous acyclic 1,1-dimethoxy-2,4-dinitronaphthalene complex (XXVI) and further indicate that the driving force for the formation of (XXV) is much more entropy dependent than that for (XXVI).

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The enhanced stability of the spiro complexes is also shown by their failure to undergo rearrangement or deuterium exchange with solvent; solutions of the complexes in dimethyl sulfoxide-d_6 do not show detectable pmr changes over a period of months. It has been shown that 1,1-dialkoxy complexes of 2,4-dinitrobenzenes undergo both rearrangement to 1,2-complexes and exchange of cyclohexadienylide protons for solvent deuterium.[50,51] Also, in the presence of excess methoxide ion, 1,1-dialkoxy complexes of 2,4-dinitronaphthalene systems undergo rapid and complete exchange of ring protons with solvent (dimethylsulfoxide-d_6) deuterium; no comparable reaction is observed with the more stable spiro complex (XXV).

Purpose of this Thesis

The purpose of this thesis is to elucidate the mechanism of the nucleophilic aromatic substitution by amines, mainly piperidine, on 6-chloro-9-methoxymethylpurine. The study will be divided in two broad areas: (1) solvent effects on the reaction rate constant and (2) the direct observation of the addition (Meisenheimer) complex in the purine system by means of n.m.r. and u.v. spectroscopy.

The subject of nucleophilic aromatic substitution in polar, protic solvents has been widely investigated by many workers; however, studies of such reactions in aprotic, non-polar solvents are somewhat limited. It is in this latter media that catalysis by acids, bases or "bifunctional" species can be examined without complications by polar effects or hydrogen bonding with solvents.

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The stability of Meisenheimer complexes depends significantly on the ability of the ring substituents to accept a negative charge. As a general rule, a nitro group attached directly to an aromatic system is equivalent to a nitrogen atom forming part of such a system, in its ability to stabilize, by resonance, a negative charge introduced into the aromatic ring. Several investigators have reported evidence for the formation of Meisenheimer complexes in nucleophilic displacement reactions of N-heteroaromatic substrates. Up to this date none has been reported in the purine system.

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CHAPTER II

EXPERIMENTAL

All boiling points, melting points and reaction temperatures reported herein are uncorrected.

Reagents

Cyclohexane

A.C.S. Spectranalyzed cyclohexane obtained from Fisher Scientific Company, Fair Lawn, New Jersey, was distilled over sodium metal (5 g. of sodium per liter of cyclohexane). The fraction boiling at 78.2°C was collected in a brown bottle.

Benzene

A.C.S. Spectranalyzed benzene, Fisher, was distilled over sodium (3 g. of sodium per liter of benzene). The material boiling at 80.1°C was collected in a brown bottle and stored until needed.

Dioxane

Certified grade 1,4-dioxane, Fisher, was distilled from lithium aluminum hydride according to the procedure outlined by R. L. Augustine.[52] The fraction boiling at 99.0°C was collected in a brown bottle and stored out under dry nitrogen.

Methanol

Baker analyzed reagent grade methanol was distilled from magnesium metal-turnings (3 g. of magnesium per 500 ml. of methanol). The fraction boiling at 64.1°C was collected and stored until further use.

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DMSO

Dimethyl sulfoxide, Fisher Certified Reagent Grade, was purified by adding 5 g. of calcium hydride per 500 ml. of DMSO. Upon distillation the first 30 ml. of distillate was discarded. The fraction boiling at 52.5 - 54.0°C at 15 mm. pressure was stored in a brown bottle under nitrogen.

Water

Distilled water was distilled once from basic potassium permanganate (4 g. of $KMnO_4$ - 1 g. of NaOH per liter of water). After refluxing the contents for 24 hours, the first 100 ml. of distillate were discarded. The purified water was collected and stored until needed.

60 percent-dioxane - 40 percent-water (v/v)

A mixture of dioxane-water was prepared using the previously purified materials. The mixture was made up by volume.

Tert-butyl alcohol

Fisher Certified t-butyl alcohol was distilled from sodium metal (1 g. of sodium per 500 ml. of the alcohol). The fraction boiling at 81.5°C was collected.

Piperidine

Purification of piperidine, Fisher, was carried out by distillation from sodium.[53] About 500 ml. of piperidine (5.05 moles) and 12 g. (0.52 g-atoms) of sodium metal were placed in a 1000 ml. round-bottomed flask and refluxed for ten hours in a two-foot Vigreux distilling column. The distillate was collected in a brown bottle, b.p. 105.1°C, and stored under nitrogen.

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Piperidine-N-d

The method used by M.F. Hawthorne[54] was followed to prepare piperidine-N-d. A mixture of 102 grams of piperidine (1.2 moles), 30 ml. (1.5 moles) of 99.8 percent deuterium oxide and 3 ml. of deuterated phosphoric acid (prepared by the reaction of phosphorous pentoxide and deuterium oxide) was refluxed for 18 hours. The piperidine-N-d was distilled, the fraction boiling at 107.0°C was collected. The amine was again treated with deuterated phosphoric acid and refluxed for 15 hours. The distillate boiling at 107.0°C was collected in a 500 ml. round-bottomed flask. Sodium metal, 3 g. (0.13 g-atoms) was added slowly. After the sodium was dissolved, the contents of the flask were refluxed for four hours. Finally the piperidine-N-d was distilled. The boiling point of the distillate was 107.0°C at atmospheric pressure. The n.m.r. spectrum of the product showed no N-h peak.

Pyrrolidine

The method previously used to purify piperidine was adopted for the purification of pyrrolidine. The fraction boiling at 87.5°C was collected and stored under nitrogen. The amine was obtained from Eastman Kodak.

Morpholine

Fisher Certified morpholine was purified using the procedure applied in the purification of piperidine. The boiling point of the distillate was 125.8 - 126.0°C at atmospheric pressure.

6-Chloro-9-methoxymethylpurine

This compound was obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. Its melting point was 116 - 118°C and it had a

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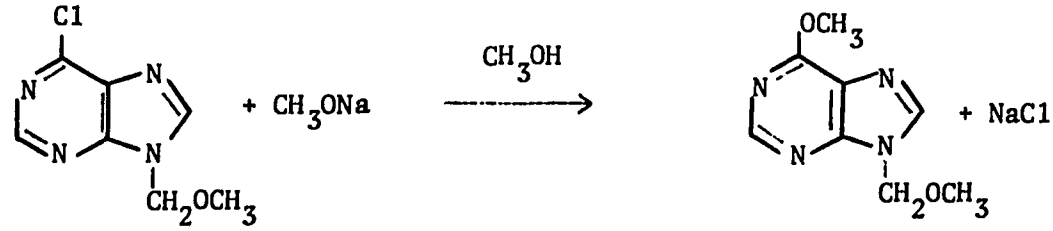
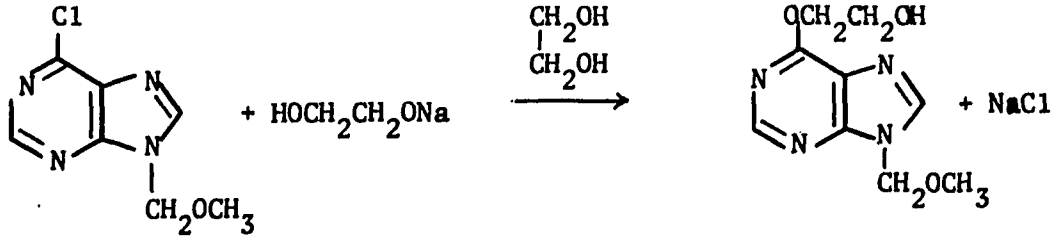
slight yellow color. After recrystallization from n-hexane it showed a m.p. of 116.8 - 117.0°C and it was white in color. The reported melting point in the literature is 117°C.[55]

6-Methoxy-9-methoxymethylpurine



Sodium methoxide was prepared by dissolving 0.75 g. (0.0163 moles) of sodium metal in 50 ml. of dry methanol. Then 1.621 g. (0.00816 moleS) of 6-chloro-9-methoxymethylpurine dissolved in 10 ml. of methanol were added. The mixture was refluxed for four hours at 63.0°C. Methanol was removed using a flash evaporator. After recrystallization from n-hexane, 6-methoxy-9-methoxymethylpurine, a new compound, had a melting point of 91.4 - 91.7°C. The mass spectrum showed a parent ion at mass 194.0257 (calculated for $C_8H_{10}N_4O_2$ 194.0280). The u.v. spectrum showed a λmax. at 248 millimicrons (ε = 11,700) in distilled water. A total of 1.208 g. of 6-methoxy-9-methoxymethylpurine were obtained. This corresponds to a 77 percent yield from the starting chloropurine.

6-(2-Hydroxyethoxy)-9-methoxymethylpurine



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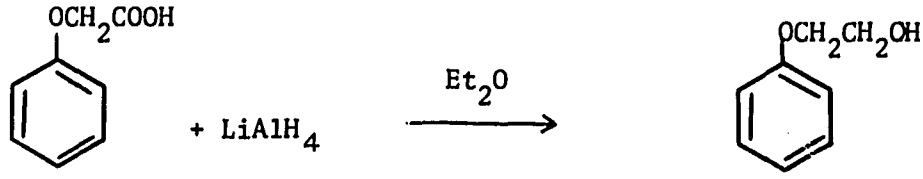
The sodium salt of ethylene glycol was prepared by the action of 0.161 g. (0.007 moles) of sodium metal on 30 ml. of dry ethylene glycol. After the metal was dissolved, 1.385 g. (0.0069 moles) of 6-chloro-9-methoxymethylpurine dissolved in 10 ml. of dry ethylene glycol were added. The mixture was stirred for 18 hours at 27°C. After removing the ethylene glycol with a vacuum pump, at 26°C over a period of three days, the purine was recrystallized from iso-octane. The white material melted at 130.2 - 130.8°C. 6-(2-hydroxyethoxy)-9-methoxymethylpurine is a new compound. The mass spectrum showed a peak at mass 224.0556 (calculated for $C_9H_{12}N_4O_3$ 224.0591) corresponding to the parent ion. The u.v. spectrum had a λmax. at 252 millimicrons (ε = 11,000) in distilled water. A yield of 1.345 g. (86 percent) of 6-(2-hydroxyethoxy)-9-methoxymethylpurine was obtained, based on the starting chloropurine.

2-Phenoxyethanol



The synthesis of 2-phenoxyethanol was accomplished by reducing 4.16 g. (0.027 moles) of phenoxyacetic acid, purchased from Eastman Organich Chemicals, with excess lithium aluminum hydride, 3.28 g. (0.08 moles) in dry ethyl ether. The acid was first dissolved in 50 ml. of dry ether and then added dropwise to the lithium aluminum hydride-etheral solution. The contents of the flask were stirred at 25°C for

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42 hours. Using a dropping funnel, 10 ml. of water were added. Immediately, 3 ml. of a 15 percent (by weight) solution of sodium hydroxide in water were added slowly. The etheral solution was filtered and dried with magnesium sulfate. After filtration the ether was removed by distillation. The crude 2-phenoxyethanol was then distilled under vacuum. The distillate had a boiling point of 135°C at 23 mm of pressure. The reported b.p. is 137°C at 25 mm. of pressure.[56] The yield of 2-phenoxyethanol from phenoxyacetic acid was 1.02 g. or 30 percent.

Potassium t-butoxide

About 3.9 g. of potassium metal were added to 100 ml. of dry t-butyl alcohol. The mixture was stirred with a magnetic bar for 15 hours. Determination of the exact concentration of potassium t-butoxide was accomplished by titration with standard HCl solution. 5.0 ml. of potassium t-butoxide in t-butyl alcohol were diluted with distilled water to 50 ml. in a volumetric flask. Titration was carried out using 1.432 N HCl and a Beckman Zeromatic II pH meter. The concentration of potassium t-butoxide in t-butyl alcohol was calculated to be 0.5642 N.

Dimethyl sulfoxide-d_6

This chemical was obtained from Matheson Coloman and Bell, Norwood, Ohio, and it contained a maximum isotopic purity of 99.5 atom percent deuterium. DMSO-d_6 was used as received without further purification.

Methanol-d_4

This chemical was purchased from Matheson Coleman and Bell. It contained a minimum isotopic purity of 99.0 percent deuterium.

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Instrumentation

Weighing Balance

All materials used throughout this research were weighed out on a Mettler type H 15 balance.

Melting Point Apparatus

A Mel-temp unit was used to obtain melting points. Fisher capillary tubes were used.

Constant Temperature Baths

A thick-walled pyrex glass bath, obtained from Fisher Scientific Company, equipped with a Sargent thermonitor unit (Model NSI-12) was used along with a NBS calibrated 0° - 100°C thermometer. Also a Precision Scientific water bath, Model 161 was employed. In these baths solutions were prepared and slow reactions were studied.

Ultraviolet Spectrophotometer

The u.v. spectra and kinetic measurements were run on a Cary 14 Recording Spectrophotometer equipped with a Lauda Thermostated Constant Temperature Unit, range 0° to 100°C. The temperature of the reactions was measured with a NBS calibrated 0° - 100°C thermometer inserted into the sample compartment of the spectrophotometer. The cuvettes used were 1.0 cm. Beckman standard silica cells. For slow reactions the cuvettes used were equipped with Teflon stopcocks to prevent evaporation.

Nuclear Magnetic Resonance Spectrometer

All n.m.r. data were obtained on a Varian A-60D spectrometer.

Mass Spectrometer

All mass spectra data were obtained using a Varian M 66 instrument.

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Electronic Calculator

The slopes used in calculating the pseudo or second-order rate constants and the slopes used in obtaining energy of activations were determined using the standard linear regression program of the Wang 700 A/B Electronic Calculator.

pH Meter

Titrations were carried out using a Beckman Zeromatic II pH meter.

Miscellaneous Equipment

Slow reactions were timed using a Precision Scientific Company timer model Minute, graduated in one hundredth of a minute.

Kinetic Procedure

The following procedure was used for the preparation of the piperidine solutions. The stock solution was made up in a 100 ml. volumetric flask. The flask was weighed and then piperidine was weighed into the flask. Approximately 80 ml. of the solvent was added and the contents of the flask were equilibrated in a water bath at 25.0°C for 15 minutes. The flask was filled to the mark with the solvent, also at 25.0°C. Using 10 ml. volumetric flasks and 5 ml. pipettes the piperidine stock solution was used to prepare all concentrations of piperidine solutions by a series of dilutions with the solvent at 25.0°C.

The above procedure was also used in the preparation of the stock solutions of morpholine and pyrrolidine in cyclohexane.

The purine stock solution was prepared by first weighing a 10 ml. volumetric flask on the balance. Then the purine was weighed into the flask. About 9 ml. of the solvent was added to dissolve the purine and

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the contents of the flask were poured into a 250 ml. volumetric flask. The 10 ml. flask was washed five times with 10 ml. portions of the solvent and the washings were combined with the contents of the 250 ml. volumetric flask. The contents of this flask were then equilibrated in the water bath for 15 minutes at 25.0°C, followed by addition of the solvent at the same temperature to the mark on the flask. The concentration of the purine stock solution was 10×10^{-5} moles liter^{-1}.

All reaction rates were followed by means of u.v. spectroscopy. First, a sample solution was run to determine the u.v. spectrum and the extintion coefficient of the product of the reaction of the purine with the nucleophile in question. Ten milliliters of the purine (10×10^{-5} M) and 10 ml. of the nucleophile (usually 0.5 M) were mixed at room temperature and allowed to react for at least 18 hours. During this time periodic checks on the amount of the product formed were determined by examining the u.v. spectrum. When the spectrum of the product remained unchanged for several hours it was assumed that the reaction was complete. Based on the concentration of the starting purine, the u.v. spectral characteristics of the product could be determined using Beer's and Lambert's Laws,[57] mainly that $\varepsilon = A/cl$, where ε is the molar extintion coefficient, c is the molar concentration and l is the path length of the cell in centimeters. The absorbance A was obtained directly from the spectrum, the path length was 1 cm. and the concentration of the product was 5×10^{-5} M. In order to have a good base line in the determination of the spectra, the reference cell always contained the same amount of nucleophile as the sample cell.

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The following procedure was employed for all the kinetic runs reported in this thesis. The u.v. spectrophotometer was set at a fixed wave length, corresponding to a region in which the spectrum of the starting purine and the spectrum of the product would not overlap. Also the wave length was chosen as close as possible to the λmax. of the product. The reference cell was prepared by adding the amount of nucleophile that was needed to balance the nucleophile in the reaction cell. The empty reaction cell and the reference cell were placed in the thermostated compartment of the u.v. instrument. A 2.5 ml. gas syringe with a Chaney adaptor was adjusted to deliver 1.7 ml. of solution (enough to half-fill the cuvette). About 1.8 ml. of the purine solution were drawn into the syringe. The syringe was placed in the sample compartment. After equilibration of the temperature for at least 15 minutes (the reactions run at 44.5°C were equilibrated for about 30 minutes) the amount of solution in the syringe was adjusted to 1.7 ml. and injected to the sample cell. The cuvette was tightly stoppered and the compartment lid replaced. The syringe was washed twice with acetone and twice with anhydrous ether and finally dried with dry nitrogen gas. Then about 1.8 ml. of the nucleophile solution was drawn into the syringe and the latter placed in the sample compartment to equilibrate the temperature. After 15 minutes, the amount of solution in the syringe was adjusted to 1.7 ml. and quickly injected into the purine solution in the reaction cell. The force of the injection proved to be sufficient to cause complete mixing of the solutions. The compartment cover was quickly replaced and the master switch was turned on. This last mixing of the solutions and adjust-

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ment of the instrument required about ten seconds to perform.

The kinetic studies were followed by observing the rate of formation of the purine-product in the u.v. spectrum. The spectrophotometer was equipped with a recorder in which the chart speed could be regulated from eight inches per minute to 1.4 inch per minute, depending on the speed of the reaction being followed. Therefore, one was able to follow the amount of product formed as a function of time.

All kinetic studies were run under pseudo-first order conditions. The nucleophile was always at least 100 times in excess of the purine. The kinetic data were treated using the Guggenheim methode[58] which permits the determination of the rate constant without knowing the absorbance at infinite time. Readings were taken at times t_1, t_2, t_3, etc., and at times $t_1 + \Delta$, $t_2 + \Delta$, $t_3 + \Delta$, etc., where Δ is a constant increment of time equal to at least to two half-lives. The Guggenheim equation can be written as $\ln(A_{t+\Delta} - A_t) = -kt + \text{constant}$, where $A_{t+\Delta}$ is the absorbance at time $(t + \Delta)$ and A_t is the absorbance at time t. A plot of log $(A_{t+\Delta} - A_t)$ against time gives a straight line of slope $-k/2.303$. Since the nucleophile is at least 100 times greater than the purine the k obtained from the plot is k_{pseudo}. This rate constant is then divided by the nucleophile concentration to obtain the second order rate constant.

The following sample calculation demonstrates this procedure. The plot of log $(A_{t+\Delta} - A_t)$ vs time t is illustrated in Figure 3.

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Table 9. The Reaction of Piperidine (0.1010 M) with 6-Chloro-9-methoxymethylpurine (5 x 10^{-5} M) in Cyclohexane, at 23.0°C. Calculation of the Second Order Rate Constant.

Time (t, min.)	A_t	$t + \Delta$	$A_{t+\Delta}$	$\log (A_{t+\Delta} - A_t)$
0	0.036	48	0.631	-0.2254
1	0.055	49	0.639	-0.2335
2	0.075	50	0.646	-0.2433
3	0.093	51	0.652	-0.2526
4	0.112	52	0.659	-0.2620
5	0.130	53	0.665	-0.2716
6	0.148	54	0.670	-0.2823
7	0.166	55	0.678	-0.2907
8	0.182	56	0.685	-0.2984
9	0.198	57	0.691	-0.3071
10	0.213	58	0.695	-0.3169
11	0.228	59	0.701	-0.3251
12	0.243	60	0.707	-0.3334
13	0.260	61	0.712	-0.3448
14	0.275	62	0.718	-0.3536
15	0.288	63	0.721	-0.3635

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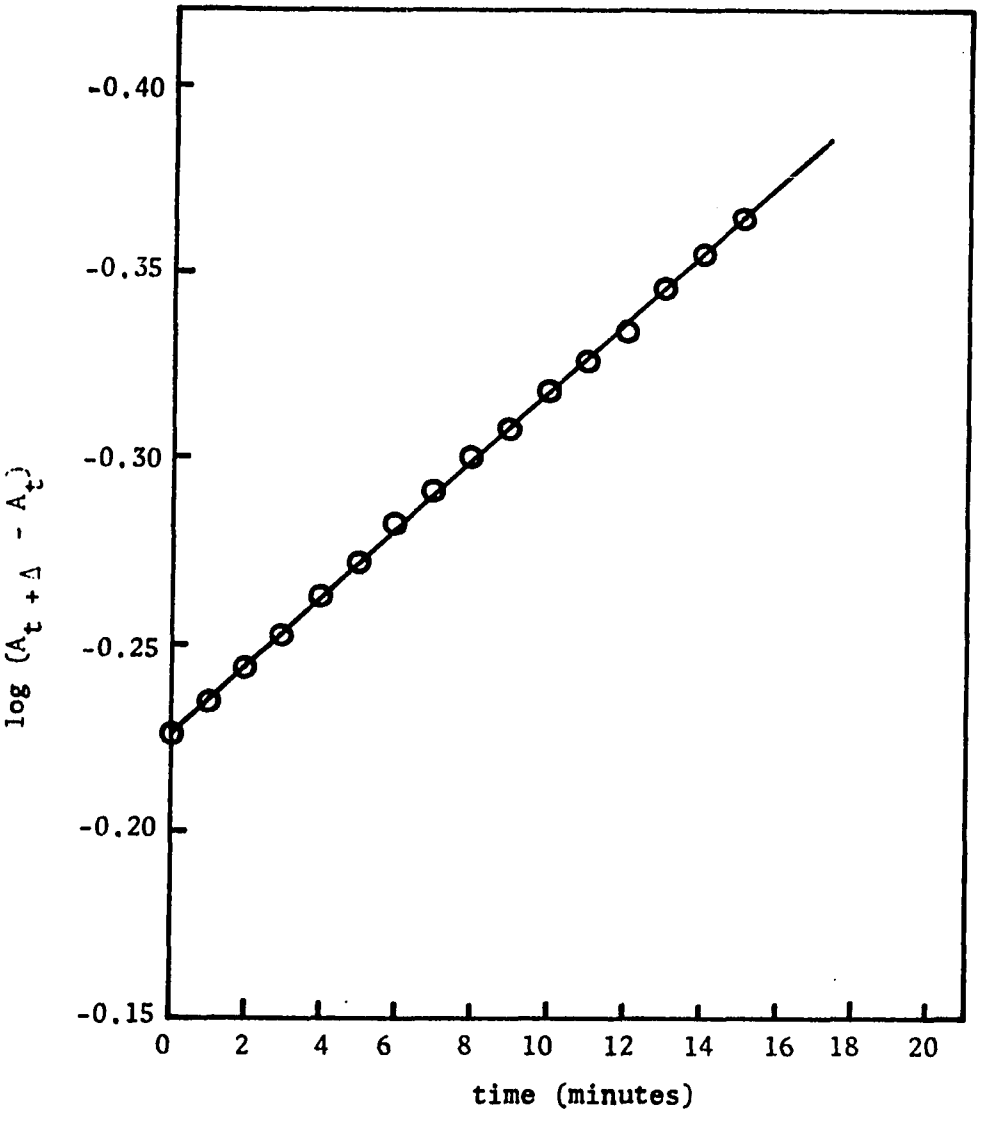

Figure 3. Reaction of Piperidine (0.1010 M with 6-Chloro-9-methoxymethylpurine (5 x 10^{-5} M) in Cyclohexane at 23.0 ± 0.2°C.

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Slope = -1.021×10^{-2} min^{-1}

$k_{pseudo} = -(-1.021) \times 10^{-2}$ $min^{-1} \times 2.303) = 2.355 \times 10^{-2}$ min^{-1}

$k_{obs} = k_{pseudo}/piperidine = 2.355 \times 10^{-2}$ $min^{-1}/0.1010$ M

$K_{obs} = 0.2328$ min^{-1} M^{-1}; $k_{obs} = 0.0038$ sec^{-1} M^{-1}

Identification of 6-Piperidino-9-methoxymethylpurine

The product of the reaction of piperidine with 6-chloro-9-methoxymethylpurine is 6-piperidino-9-methoxymethylpurine. This compound, previously unknown, was identified by comparing its u.v. spectrum with that of the closely related 6-dimethylamino-9-ethylpurine. The u.v. of these two purines are very similar. 6-dimethylamino-9-ethylpurine has a λmax.= 275 millimicrons (ε = 20,600 in water).[59] 6-piperidino-9-methoxymethylpurine has a λmax. = 280 millimicrons (ε = 20,000).

Preparation of the Meisenheimer Complex of 6-(2-hydroxyethoxy)-9-methoxymethylpurine

In a 5 ml. volumetric flask 0.0426 g. (1.91×10^{-4} moles) of 6-(2-hydroxyethoxy)-9-methoxymethylpurine were dissolved in 0.25 ml. of dry t-butyl alcohol. To this solution 0.34 ml. of 0.5642 M (1.93×10^{-4} moles) of potassium t-butoxide in t-butyl alcohol were added. The solution was colorless at this point. Immediately it was transferred to a n.m.r. sample tube by means of a syringe. Dry nitrogen gas was introduced from the top of the sample tube for about 20 seconds. The n.m.r. tube lid was quickly placed and sealed with molten wax. The n.m.r. of the sample was ready to be taken.

Preparation of the Meisenheimer Complex of 6-Methoxy-9-methoxymethylpurine

In a 5 ml. volumetric flask 0.2425 g. (1.25×10^{-3} moles) of 6-

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methoxy-9-methoxymethylpurine were weighed and 0.25 ml. of dry t-butyl alcohol were added to dissolve the purine. In another 5 ml. volumetric flask, which had been previously weighed, 0.040 g. (1.25×10^{-3} moles) of dry methanol were introduced. To this flask 2.25 ml. of 0.5642 M (1.28×10^{-3} moles) of potassium t-butoxide were added. The mixture was shaken for five minutes and finally transferred to the flask containing the purine. From here on the procedure used in the case of the preparation of the Meisenheimer complex of 6-(2-hydroxyethoxy)-9-methoxymethylpurine was followed. The solution at this point was colorless.

Preparation of the Buffer Solution

In order to keep the pH and ionic strength constants during the kinetic runs of the reaction of piperidine with 6-chloro-9-methoxymethylpurine and 6-methoxy-9-methoxymethylpurine in water, a buffer solution had to be used.

Making use of the relationship $pH = pK_a + \log [B]/[BH^+]$, where $pK_a = -\log K_a$ and $K_a = [H^+][B]/[BH^+]$ or the equilibrium expression for the conjugate acid of the base,[60] [B] is the molar concentration of the base and $[BH^+]$ is the molar concentration of the conjugate acid of the base.

The pK_a for piperidine was determined by titration against $HClO_4$, and it was found to be equal to 11.25 at 25.0°C and ionic strength of 0.5 M.

In a 100 ml. volumetric flask, 10.0739 g. (0.11830 moles) of piperidine were added along with about 50 ml. of distilled water. Next, 34.23 ml. of 1.4320 N HCl were added. Keeping in mind that the final volume of the buffer solution would be 100 ml., the concentration of HCl

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would be (34.23 ml.) (1.4320 N)/(100 ml.) = 0.4901 N which would also be the concentration of piperidinium hydrochloride. The concentration of free piperidine would be (1.1830 - 0.4901) = 0.6929 M. The ionic strength was then adjusted to 0.5011 M by adding 0.0110 g. of potassium chloride.

The calculated pH was equal to 11.40. The experimental pH, measured with the pH meter, was 11.50.

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CHAPTER III

RESULTS AND DISCUSSION

The following kinetic study is an attempt to elucidate the mechanism of the nucleophilic aromatic substitution by amines. mainly piperidine, on 6-chloro-9-methoxymethylpurine. The work herein described can be divided in two broad areas: (1) solvent effects on the reaction rate constant and (2) the direct observation of the addition (Meisenheimer) complex by means of n.m.r. and u.v. spectroscopy.

The solvents used in the reaction of piperidine with 6-chloro-9-methoxymethylpurine were cyclohexane, benzene, 1,4-dioxane, water, 60 percent 1,4-dioxane - 40 percent water (by volume), dimethyl sulfoxide and anhydrous methanol.

Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine in Cyclohexane

Table 10 gives the second order rate coefficients (k_{obs}) as obtained from the pseudo-first order rate constants for the reaction of piperidine-N-h with 6-chloro-9-methoxymethylpurine in cyclohexane at 10.5°, 23.0° and 44.5°C. The maximum error in the rate constants is approximately $\pm$ 3 percent for the higher concentrations of piperidine (greater than 0.4 M) and $\pm$ 2 percent for the lower concentrations (less than 0.4 M). The plot of k_{obs} versus the piperidine concentrations is illustrated for the three experimental temperatures in Figures 4, 5 and 6.

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Table 10. Second Order Rate Constants for the Reaction of Piperidine-N-h with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M), in Cyclohexane.

Piperidine-N-h(M)	$k_{obs}(sec^{-1}M^{-1})$	1/Piperidine	$1/k_{obs}$
	10.5 ± 0.2°C		
0.0998	0.00295	10.02	338.98
0.1497	0.00411	6.68	243.30
0.1998	0.00506	5.00	197.63
0.2987	0.00746	3.34	134.04
0.3988	0.00915	2.50	109.28
0.6325	0.01185	1.58	84.38
0.7590	0.01365	1.31	73.26
0.8855	0.01481	1.13	63.52
1.0120	0.01635	0.98	61.16
1.2651	0.01820	0.79	54.94
	23.0 ± 0.2°C		
0.0250	0.00181	40.00	552.48
0.0501	0.00243	19.96	411.52
0.1010	0.00388	9.90	257.73
0.2020	0.00671	4.95	149.03
0.3030	0.00915	3.30	109.28
0.4040	0.01173	2.47	85.25
0.6325	0.01700	1.58	58.82
0.7590	0.01871	1.32	53.44
0.8855	0.02102	1.13	47.57
1.0120	0.02283	0.98	43.80
1.2651	0.02595	0.79	38.53
	44.5 ± 0.5°C		
0.0500	0.00523	20.00	191.20
0.0990	0.00710	10.10	140.84
0.1981	0.01180	5.04	84.74
0.2510	0.01341	3.98	74.57
0.2981	0.01628	3.35	61.42
0.3511	0.01820	2.84	54.94
0.3980	0.02056	2.51	48.63
0.6325	0.03033	1.58	32.97
0.7590	0.03401	1.31	29.40
0.8855	0.03750	1.13	26.66
1.0120	0.04083	0.98	24.49
1.2651	0.04470	0.79	22.37

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Table 11. Second Order Rate Constants for the Reaction of Piperidine-N-d with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M), in Cyclohexane.

Piperidine-N-d(M)	k_{obs} ($sec^{-1}M^{-1}$)	1/Piperidine	$1/k_{obs}$
	10.5 ± 0.2°C		
0.0501	0.00221	19.96	452.48
0.0960	0.00315	10.41	317.46
0.2001	0.00555	4.99	180.18
0.3011	0.00821	3.32	121.80
0.4012	0.01051	2.49	95.14
0.5503	0.01390	1.81	71.94
0.7704	0.01683	1.29	59.41
0.8805	0.01840	1.13	54.34
1.1007	0.02083	0.90	48.00
	23.0 ± 0.2°C		
0.1010	0.00406	9.90	246.30
0.2010	0.00768	4.97	130.20
0.3010	0.01061	3.32	94.25
0.4020	0.01408	2.48	71.02
0.5503	0.01176	1.81	56.62
0.7704	0.02183	1.29	45.80
0.8805	0.02346	1.13	42.62
1.1007	0.02660	0.91	37.59
	44.5 ± 0.5°C		
0.0515	0.00548	19.41	182.48
0.1030	0.00740	9.71	135.13
0.2070	0.01278	4.83	78.24
0.3100	0.01745	3.22	57.30
0.4140	0.02283	2.41	43.80
0.5503	0.02826	1.81	35.38
0.7704	0.03705	1.29	26.99
0.8805	0.04166	1.13	24.00
1.1007	0.04630	0.91	21.59

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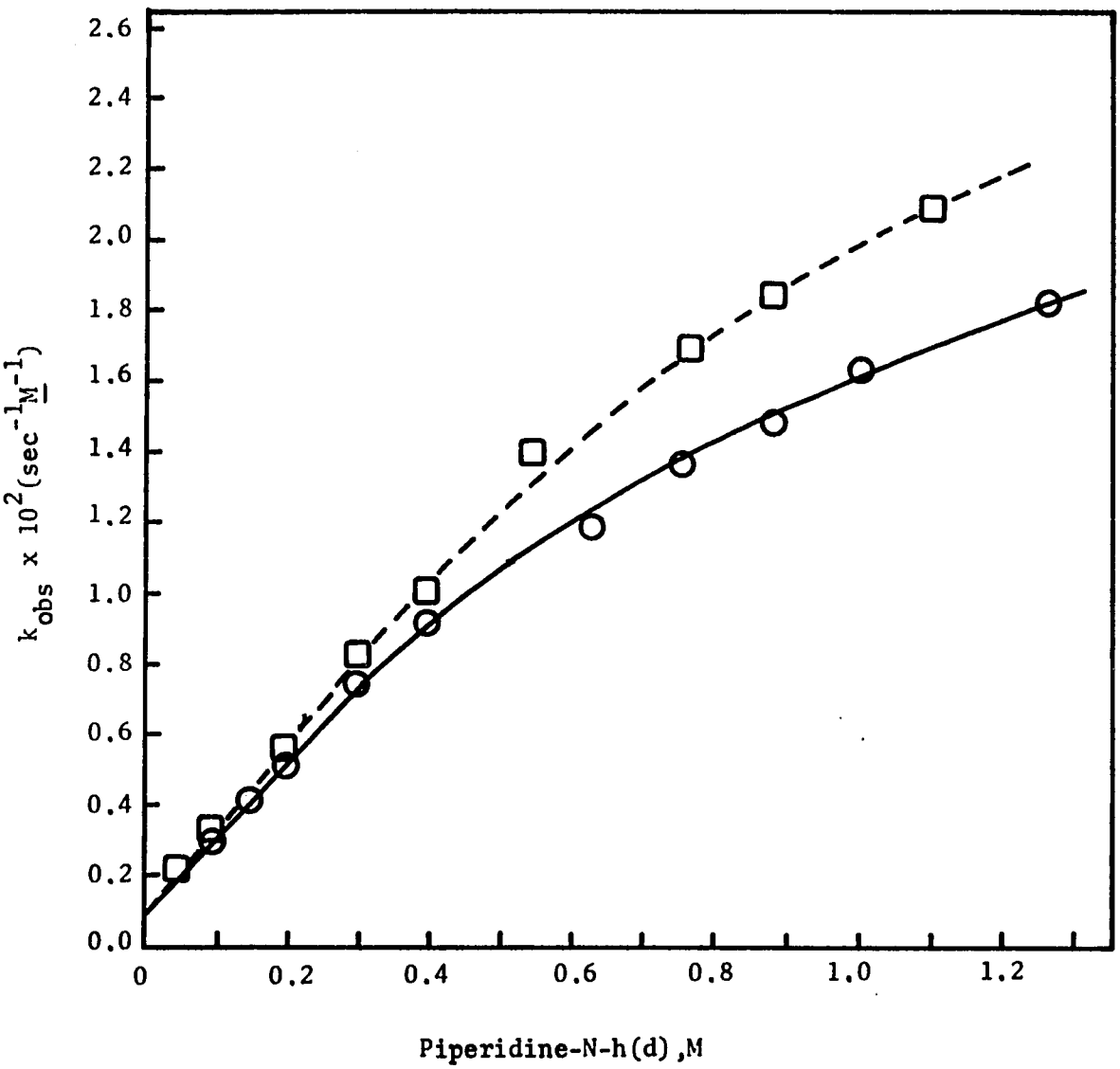

Figure 4. Piperidine-N-h(d) - Purine Reactions at 10.5°C, in Cyclohexane.

——— Piperidine-N-h

– – – – Piperidine-N-d

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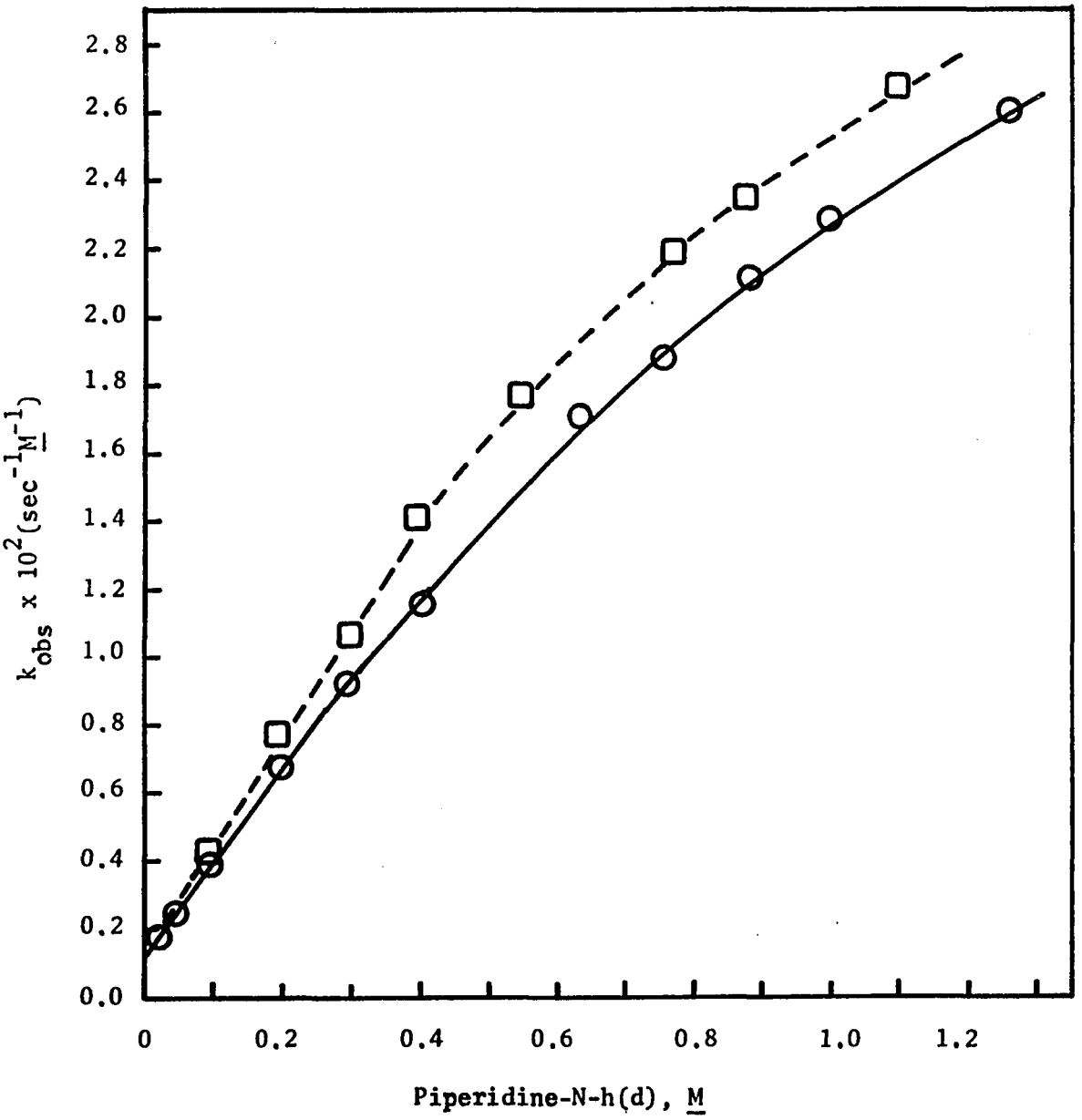

Figure 5. Piperidine-N-h(d) - Purine Reactions at 23.0°C, in Cyclohexane.

_____ **Piperidine-N-h**

_ _ _ _ _ **Piperidine-N-d**

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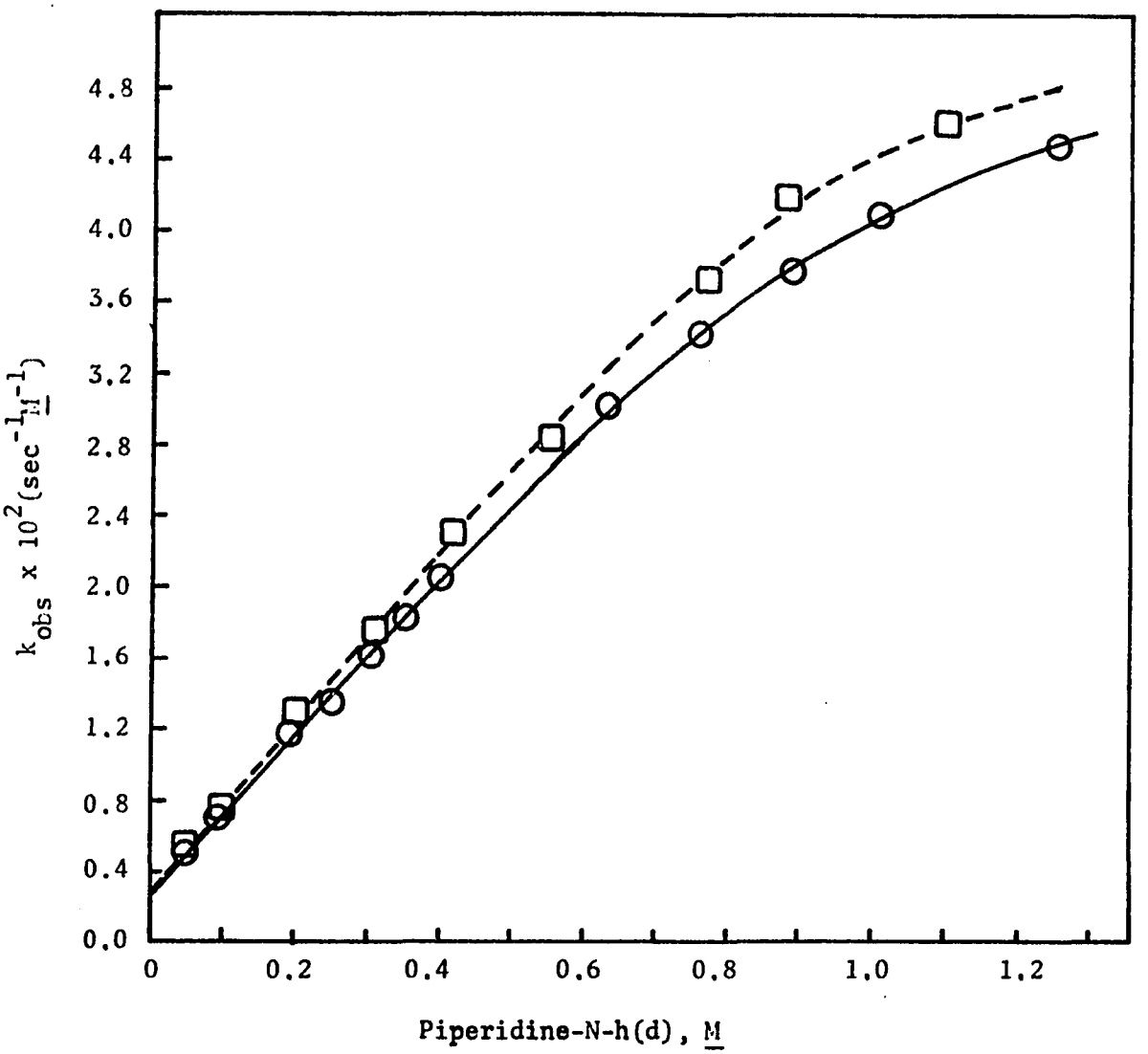

Figure 6. Piperidine-N-h(d) - Purine Reactions at 44.5°C, in Cyclohexane.

———— Piperidine-N-h

– – – – – Piperidine-N-d

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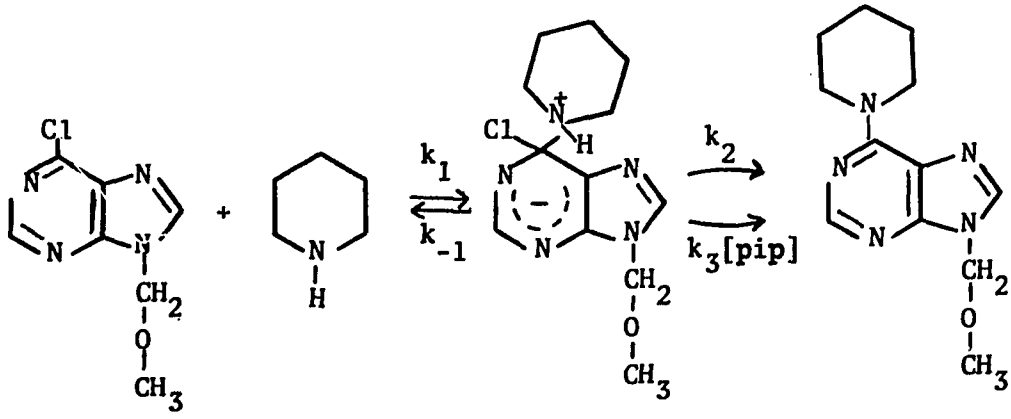
These figures also contain the plots of k_{obs} vs piperidine-N-d. The kinetic isotope effect will be discussed later.

On examination of these data, it is obvious that the second order rate constant (k_{obs}) is changing with a variation in the concentration of piperidine. Figures 4, 5 and 6 demonstrate that at low piperidine concentration there is an increasing linear dependence of the second order rate constant on the concentration of piperidine. However, at higher piperidine concentrations curvature develops. This behavior is reminiscent of the catalysis described by Bunnet[2] and other workers in the nucleophilic aromatic substitution of amines on substituted nitrobenzenes and nitronaphthalenes. This mechanism was discussed in the Introduction.

This multi-step, addition-elimination mechanism, based on the kinetic treatment of the data, also appeared most attractive for the reaction of piperidine with 6-chloro-9-methoxymethylpurine (XXVII).



XVII XVIII XXIX

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Using the steady-state approximation, the following relationship is obtained:

$$k_{obs} = \frac{k_1k_2 + k_1k_3[B]}{k_{-1} + k_2 + k_3[B]} \qquad (9)$$

where B is in this case piperidine. For the complete derivation of this relationship, see the Introduction chapter.

Now, if at low concentrations of piperidine [B] $k_{-1} >> k_2 + k_3[B]$, that is, the initial formation of the intermediate complex (XXVIII) is fast and the decomposition of it is slow then,

$$k_{obs} = \frac{k_1k_2}{k_{-1}} + \frac{k_1k_3[B]}{k_{-1}} \qquad (11)$$

A plot of k_{obs} vs the concentration of B is linear,with a slope = k_1k_3/k_{-1} and the intercept = k_1k_2/k_{-1}, i.e., the catalyzed k_3' and the uncatalyzed k_2', rate constants, respectively.

At higher concentrations of piperidine, (B), $k_{-1} \simeq k_2 + k_3[B]$, resulting in curvature of the plot. Bunnett[61] has postulated that acid catalysis is responsible for the change in the second order rate constant, k_{obs}, with increasing amine concentrations. Earlier[62] and more recent[63] reports concerning the explanation of these effects have dealt with "base" or "bifunctional" catalysis. From a linear Hammett plot[64] constructed from the catalytic rate coefficients of a series of meta and para substituted benzyl alcohols it can be shown that there is a "bifunctional" catalysis with only a small emphasis on the acidity of

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the catalyst. The ρ (rho) value obtained as +0.22.

The rate coefficients and activation data for the reaction of piperidine-N-h and -N-d with 6-chloro-9-methoxymethylpurine in cyclohexane are listed in Table 12. The data were treated by using the nulti-step, addition-elimination mechanism as proposed by Bunnett and co-workers.[2-17] The slope of the linear portion of the plot of k_{obs} vs the concentration of piperidine is the rate coefficient for the "catalyzed" reaction and the intercept is the rate coefficient for the "uncatalyzed" reaction.

Table 12. Rate and Activation Data for the Reaction of Piperidine-N-h and -N-d with 6-Chloro-9-methoxymethylpurine in Cyclohexane.

Reaction Step Considered	Rate Coefficient* 10.5°	23.0°	44.5°C	ΔE_{act} (kcal/mole)	$\Delta S^{\ddagger}$(23°C) (cal/deg/mole)
(a) Piperidine-N-h					
Catalyzed	0.0225	0.0278	0.0419	3.29 ± 0.10	-56.5 ± 1.7
Uncatalyzed	0.0007	0.0011	0.0031	8.07 ± 3.23	-46.8 ± 18.7
(b) Piperidine-N-d					
Catalyzed	0.0239	0.0327	0.0471	3.52 ± 0.10	-55.4 ± 1.6
Uncatalyzed	0.0009	0.0012	0.0028	7.59 ± 3.03	-48.1 ± 19.2

*The units of the catalyzed step are ($sec^{-1}\underline{M}^{-2}$) and the units of the uncatalyzed step are ($sec^{-1}\underline{M}^{-1}$).

The ratio of the slopes in Figures 4, 5 and 6 for the concentration range zero to 0.3 M piperidine-N-h and -N-d indicates a K_H/K_D = 0.94 ± 0.03, 0.86 ± 0.03 and 0.89 ± 0.03 at 10.5°, 23.0° and 44.5°C,

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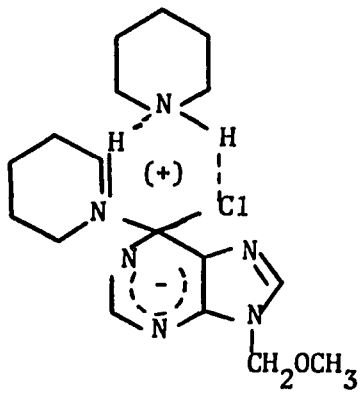
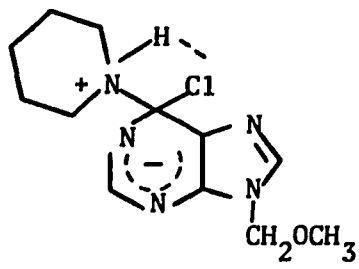
respectively. The behavior may be interpreted as a secondary deuterium isotope effect similar to that described by Bernasconi and Zollinger[65] in the study of the reaction of p-anisidine with dinitro halobenzenes in benzene solution. A K_H/K_D = 1.27 has been reported[66] for the nucleophilic displacement of the ether group in 2,4-dinitrophenyl phenyl ether by piperidine.

Perhaps a more suitable explanation lies in the possible bifunctional nature of the catalyst (piperidine) which can assist in proton and halide removal in a more or less synchronous manner (XXX).



XXX

The K_H/K_D for the "uncatalyzed" step is 1.05 ± 0.03 at 23.0°C. For all practical purposes there is no isotope effect. The transition state could be depicted as a four-center one, such as (XXXI).



XXXI

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This explanation has been suggested by Bernasconi and Zolliner[27] and Pietra[67] in their nucleophilic aromatic substitution studies in benzene solution.

Inverting both sides of equation (9) we obtain

$$\frac{1}{k_{obs}} = \frac{k_{-1} + k_2 + k_3[B]}{k_1k_2 + k_1k_3[B]} \tag{14}$$

or

$$\frac{1}{k_{obs}} = \frac{k_{-1}}{k_1k_2 + k_1k_3[B]} + \frac{k_2 + k_3[B]}{k_1k_2 + k_1k_3[B]} \tag{15}$$

Now, if $k_1k_3[B] >> k_1k_2$ (at high concentrations of piperidine), then

$$\frac{1}{k_{obs}} = \frac{k_{-1}}{k_1k_3}\left[\frac{1}{[B]}\right] + \frac{1}{k_1} \tag{16}$$

Therefore, a plot of $1/k_{obs}$ versus 1/[piperidine] gives a linear plot of slope = k_{-1}/k_1k_3 and intercept = $1/k_1$. The value of k_1 for the reaction of piperidine and 6-chloro-9-methoxymethylpurine can be extracted from this relationship.

Table 13. Values of k_1 for the Reaction of Piperidine-N-h and -N-d with 6-Chloro-9-methoxymethylpurine in Cyclohexane.

Amine	10.5°C	$k_1(sec^{-1}M^{-1})$ 23.0°C	44.5°C
Piperidine-N-h	0.0417 ± 0.0166	0.0548 ± 0.0219	0.0874 ± 0.0349
Piperidine-N-d	0.0403 ± 0.0161	0.0536 ± 0.0214	0.0860 ± 0.0344

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The error in k_1 is particularly large (40 percent) since a small variation in the slope produces a significant change in the intercept. However, the values for $k_{1(H)}$ and $k_{1(D)}$ appear to be the same, within experimental error, for the respective temperatures.

Table 14. Activation Data for k_1 in the Reaction of Piperidine-N-h and -N-d with 6-Chloro-9-methoxymethylpurine in Cyclohexane.

Amine	ΔE_{act} (kcal/mole)	$\Delta S^{\ddagger}_{act}$(23.0°C) (cal/deg/mole)
Piperidine-N-h	3.88 ± 1.55	-53.16 ± 21.26
Piperidine-N-h	3.97 ± 1.59	-52.90 ± 21.16

Again, the error in E_{act} and $\Delta S^{\ddagger}_{act}$ are large since these quantities were calculated using the values of k_1. However, the values of E_{act} and $\Delta S^{\ddagger}_{act}$ for the N-protio and N-deutero piperidine are, within experimental error, the same.

Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine in Benzene

Piperidine-N-h and -N-d were reacted with 6-chloro-9-methoxymethylpurine using benzene as the solvent. Tables 15 and 16 list the second order rate coefficients (k_{obs}) as obtained from the pseudo-first order rate constats at 23.0°, 32.0° and 44.5°C. Figures 7, 8 and 9 are a plot of the data.

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Table 15. Second Order Rate Constants for the Reaction of Piperidine-N-h with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in Benzene.

Piperidine-N-h(M)	$k_{obs}(sec^{-1}M^{-1})$	1/Piperidine	$1/k_{obs}$
	23.0 ± 0.2°C		
0.0674	0.00251	14.83	398.40
0.1349	0.00306	7.41	326.79
0.2698	0.00400	3.70	250.00
0.3777	0.00475	2.64	210.52
0.5281	0.00586	1.89	170.64
0.6175	0.00634	1.62	157.72
0.7410	0.00691	1.35	144.71
0.8646	0.00767	1.15	130.37
0.9881	0.00818	1.01	122.24
1.2351	0.00926	0.81	107.99
	32.0 ± 0.2°C		
0.0674	0.00471	14.83	212.31
0.1349	0.00554	7.41	180.50
0.2698	0.00671	3.71	149.03
0.3777	0.00786	2.64	127.22
0.5396	0.00935	1.85	106.95
0.6175	0.00975	1.62	102.56
0.7410	0.01060	1.35	94.33
0.8646	0.01140	1.15	87.72
0.9881	0.01208	1.01	82.78
1.2351	0.01300	0.81	76.92
	44.5 ± 0.5°C		
0.0674	0.00935	14.83	106.95
0.1349	0.01037	7.41	96.43
0.2698	0.01210	3.70	82.64
0.3777	0.01382	2.64	72.35
0.5396	0.01600	1.85	62.50
0.6175	0.01670	1.62	59.88
0.7410	0.01850	1.35	54.05
0.8646	0.01950	1.15	51.28
0.9881	0.02033	1.01	49.18
1.2351	0.02200	0.81	45.45

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Table 16. Second Order Rate Constants for the Reaction of Piperidine-N-d with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in Benzene.

Piperidine-N-d(M)	k_{obs} ($sec^{-1}M^{-1}$)	1/Piperidine	$1/k_{obs}$
	23.0 ± 0.2°C		
0.0895	0.00301	11.17	332.22
0.1790	0.00362	5.58	276.24
0.2506	0.00442	3.99	226.24
0.3580	0.00510	2.80	196.07
0.5307	0.00606	1.88	165.01
0.7430	0.00725	1.34	137.93
0.8400	0.00776	1.19	128.66
1.0615	0.00878	0.94	113.89
	32.0 ± 0.2°C		
0.0895	0.00517	11.17	193.42
0.1790	0.00610	5.58	163.93
0.2506	0.00680	3.99	147.05
0.3580	0.00813	2.79	123.00
0.5307	0.00952	1.88	105.04
0.7430	0.01110	1.34	90.09
0.8400	0.01170	1.19	85.47
1.0615	0.01270	0.94	78.74
	44.5 ± 0.5°C		
0.0895	0.00975	11.17	102.56
0.1790	0.01125	5.58	88.88
0.2506	0.01233	3.99	81.10
0.3580	0.01378	2.79	72.56
0.5307	0.01635	1.88	61.16
0.7430	0.01893	1.34	52.82
0.8400	0.01984	1.19	50.40
1.0615	0.02142	0.94	46.68

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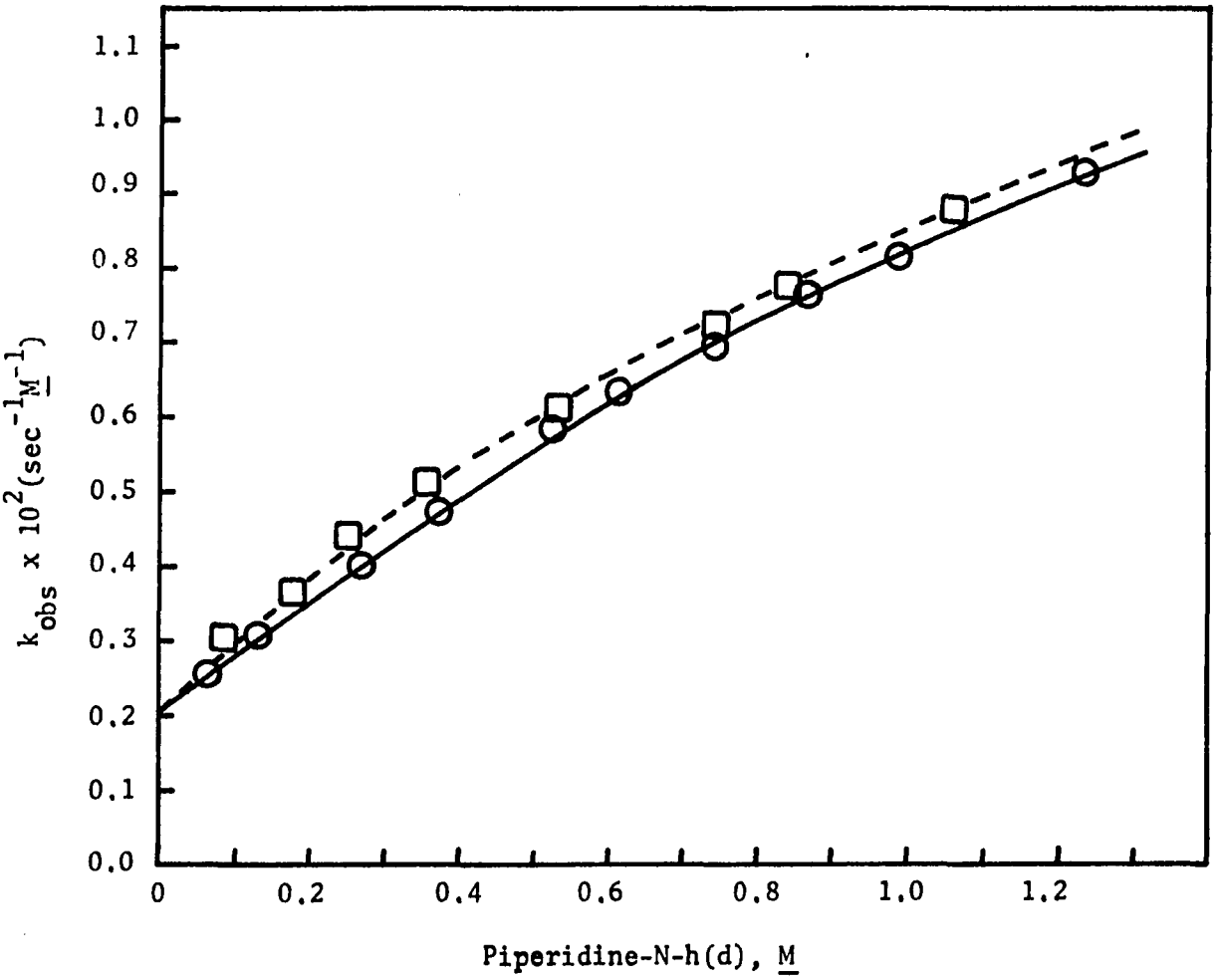

Figure 7. Piperidine-N-h(d) - Purine Reactions at 23.0°C, in Benzene.

——— Piperidine-N-h

– – – Piperidine-N-d

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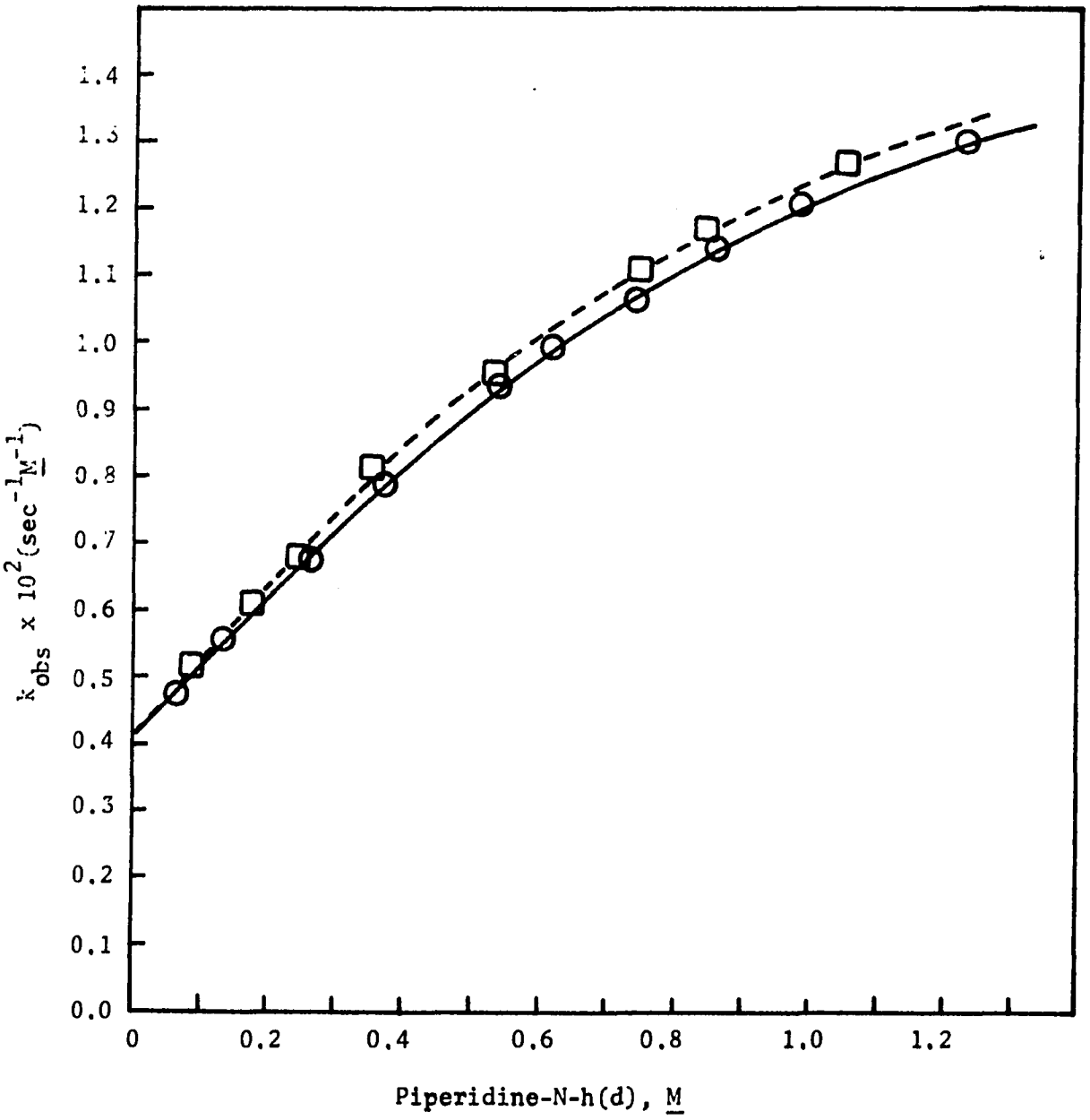

Figure 8. Piperidine-N-h(d) - Purine Reactions at 32.0°C, in Benzene.

——— Piperidine-N-h

— — — Piperidine-N-d

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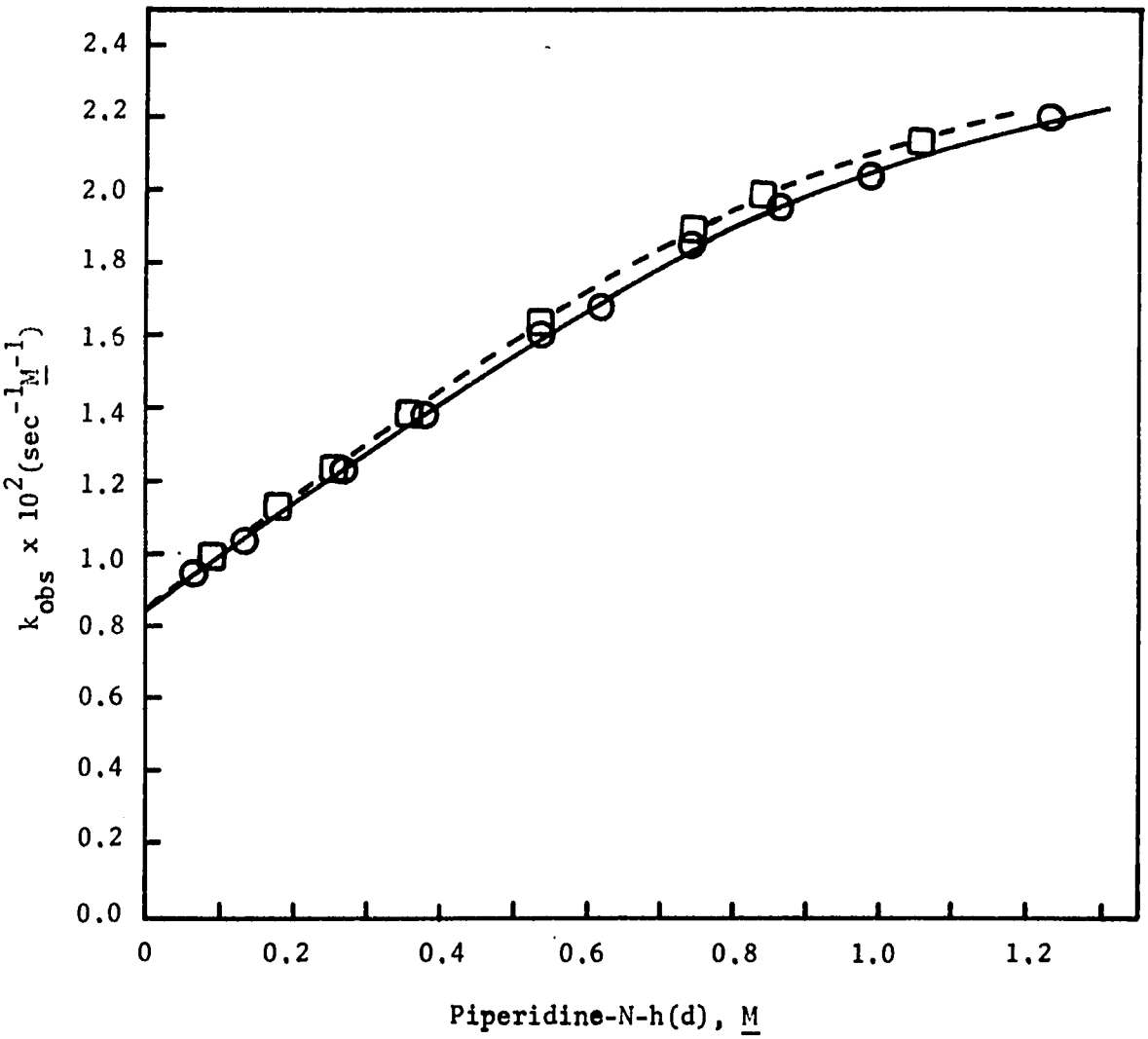

Figure 9. Piperidine-N-h(d) - Purine Reactions at 44.5°C, in Benzene.

——— Piperidine-N-h

– – – Piperidine-N-d

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On examination of these data, it can be seen that the second-order rate coefficient (k_{obs}) is changing with a variation in the concentration of the amine. This behavior is just like the one previously discussed for the same reaction but using cyclohexane as the solvent.

Table 17. Rate and Activation Data for the Reaction of Piperidine-N-h and -N-d with 6-Chloro-9-methoxymethylpurine in Benzene.

Reaction Step Considered	Rate Coefficient* 23.0°	32.0°	44.5°C	ΔE_{act} (kcal/mole)	$\Delta S^{\ddagger}$(23°C) (cal/deg/mole)
(a) Piperidine-N-h					
Catalyzed	0.0071	0.0099	0.0141	5.49 ± 0.16	-51.8 ± 1.5
Uncatalyzed	0.0025	0.0041	0.0084	10.29 ± 4.11	-37.6 ± 15.0
(b) Piperidine-N-d					
Catalyzed	0.0080	0.0101	0.0149	5.56 ± 0.16	-51.3 ± 1.5
Uncatalyzed	0.0022	0.0042	0.0084	10.98 ± 4.39	-35.5 ± 14.2

*The units of the catalyzed step are ($sec^{-1}\underline{M}^{-2}$) and the units of the uncatalyzed step are ($sec^{-1}\underline{M}^{-1}$).

The rate coefficients and activation data for the reaction of piperidine-N-h and -N-d with 6-chloro-9-methoxymethylpurine in benzene are listed in Table 17. The data were treated by using the multi-step, addition-elimination mechanism as applied before to the same reaction in the solvent cyclohexane. The rate coefficients, ΔE_{act} and $\Delta S^{\ddagger}_{act}$ for the reaction are of comparable magnitudes in both solvents, benzene and cyclohexane.

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The ratio of the slopes in Figure 7, 8 and 9 for the concentration range 0 to 0.3 M piperidine-N-h and -N-d indicates a K_H/K_D = 0.89 ± 0.03, 0.97 ± 0.03 and 0.95 ± 0.03 at 23.0°, 32.0°and 44.5°C.

Inverse plots ($1/k_{obs}$ vs 1/piperidine) give a line with slope = k_{-1}/k_1k_3 and intercept = $1/k_1$. The values of k_1 for the reaction of piperidine-N-h and -N-d with 6-chloro-9-methoxymethylpurine in benzene were calculated this way and are listed in Table 18.

Table 18. Values of k_1 for the Reaction of Piperidine-N-h and -N-d with 6-Chloro-9-methoxymethylpurine in Benzene.

Amine	$k_1(sec^{-1}M^{-1})$		
	23.0°C	32.0°C	44.5°C
Piperidine-N-h	0.0183±0.0073	0.0195±0.0078	0.0317±0.0126
Piperidine-N-d	0.0174±0.0069	0.0192±0.0076	0.0310±0.0124

The values for $k_{1(H)}$ and $k_{1(D)}$ are the same, within experimental error, for the respective temperatures.

The activation energy, ΔE_{act}, and the activation entropy, $\Delta S^{\ddagger}_{act}$, calculated using the values of k_1 listed in Table 18, are shown in Table 19. The similarity of all of the kinetic data for the reaction of piperidine-N-h and -N-d with 6-chloro-9-methoxymethylpurine in benzene and in cyclohexane indicates that the same reaction mechanism is operating in both solvents. The ratio of (catalyzed/uncatalyzed) reaction in cyclohexane to that in benzene is 9.3, for piperidine-N-h at 23.0°C.

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Table 19. Activation Data for k_1 in the Reaction of Piperidine-N-h and -N-d with 6-Chloro-9-methoxymethylpurine in Benzene.

Amine	ΔE_{act} (kcal/mole)	$\Delta S^{\ddagger}_{act}$(23.0°C) (cal/deg/mole)
Piperidine-N-h	4.87 ±1.95	-51.98 ± 20.79
Piperidine-N-d	5.02 ±2.04	-51.32 ± 20.53

Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine

1,4-Dioxane

Piperidine-N-h and -N-d were reacted with 6-chloro-9-methoxymethylpurine using 1,4-dioxane as the solvent. Tables 20 and 21 give the second order rate constants (k_{obs}) as obtained from the pseudo-first order rate constants. Figure 10, 11 and 12 are a graphical representation of these data, at 23.0°, 32.0° and 44.5°C.

It is seen from these results that at 23.0° there is a small increase in k_{obs} with an increase in the amine concentration from 0 to about 0.4 M, i.e., the reaction is slightly catalyzed by the amine. See Figure 10.

From a plot of $1/k_{obs}$ vs 1/piperidine, k_1 can be obtained. At 23.0°C it has a value of 0.0096 $sec^{-1}M^{-1}$ for piperidine-N-h and 0.0104 $sec^{-1}M^{-1}$ for piperidine-N-d. The value of $K_{1(H)}/K_{1(D)}$ is equal to 0.96 and it is within experimental error of 1.0.

At 32.0° and 44.5°C there is no catalysis by piperidine-N-h and

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Table 20. Second Order Rate Constants for the Reaction of Piperidine-N-h with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in 1,4-Dioxane.

Piperidine-N-h	k_{obs} ($sec^{-1}M^{-1}$)	1/Piperidine	$1/k_{obs}$
	23.0 ± 0.2°C		
0.0495	0.00731	20.20	136.79
0.1003	0.00758	9.97	131.92
0.1490	0.00785	6.71	127.38
0.2005	0.00821	4.98	121.80
0.2490	0.00833	4.01	120.04
0.2998	0.00870	3.33	114.95
0.3995	0.00895	2.50	111.73
0.6239	0.00908	1.60	110.13
0.7487	0.00918	1.33	108.93
0.8735	0.00916	1.14	109.17
0.9983	0.00930	1.00	107.52
1.2479	0.00930	0.80	107.52
	32.0 ± 0.2°C		
0.0510	0.01165	19.60	85.83
0.1020	0.01183	9.80	84.53
0.2056	0.01184	4.86	84.45
0.3070	0.01184	3.25	84.45
0.4303	0.01200	2.32	83.33
0.6239	0.01197	1.60	83.54
0.7487	0.01204	1.33	83.05
0.8735	0.01201	1.14	83.26
0.9983	0.01200	1.00	83.33
1.2479	0.01200	0.80	83.33
	44.5 ± 0.5°C		
0.0490	0.02200	20.40	45.45
0.0905	0.02210	11.04	45.24
0.2010	0.02216	4.97	45.12
0.2702	0.02233	3.70	44.78
0.3602	0.02241	2.77	44.62
0.6239	0.02227	1.60	44.90
0.7487	0.02220	1.33	45.04
0.8735	0.02242	1.14	44.60
0.9983	0.02243	1.00	44.58
1.2479	0.02252	0.80	44.40

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Table 21. Second Order Rate Constants for the Reaction of Piperidine-N-d with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in 1,4-Dioxane.

Piperidine-N-d	k_{obs} ($sec^{-1}M^{-1}$)	1/Piperidine	$1/k_{obs}$
	23.0 ± 0.2°C		
0.0502	0.00859	19.92	116.41
0.1002	0.00872	9.98	114.67
0.1480	0.00893	6.75	111.98
0.2001	0.00915	4.99	109.28
0.2490	0.00943	4.01	106.04
0.3002	0.00960	3.33	104.16
0.4004	0.00975	2.49	102.56
0.5070	0.01001	1.97	99.90
0.7098	0.01010	1.41	99.90
0.8112	0.01021	1.23	97.94
1.0140	0.01020	0.98	98.03
	32.0 ± 0.2°C		
0.0490	0.01136	20.40	88.02
0.1002	0.01168	9.98	85.61
0.2004	0.01165	4.99	85.83
0.3005	0.01203	3.32	83.12
0.4100	0.01207	2.44	82.85
0.5070	0.01207	1.97	82.85
0.7098	0.01223	1.41	81.76
0.8112	0.01231	1.23	81.23
1.0140	0.01236	0.98	80.90
	44.5 ± 0.5°C		
0.1004	0.02136	9.96	46.81
0.2010	0.02158	4.97	46.33
0.3010	0.02163	3.32	46.23
0.4010	0.02196	2.49	45.53
0.5070	0.02193	1.97	45.59
0.7098	0.02158	1.40	46.33
0.8112	0.02193	1.23	45.59
1.0140	0.02216	0.98	45.12

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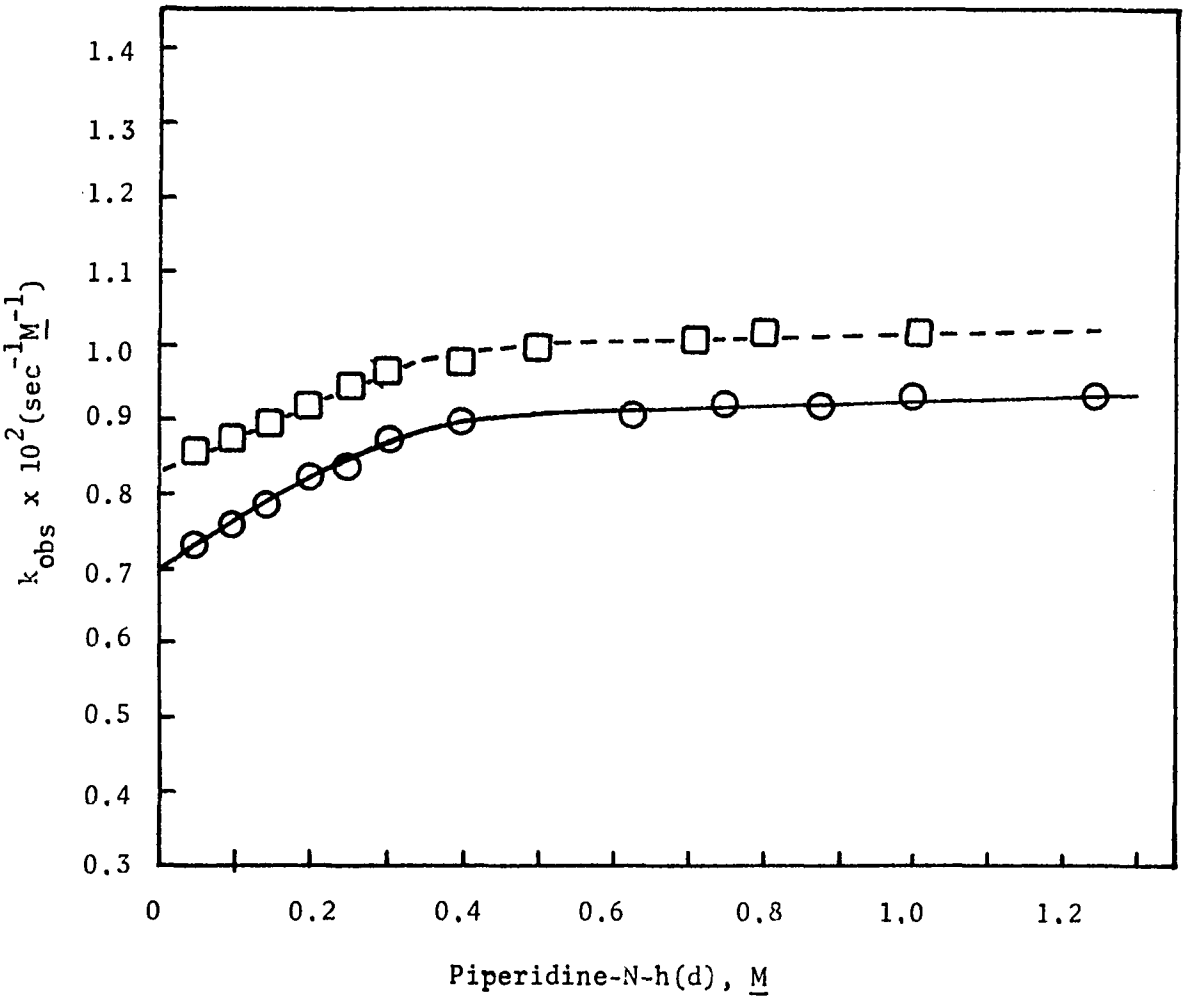

Figure 10. Piperidine-N-h(d) - Purine Reactions at 23.0°C, in 1,4-Dioxane.

——— Piperidine-N-h

— — — Piperidine-N-d

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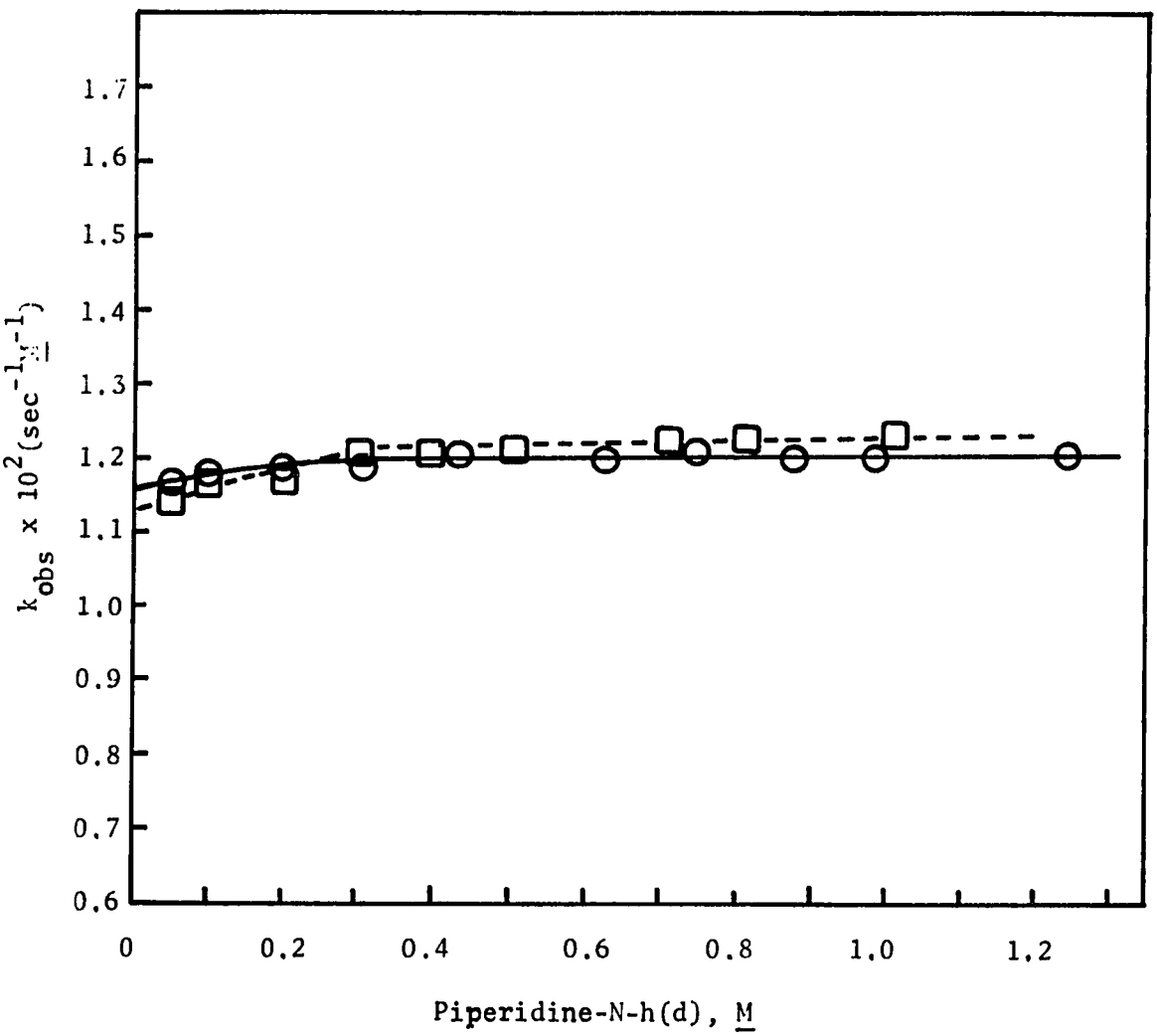

Figure 11. Piperidine-N-h(d) - Purine Reactions at 32.0°C, in 1,4-Dioxane.

——— Piperidine-N-h

— — — Piperidine-N-d

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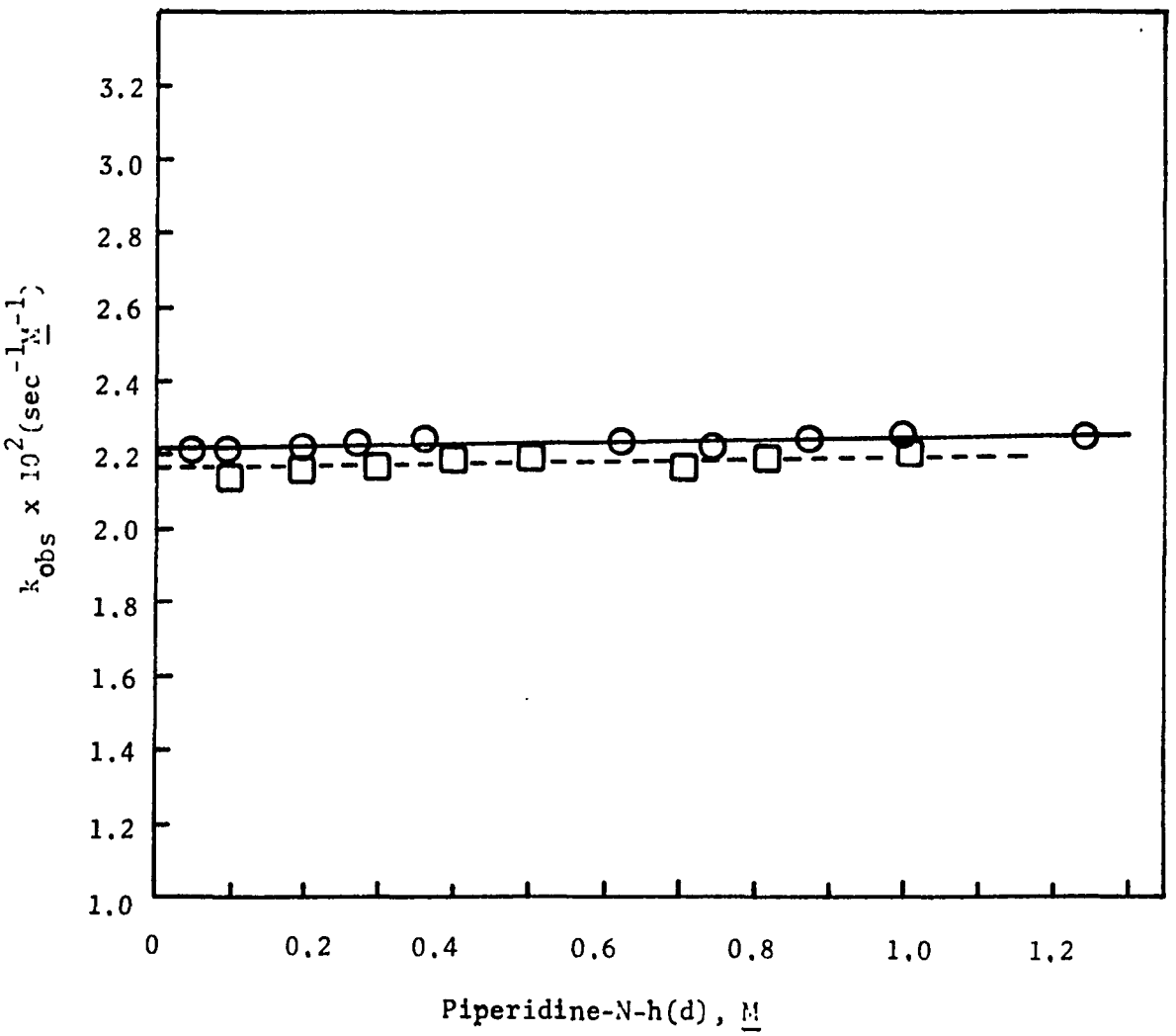

Figure 12. Piperidine-N-h(d) - Purine Reactions at 44.5°C, in 1,4-Dioxane.

——— Piperidine-N-h

— — — Piperidine-N-d

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-N-d. The rate constants, for all concentrations of the amine studied, at either one of these two temperatures are well within the experimental error (3 percent). Therefore, the second order rate coefficient (k_{obs}) is no longer dependent on the concentration of piperidine.

Although the dielectric constant of 1,4-dioxane corresponds almost exactly to that of benzene, 2.21 and 2.27 at 25.0°C respectively, the ether possess a bonding dipole between the carbon and oxygen. The C-O bonding dipole can interact with the transition state of the reaction. This seems to be the case in the reaction of piperidine with 6-chloro-9-methoxymethylpurine in 1,4-dioxane. At 0.5 M piperidine the second order rate coefficient for the reaction in benzene is 0.009 $sec^{-1}M^{-1}$ at 32.0°C, and the rate constant is dependent on the concentration of piperidine. In 1,4-dioxane at the same concentration of the amine, the rate constant is 0.0120 $sec^{-1}M^{-1}$, at the same temperature, but there is no catalysis by piperidine.

It is interesting to note that at 23.0°C piperidine-N-d reacts a little faster with 6-chloro-9-methoxymethylpurine than piperidine-N-h, for all concentrations studied, as shown in Figure 10. This is also the case when the reaction is run at 32.0°C, see Figure 11, but only from 0 to about 0.25 M piperidine. From 0.25 to 1.3 M piperidine-N-h reacts a little faster than piperidine-N-d. At 44.5°C, see Figure 12, piperidine-N-h reacts faster than piperidine-N-d for all concentrations of the amine studied.

The K_H/K_D is 0.89 ± 0.03, 1.01 ± 0.03 and 1.01 ± 0.03 at 23.0°, 32.0° and 44.5°C, respectively.

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Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine in Water, 60 Percent 1,4-Dioxane - 40 Percent Water (v/v), Dimethyl Sulfoxide and Methanol

In this section the kinetics of the reaction of piperidine with 6-chloro-9-methoxymethylpurine in the solvents water, 60 percent 1,4-dioxane - 40 percent (v/v), dimethyl sulfoxide and methanol will be discussed together because, as it turned out, in these four solvents there is no catalysis by piperidine, i.e., the second order rate constant (k_{obs}) is independent of the concentration of the amine.

Tables 22, 23, 24 and 25 give k_{obs} as obtained from the pseudo-first order rate constants for the reactions at 25.0°, 32.0°and 44.5°C. The experimental error in the rate constants is approximately 5 percent.

A plot of the second order rate constants versus the dielectric constant of the solvents, see Figure 13, shows that k_{obs} is directly related to the dielectric of the reaction medium. In the case of pure solvents, e.g., water, methanol and dimethyl sulfoxide the points fall nicely on a straight line. In the case of a mixed solvent, such as 60 percent 1,4-dioxane - 40 percent water (v/v/), the point is off the line. This could be due to an increase solvating power of the reaction medium by the interaction of piperidine with water as shown by the following equation:

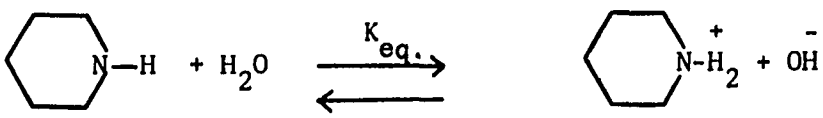

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Table 22. Second Order Rate Constants for the Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in Water (pH = 11.40, ionic strength = 0.5011 M).

Piperidine(M)	$k_{obs}(sec^{-1}M^{-1})$
25.0 ± 0.2°C	
0.0433	0.1878
0.0866	0.1851
0.1732	0.1885
0.3464	0.1901
32.0 ± 0.2°C	
0.0433	0.2668
0.0866	0.2651
0.1177	0.2683
0.1732	0.2700
44.5 ± 0.5°C	
0.0433	0.4891
0.0866	0.4928
0.1177	0.4908
0.1732	0.4940

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Table 23. Second Order Rate Constants for the Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in 60 Percent 1,4-Dioxane - 40 Percent Water (v/v).

Piperidine(M)	$k_{obs}(sec^{-1}M^{-1})$
25.0 ± 0.2°C	
0.0704	0.0551
0.1354	0.0597
0.2708	0.0576
0.5416	0.0581
32.0 ± 0.2°C	
0.0634	0.0748
0.1268	0.0783
0.1902	0.0799
0.3805	0.0738
44.5 ± 0.5°C	
0.0634	0.1246
0.1268	0.1233
0.1902	0.1261
0.3805	0.1222

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Table 24. Second Order Rate Constants for the Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in Dimethyl Sulfoxide.

Piperidine(M)	$k_{obs}(sec^{-1}M^{-1})$
25.0 ± 0.2°C	
0.0501	0.0597
0.1001	0.0598
0.2002	0.0604
0.3003	0.0598
0.4004	0.0597
32.0 ± 0.2°C	
0.0501	0.0732
0.1001	0.0718
0.2002	0.0738
0.3003	0.0718
44.5 ± 0.5°C	
0.0501	0.1156
0.1001	0.1177
0.2002	0.1172
0.3003	0.1155

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Table 25. Second Order Rate Constants for the Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in Anhydrous Methanol.

Piperidine(M)	$k_{obs}(sec^{-1}M^{-1})$
25.0 ± 0.2°C	
0.0572	0.0185
0.1002	0.0185
0.1718	0.0186
0.2864	0.0188
32.0 ± 0.2°C	
0.0574	0.0265
0.1002	0.0263
0.1719	0.0271
0.2864	0.0269
44.5 ± 0.5°C	
0.0572	0.0508
0.1004	0.0531
0.1718	0.0524
0.2864	0.0510

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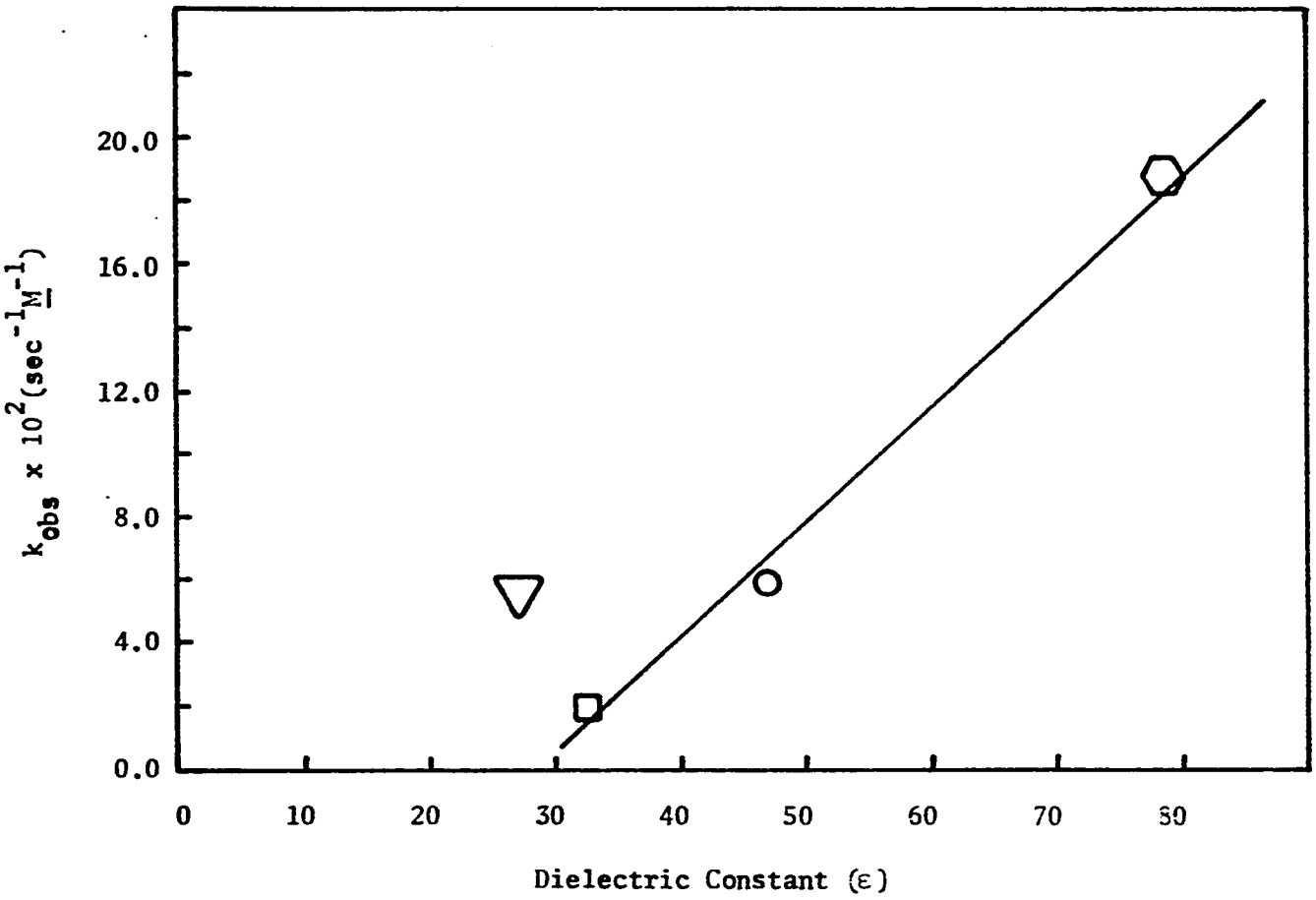

Figure 13. Rate Constants as a Function of the Dielectric Constant of the Solvent for the Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine at 25.0°C.

⬡ ....Water
□ ....Methanol
○ ....Dimethyl Sulfoxide
▽ ....60 Percent Dioxane 40 Percent Water (v/v).

Table 26. Rate Constants for the Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine at 25,0°C, and the Dielectric Constant of the Solvents.

Solvent	Dielectric Constant(ε)	k_{obs} ($sec^{-1}\underline{M}^{-1}$)
60 Percent 1,4-Dioxane-40 Percent Water (v/v)	27.21 (70)	0.0566
Methanol	32.63 (69)	0.0186
Dimethyl Sulfoxide	46.40 (71)	0.0596
Water	78.50 (69)	0.1883

The equilibrium seems to lie far to the left when the solvent is 60 percent 1,4-dioxane - 40 percent water (v/v). According to Bunnett[63] this equilibrium is negligible. The fact that k_{obs} increases as the dielectric constant of the reaction medium increases is in agreement with the idea that the reaction of piperidine with 6-chloro-9-methoxymethylpurine goes through a charged intermediate-complex species. Solvents with a greater ability to stabilize this intermediate should accelerate the reaction rate.

Table 27 is a list of the activation data for the reaction of piperidine with 6-chloro-9-methoxymethylpurine in the four solvents just discussed.

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Table 27. Activation Data for the Reaction of Piperidine with 6-Chloro-9-methoxymethylpurine in Water, 60 Percent 1,4-Dioxane - 40 Percent Water, Dimethyl Sulfoxide and Methanol.

Solvent	ΔE_{act} (kcal/mole)	ΔS_{act}(25.0°C) (cal/deg/mole)
Water	9.49 ± 0.47	-31.98 ± 1.59
60 Percent Dioxane-40 Percent Water	7.72 ± 0.38	-40.28 ± 2.01
Dimethyl Sulfoxide	6.71 ± 0.33	-43.61 ± 2.18
Methanol	9.98 ± 0.49	-34.93 ± 1.74

Reaction of 6-Chloro-9-methoxymethylpurine with Pyrrolidine and Morpholine in Cyclohexane

Reactions of 6-chloro-9-methoxymethylpurine with pyrrolidine and morpholine, in cyclohexane, were carried out separately, under the same experimental conditions used in the case of piperidine. The purpose of these experiments was to obtain a relationship between the second order rate constant (k_{obs}) and the nucleophile effecting the attack on the chloropurine.

Tables 28 and 29 list the second order rate coefficients (k_{obs}) as obtained from the pseudo-first order rate constants for the reaction of 6-chloro-9-methoxymethylpurine with pyrrolidine and morpholine in cyclohexane, respectively. The maximum error in the rate constants is approximately ± 3 percent. The plots of k_{obs} vs each of the two amine

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Table 28. Second Order Rate Constants for the Reaction of Pyrrolidine with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in Cyclohexane.

Pyrrolidine(M)	k_{obs} ($sec^{-1}M^{-1}$)
23.0 ± 0.2°C	
0.0475	0.0148
0.0951	0.0244
0.1902	0.0409
0.3604	0.0715
32,0 ± 0.2°C	
0.0445	0.0177
0.0951	0.0283
0.1902	0.0459
0.4100	0.0845
44.5 ± 0.5°C	
0.0301	0.0191
0.0603	0.0286
0.1207	0.0472
0.2414	0.0851

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Table 29. Second Order Rate Constants for the Reaction of Morpholine with 6-Chloro-9-methoxymethylpurine (5×10^{-5} M) in Cyclohexane.

Morpholine(M)	$k_{obs}(sec^{-1}M^{-1})$
23.0 ± 0.2°C	
0.0824	0.00049
0.1648	0.00082
0.3297	0.00139
0.6595	0.00262
32.0 ± 0.2°C	
0.0643	0.00052
0.1287	0.00090
0.2574	0.00157
0.4750	0.00275
44.5 ± 0.5°C	
0.0824	0.00090
0.1648	0.00152
0.3297	0.00268
0.6595	0.00522

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concentrations is illustrated, for the three experimental temperatures, in Figures 14 to 19.

On examination of these data, it is obvious that the second order rate coefficient (k_{obs}) is changing with a variation in the concentration of the amines, just like in the case of piperidine. The multistep, addition-elimination mechanism proposed for the reaction of piperidine with 6-chloro-9-methoxymethylpurine in non-polar aprotic solvents, such as cyclohexane and benzene, seems to be taking place here too.

Making a comparison of the rate constants for the reaction of 6-chloro-9-methoxymethylpurine with piperidine, morpholine and pyrrolidine, in cyclohexane (at 0.2 M concentration of amine and at 23.0°C) the order of reactivity of the amine used is pyrrolidine > piperidine > morpholine. Table 30 shows $\log(k_{obs})$ of these amines and their respective pK_b's. It is true that pK_b's are only strictly valid in aqueous solutions, nevertheless they can give a rough approximation of the order of the nucleophilicity of the amines in non-aqueous solvents if nucleophilicity is important in the reaction.

From Table 30 we can see that k_{obs} is larger for pyrrolidine and smaller for morpholine, with piperidine having an intermediate value. This is the same order followed by their respective pK_b's. Data in Table 30 was plotted and is shown in Figure 20.

The rate coefficients and activation data for the reaction of 6-chloro-9-methoxymethylpurine with pyrrolidine and morpholine in cyclohexane are listed in Table 31. The data were treated by using the multistep, addition-elimination mechanism discussed previously.

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Table 30. $Log(k_{obs})$ for the Reaction of 6-Chloro-9-methoxymethylpurine with Different Nucleophiles in Cyclohexane, at 23.0°C, and pK_b's of the Nucleophiles.

Nucleophile (0.2 M)	$Log(k_{obs})$	pK_b (72)
Morpholine	-3.0222	5.67
Piperidine	-2.1739	2.88
Pyrrolidine	-1.3615	2.73

Table 31. Rate and Activation Data for the Reaction of 6-Chloro-9-methoxymethylpurine with Pyrrolidine and Morpholine in Cyclohexane.

Reaction Step Considered	Rate Coefficient* 23.0°	32.0°	44.5°C	ΔE_{act} (kcal/mole)	$\Delta S^{\ddagger}_{act}$(23.0°C) (cal/deg/mole)
(a) Pyrrolidine					
Catalyzed	0.18021	0.21880	0.31394	4.85 ± 0.14	-47.53 ± 1.42
Uncatalyzed	0.00656	0.00899	0.00975	3.38 ± 0.10	-59.08 ± 1.77
(b) Morpholine					
Catalyzed	0.00362	0.00543	0.00746	6.20 ± 0.18	-50.73 ± 1.50
Uncatalyzed	0.00018	0.00021	0.00024	9.91 ± 0.29	-43.80 ± 1.31

*The units of the catalyzed step are $(sec^{-1}M^{-2})$ and the units of the uncatalyzed step are $(sec^{-1}M^{-1})$.

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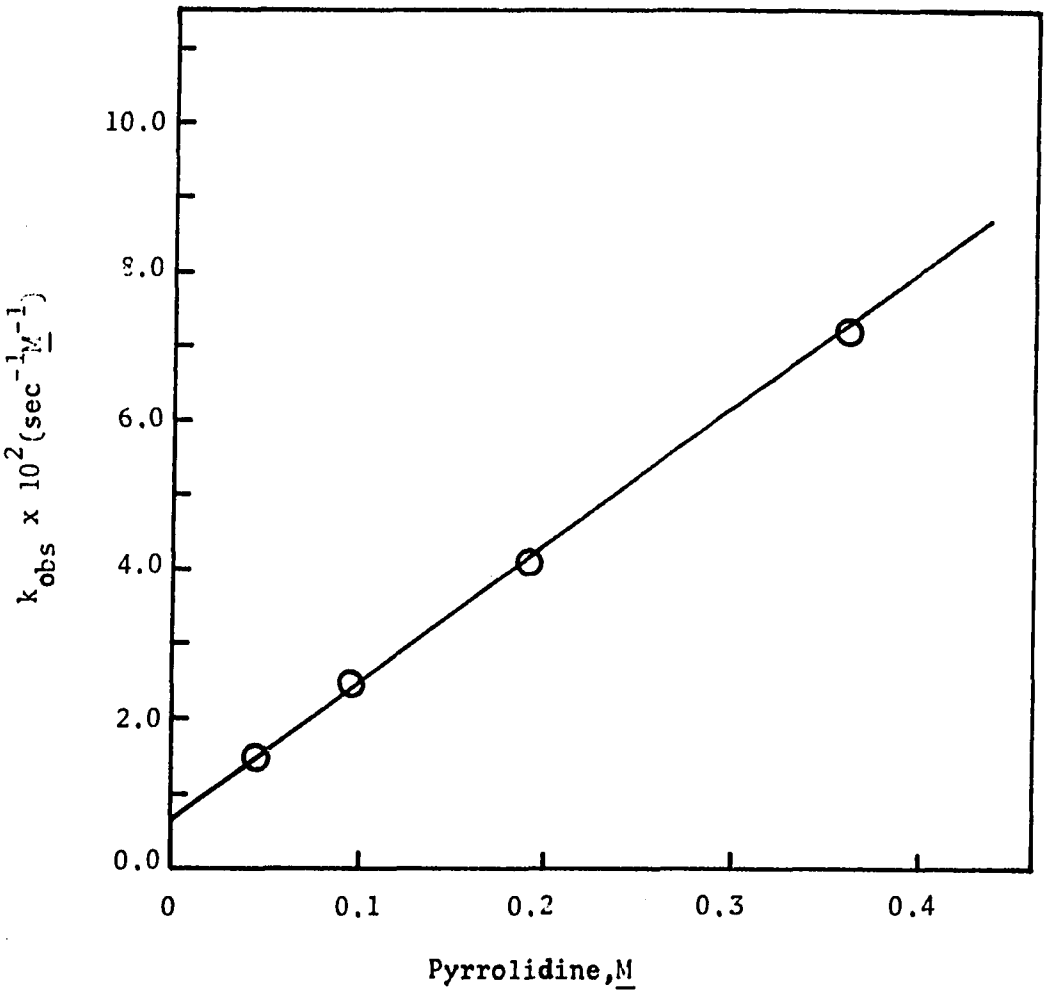

Figure 14. Pyrrolidine - Purine Reactions at 23.0°C in Cyclohexane.

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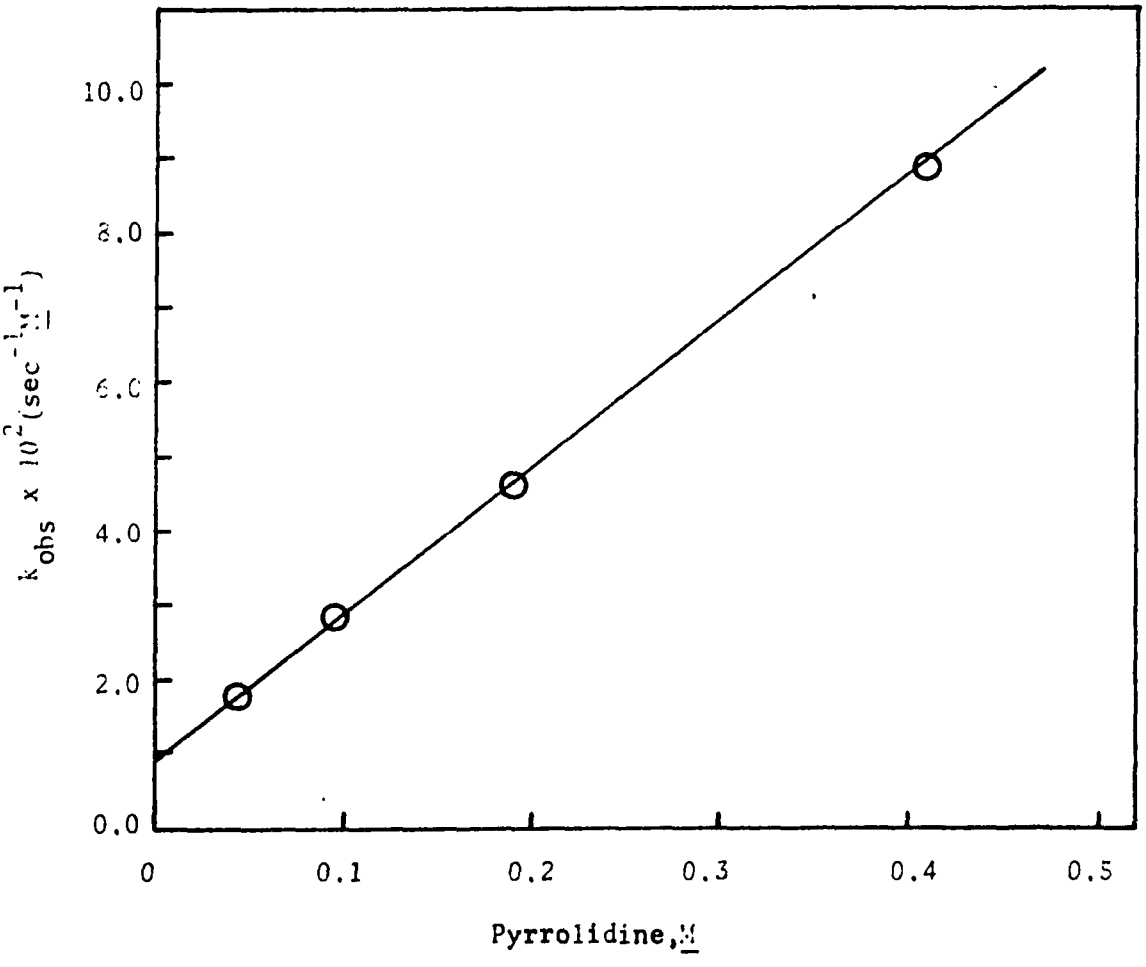

Figure 15. Pyrrolidine - Purine Reactions at 32.0°C in Cyclohexane.

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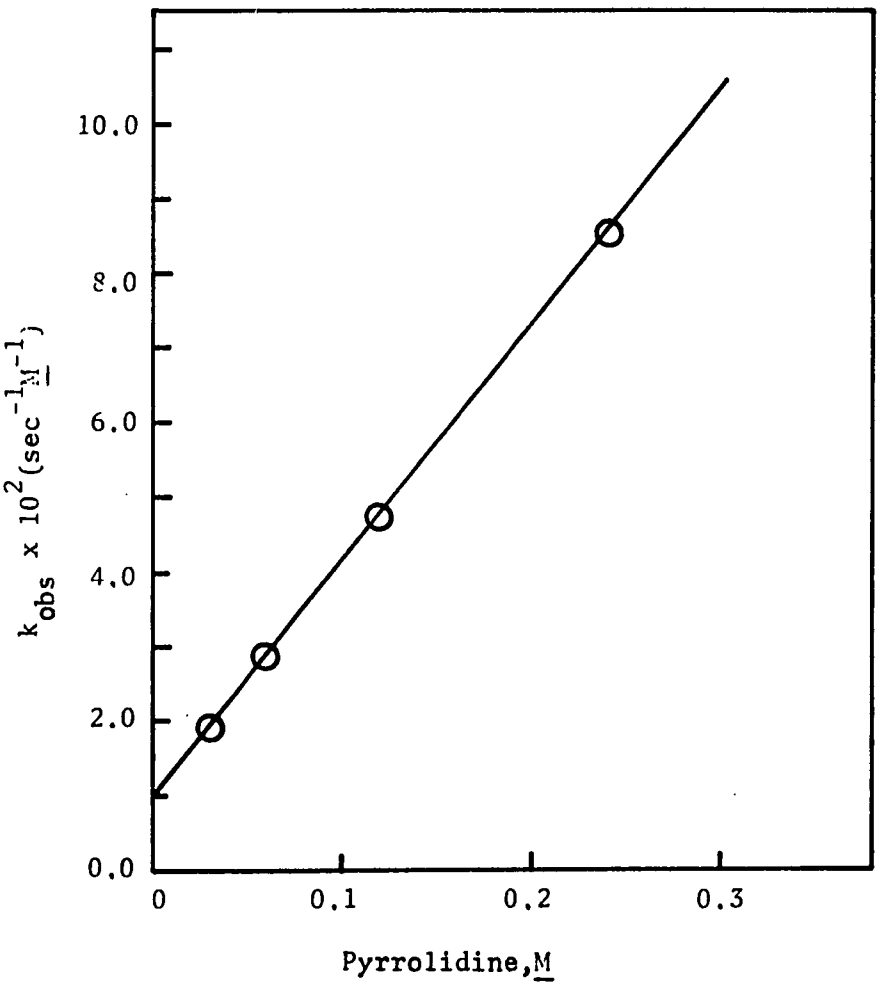

Figure 16. Pyrrolidine - Purine Reactions at 44.5°C in Cyclohexane.

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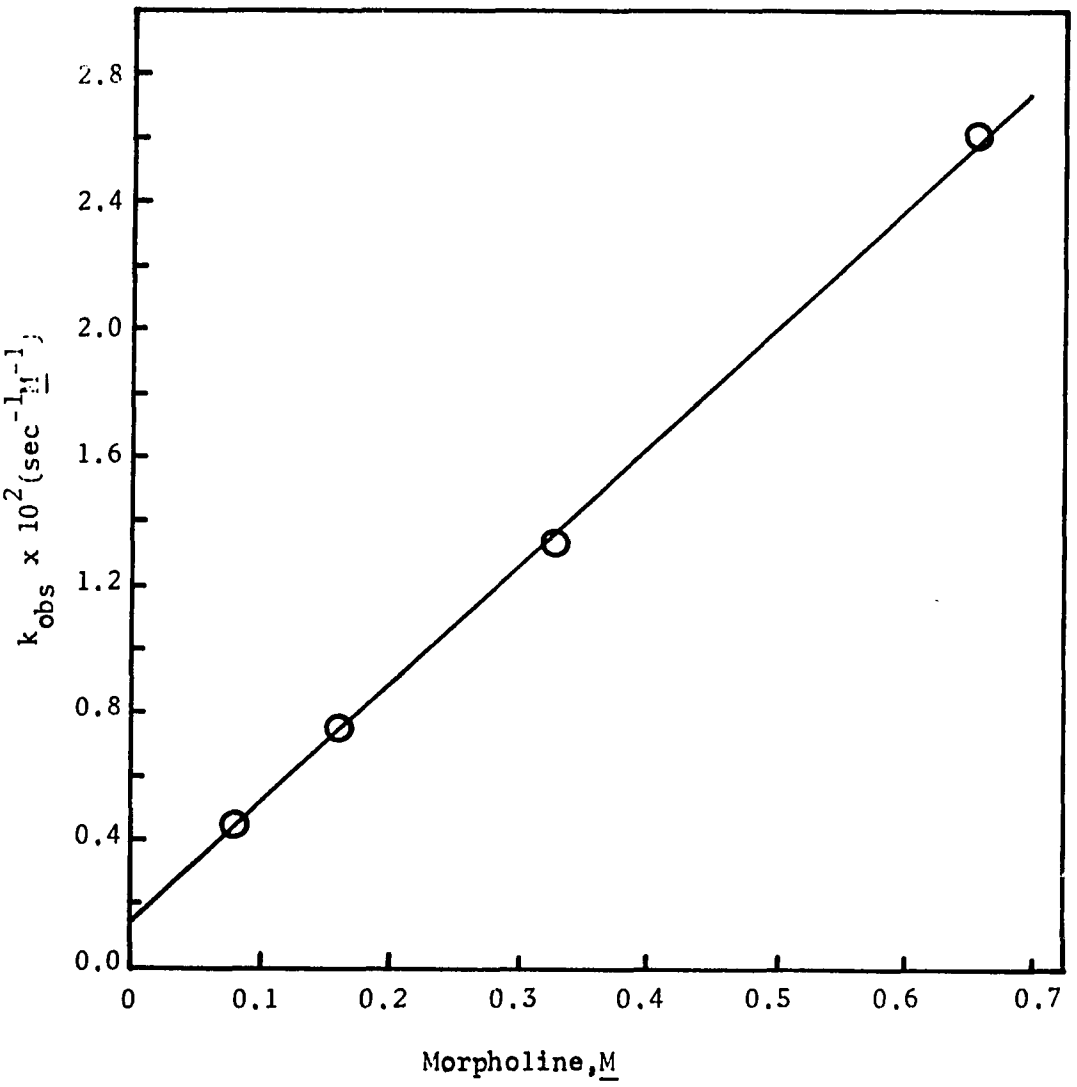

Figure 17. Morpholine - Purine Reactions at 23.0°C in Cyclohexane.

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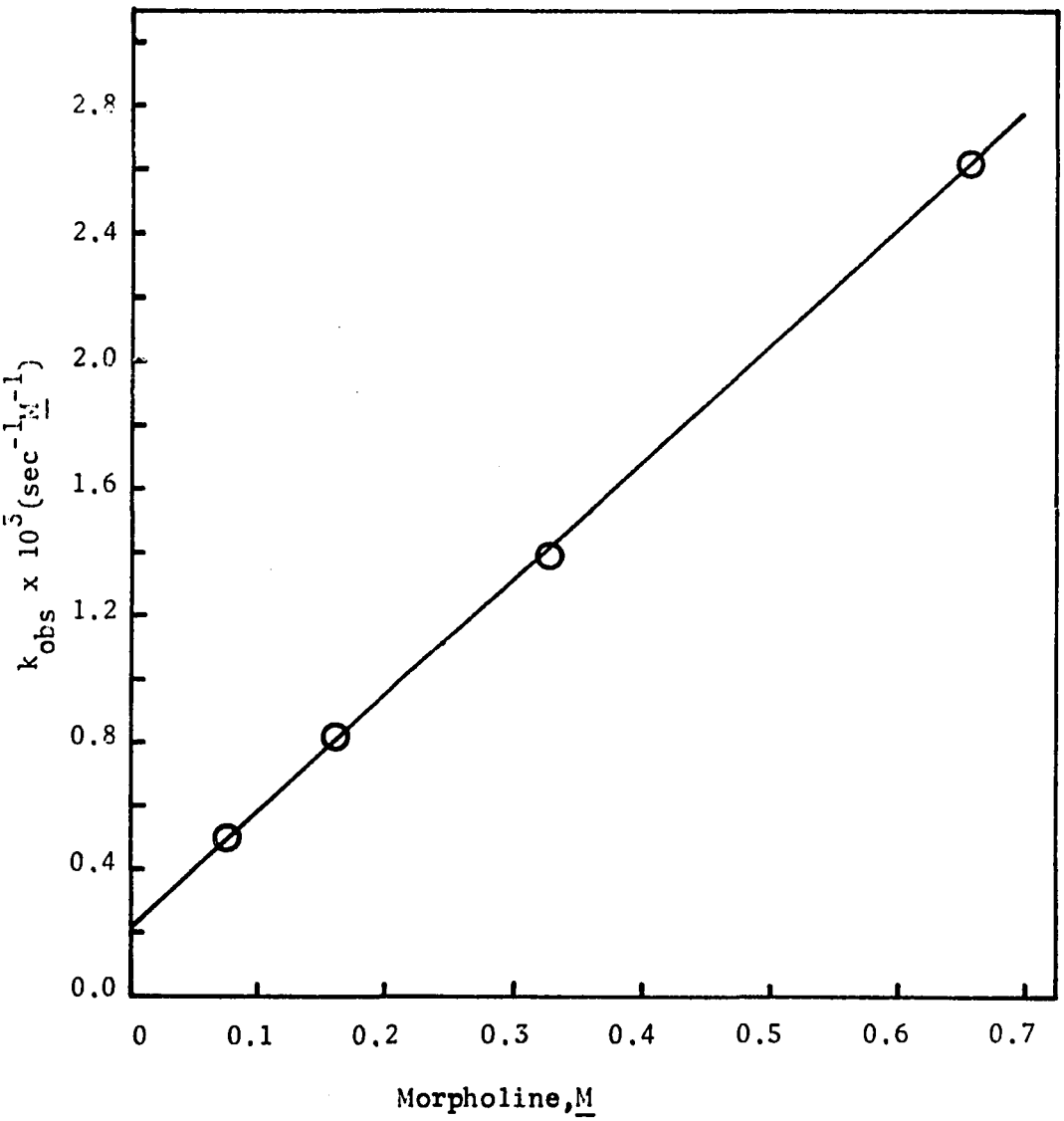

Figure 18. Morpholine - Purine Reactions at 32.0°C in Cyclohexane.

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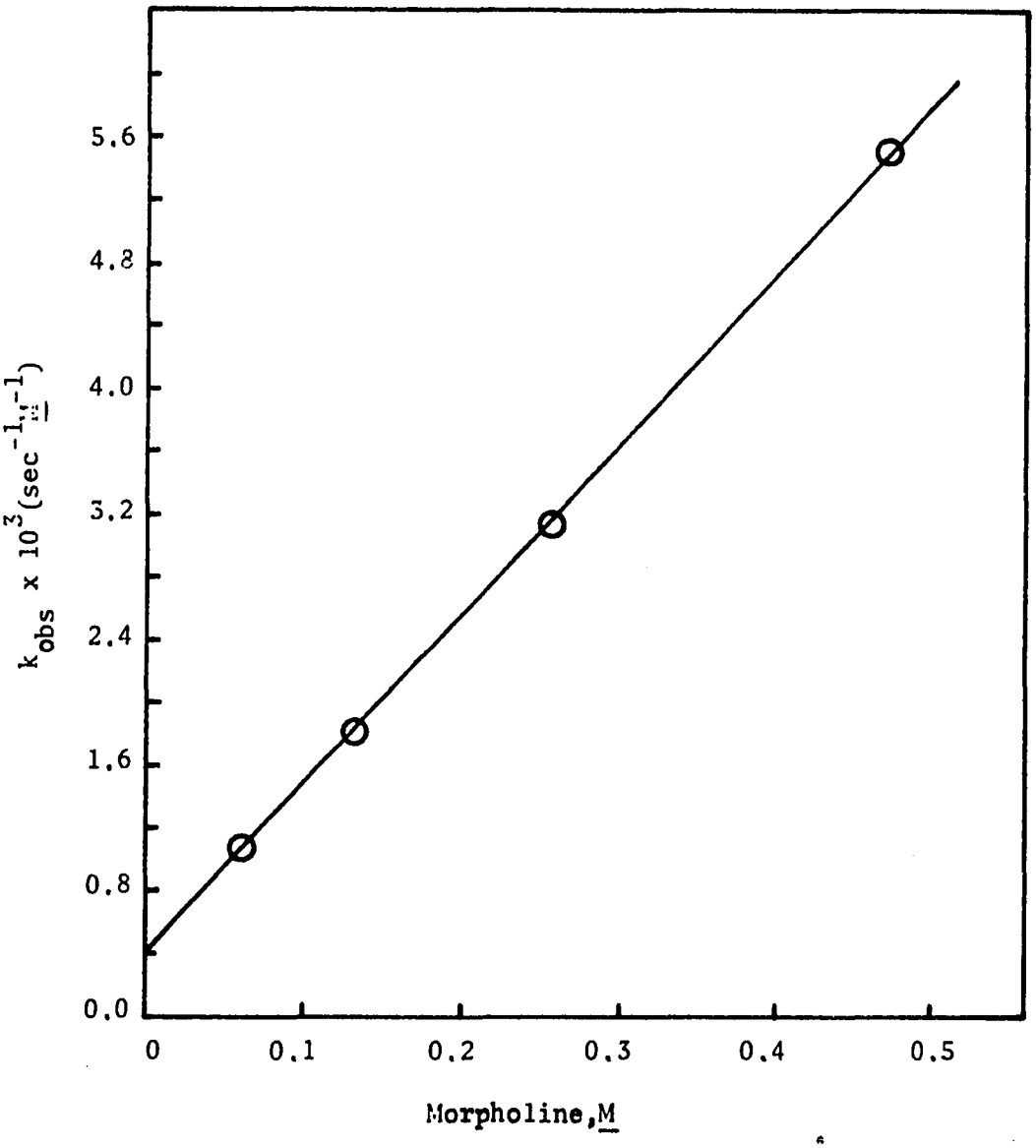

Figure 19. Morpholine - Purine Reactions at 44.5°C in Cyclohexane.

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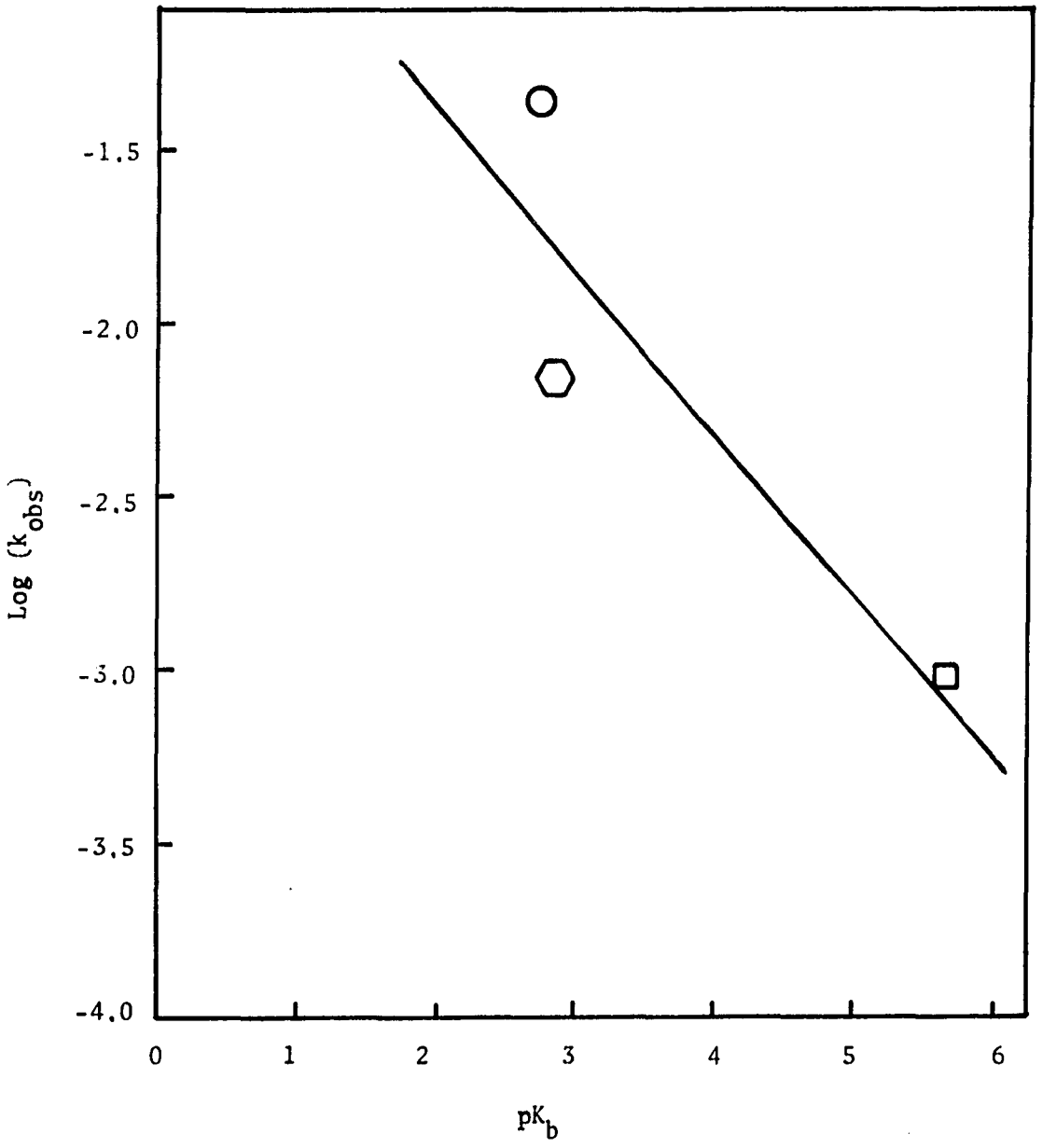

Figure 20. Log(k_{obs}) for the Reaction of 6-Chloro-9-methoxymethylpurine with Different Nucleophiles in Cyclohexane at 23.0°C, and the pK_b of the Nucleophiles.

○ .....Pyrrolidine
⬡ .....Piperidine
□ .....Morpholine

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Reaction of Piperidine with 6-Methoxy-9-methoxymethylpurine in Water

The methoxide group (OCH_3^-) is known to be displaced slower than chloride ion (Cl^-) in nucleophilic aromatic substitutions. With this idea in mind, the reaction of 6-methoxy-9-methoxymethylpurine with piperidine in water (pH = 11.40, ionic strength = 0.5011 M) was studied. Table 32 gives k_{obs} as obtained from the pseudo-first order rate constants for the reactions at 25.0° and 32.0°C. The experimental error in the rate constants in approximately ± 5 percent.

The data show that there is no catalysis by piperidine i.e., the second order rate constant (k_{obs}) is independent of the concentration of piperidine. This kind of behavior is the same as that found in the case of the reaction of 6-chloro-9-methoxymethylpurine with piperidine under the same experimental conditions. The rate constants (k_{obs}) for the reaction of piperidine with 6-chloro-9-methoxymethylpurine and 6-methoxy-9-methoxymethylpurine are 0.1860 and 0.0000068 $sec^{-1}M^{-1}$ at 25.0°C, respectively. The ratio of the above rate constants is 27,400. As expected methoxide is displaced slower than chloride ion.

The energy and entropy of activation for the reaction of piperidine with 6-methoxy-9-methoxymethylpurine in water are 15.60 ± 0.78 kcal/mole and -31.64 ± 1.58 cal/deg/mole (at 25.0°C), respectively.

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Table 32. Second Order Rate Constants for the Reaction of Piperidine with 6-Methoxy-9-methoxymethylpurine (5×10^{-5} M) in Water (pH = 11.40, Ionic Strength = 0.5011 M).

Piperidine(M)	$k_{obs}(sec^{-1}M^{-1})$
	25.0 ± 0.2°C
0.1063	0.00000683
0.1722	0.00000688
0.2836	0.00000681
0.3545	0.00000683
	32.0 ± 0.2°C
0.0866	0.00001370
0.1177	0.00001410
0.1732	0.00001300
0.2771	0.00001350

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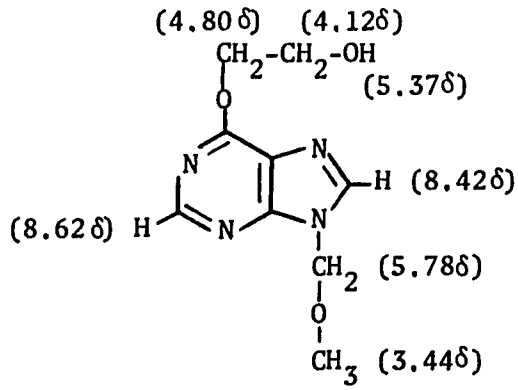
Meisenheimer Complex Formation from the Reaction of 6-(2-hydroxyethoxy)-9-methoxymethylpurine with Potassium t-Butoxide in t-Butyl Alcohol

The reaction of 6-(2-hydroxyethoxy)-9-methoxymethylpurine with potassium t-butoxide in t-butyl alcohol was followed using n.m.r. spectroscopy. Figure 21 shows the n.m.r. spectrum of 6-(2-hydroxyethoxy)-9-methoxymethylpurine in 0.32 M potassium t-butoxide in t-butyl alcohol, one minute after mixing. The peak assignments are as follows, in δ(ppm), external tetramethylsilane = 0.0δ.

The remaining peaks are due to the solvent, t-butyl alcohol. (The hydroxyl proton of the purine glycol and that of the solvent absorb in the same region, 5.37δ, due to rapid exchange.)

Twenty minutes after mixing there is a change in the n.m.r. spectrum of the purine glycol, see Figure 22. If one considers the following reaction to be taken place, the n.m.r. spectrum is easily interpreted.



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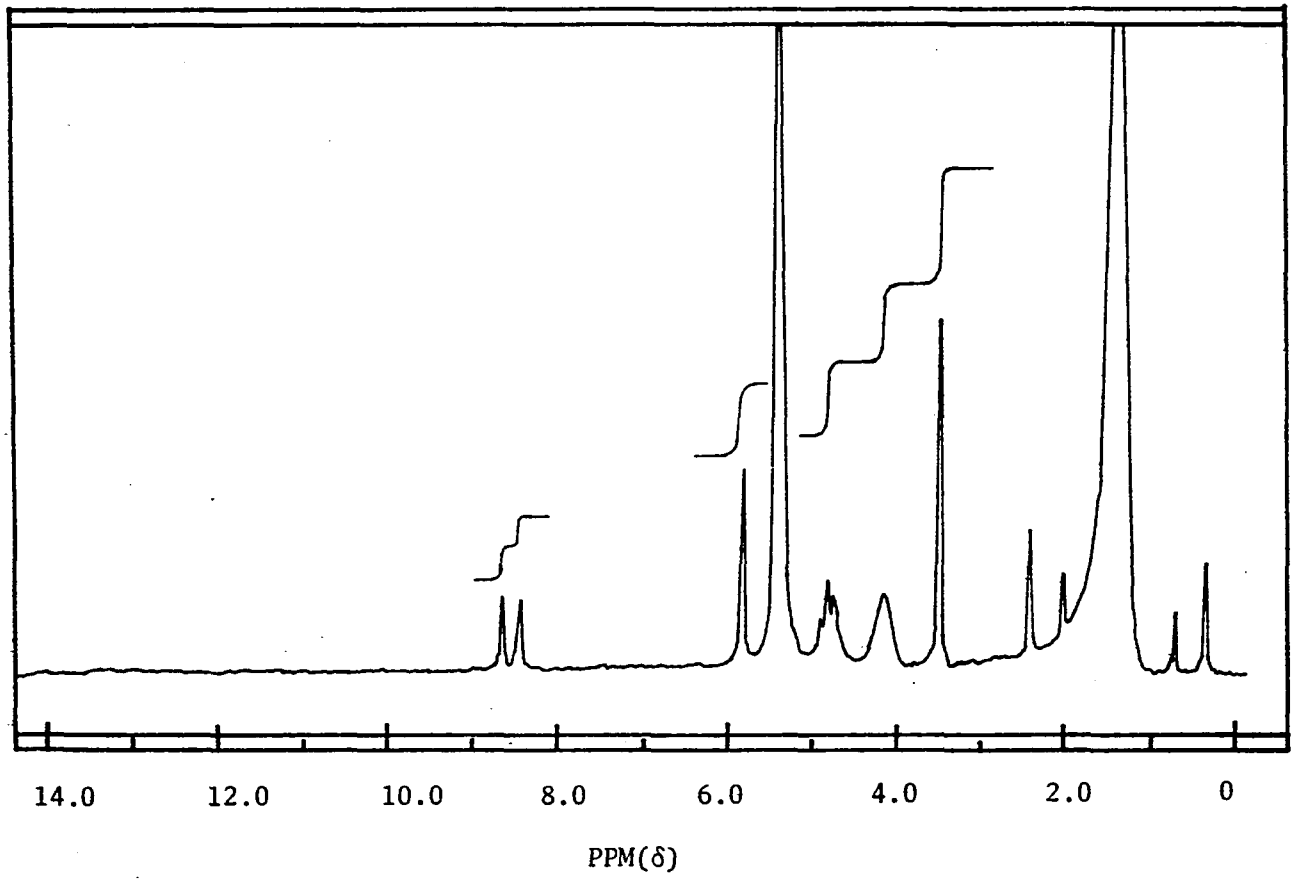

Figure 21. N.M.R. of 6-(2-hydroxyethoxy)-9-methoxymethylpurine in 0.32 M Potassium t-Butoxide in t-Butyl Alcohol (one minute after mixing), at 40°C.

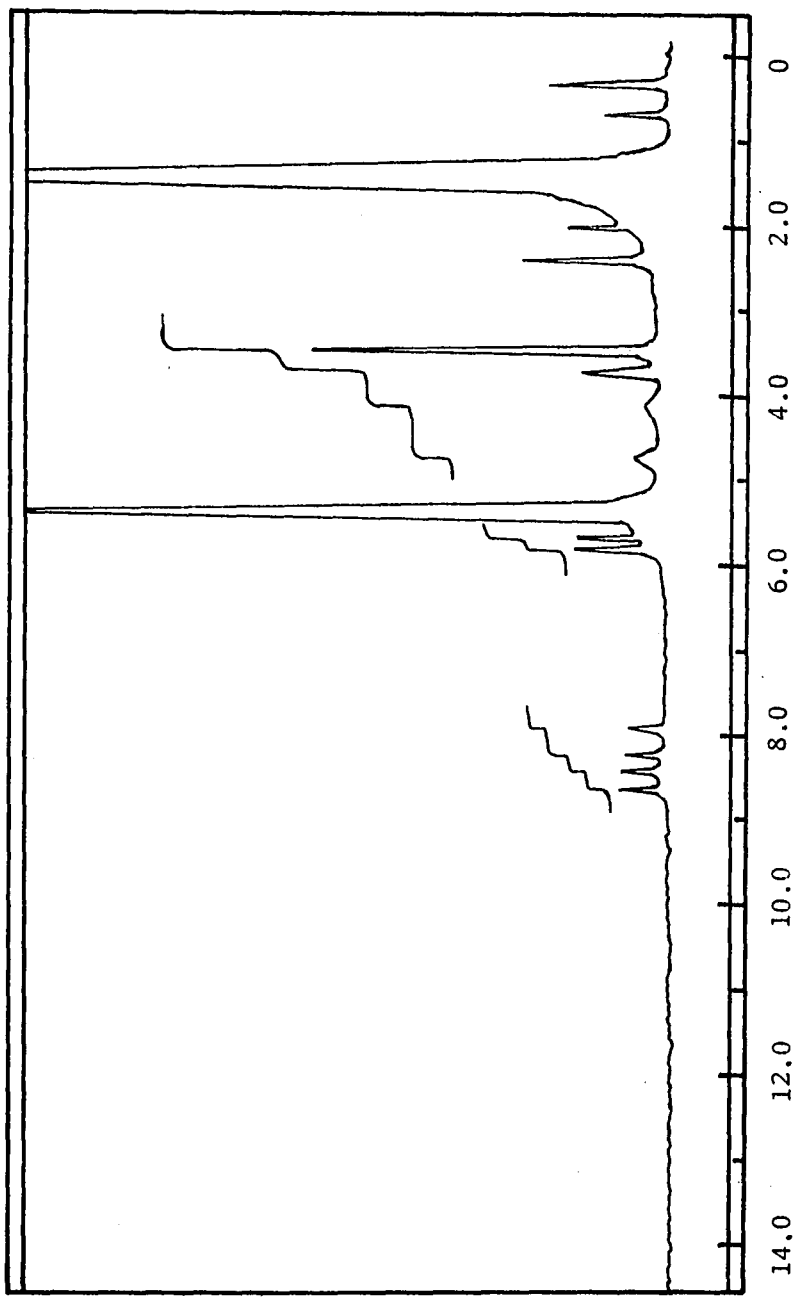

Figure 22. N.M.R. of 6(2-hydroxyethoxy)-9-methoxymethylpurine in 0.32 M Potassium t-Butoxide in t-Butyl Alcohol (20 minutes after mixing), at 40°C.

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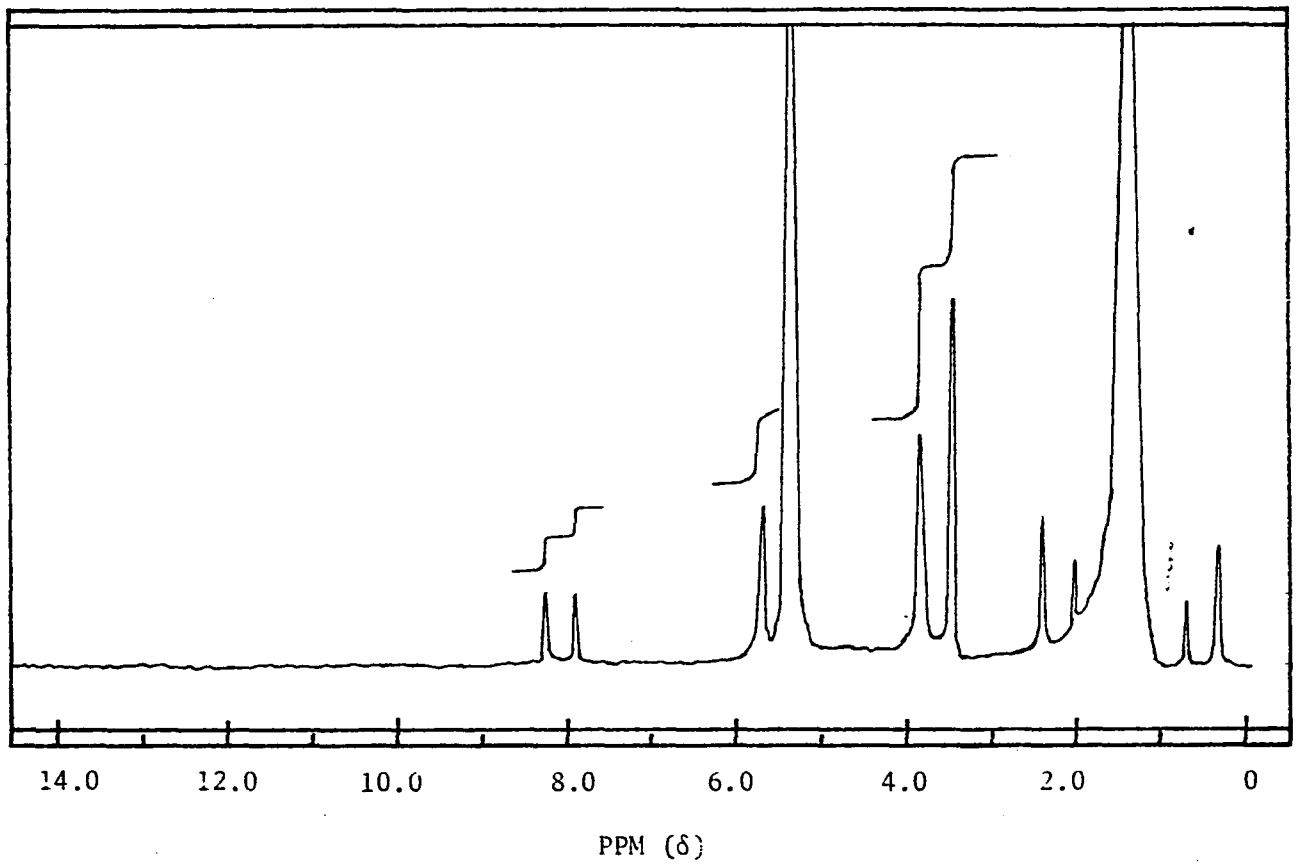
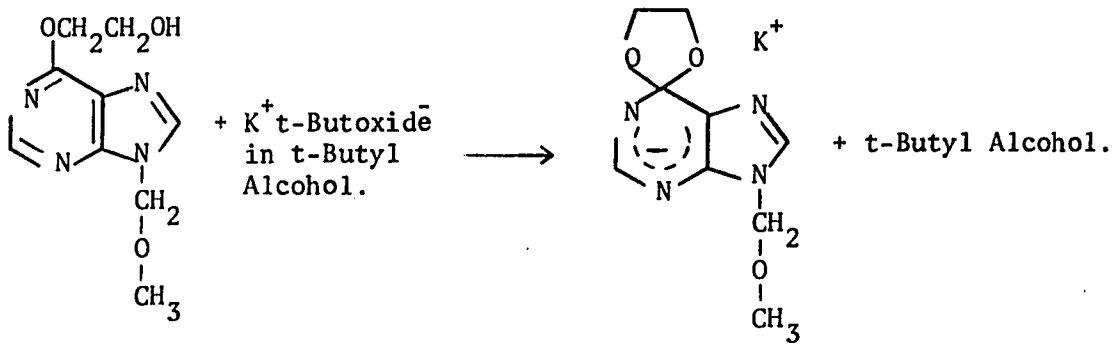
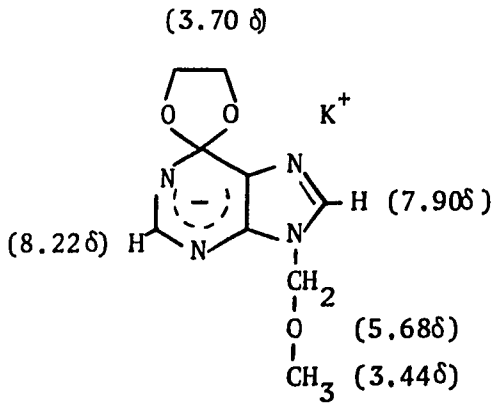


Figure 23. N.M.R. of 6-(2-hydroxyethoxy)-9-methoxymethylpurine in 0.32 M Potassium t-Butoxide in t-Butyl Alcohol (90 minutes after mixing), at 40°C.



i.e., potassium t-butoxide removes the proton of the hydroxyl group in the purine glycol to form an anion which immediately attacks the purine intromolecularly at C-6 forming the spiro Meisenheimer complex:



The n.m.r. on Figure 22 consists of a mixture of the starting purine glycol and its spiro Meisenheimer complex. Integration of the peaks shows the mixture to be about 55:45, respectively. This spectrum shows that the formation of the spiro Meisenheimer complex introduces a

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negative charge in the purine aromatic ring. This negative charge increases the electron density in the whole purine molecule. Thus, the proton attached to C-2 is shifted upfield from 8.62 to 8.22δ. The proton at C-8 is shifted from 8.42 to 7.90δ. The methylene protons of the hydroxyethoxy group in the starting purine glycol are not equivalent, as shown by their absorptions at 4.80 and 4.12δ. In the spiro Meisenheimer complex they are almost equivalent as shown by the slightly broad absorption at 3.70δ. The methylene protons of the methoxymethyl group are also affected by the introduction of the negative charge in the aromatic ring. Their absorption is shifted from 5.78 to 5.68δ. The methoxy protons are not affected by the introduction of the negative charge in the purine ring, as shown by their unchanged absorption at 3.44δ.

Ninety minutes after mixing, the n.m.r. of Figure 23 shows that all of the starting purine glycol has now been converted to the spiro Meisenheimer complex.

The solution in the n.m.r. tube changed from colorless to start with to an orange-yellow color. After about 24 hours a fine orange-yellow solid precipitated out of solution. This solid material, as will be explained later, is the spiro Meisenheimer complex salt.

The rate of formation of the spiro Meisenheimer complex of 6-(2-hydroxyethoxy)-9-methoxymethylpurine was followed using ultra-violet spectroscopy, as previously described in Chapter II. The reaction of 6-(2-hydroxyethoxy)-9-methoxymethylpurine with potassium t-butoxide in t-butyl alcohol was carried out under pseudo-first order conditions. In this case the potassium t-butoxide was present in large excess (0.0705

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to 0.2821 M) compared to the purine glycol (5×10^{-5} M). The second order rate coefficients for these reactions at 25.0°, 32.0° and 44.5°C are tabulated on Table 33. Figure 24 shows the ultraviolet spectra of 6-(2-hydroxyethoxy)-9-methoxymethylpurine and that of its spiro Meisenheimer complex.

It is obvious from the kinetic data that the second order rate coefficient is independent of the potassium t-butoxide concentration. This suggests that under pseudo-first order conditions the rate controlling step in the reaction is the formation of the spiro Meisenheimer complex i.e., the internal cyclization of the alkoxide ion.

The activation energy, ΔE_{act}, for the formation of the spiro Meisenheimer complex has a value of 19.35 ± 0.96 kcal/mole. The entropy of activation, $\Delta S^{\ddagger}_{act}$, at 25.0°C is -12.44 ± 0.62 cal/deg/mole.

Table 33. Second Order Rate Constants for the Reaction of Potassium t-Butoxide with 6-(2-hydroxyethoxy)-9-methoxymethylpurine (5×10^{-5} M) in t-Butyl Alcohol.

	Rate Constant($sec^{-1}M^{-1}$)		
Potassium t-Butoxide(M)	25.0 ± 0.2°	32.0 ± 0.2°	44.5 ± 0.5°C
0.0705	0.000204	0.000450	0.001520
0.1410	0.000219	0.000416	0.001480
0.2115	0.000191	0.000434	0.001550
0.2821	0.000206	0.000408	0.001480

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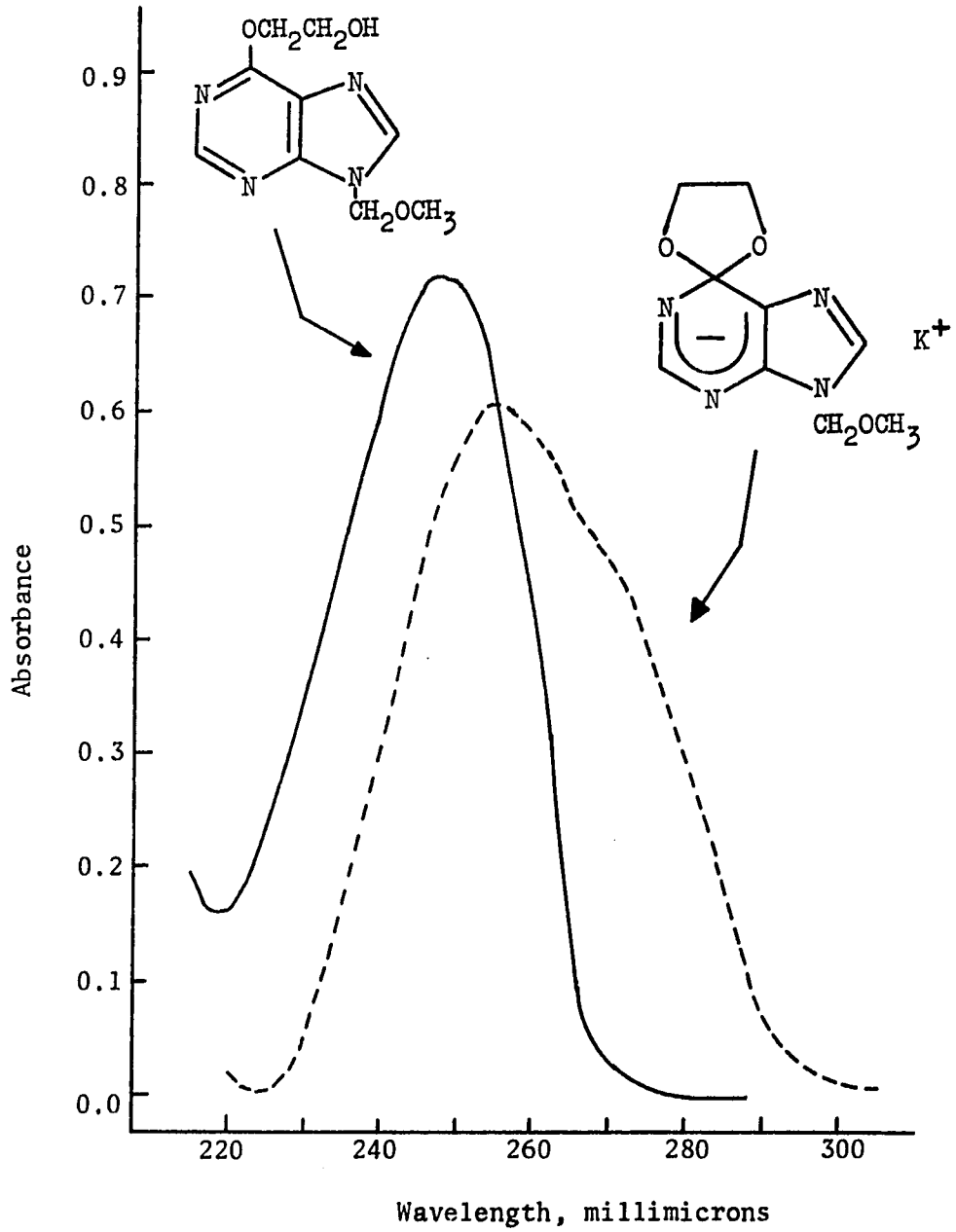

Figure 24. U.V. Spectra of 6-(2-hydroxyethosy)-9-methoxy methylpurine ($\lambda_{max.}$ 248 mμ, ε = 10,900) and of Meisenheimer Complex ($\lambda_{max.}$ mμ, ε = 9,385) in t-Butyl Alcohol.

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The following kinetic data for the compounds listed in Table 34, show some interesting relationships. (The rate constants for the chloro-compounds are for their reaction with 0.4 M piperidine.) Comparison of the rate constants for the nucleophilic attack of piperidine on the chloro-compounds shows that the naphthalene system is more reactive than the purine system (0.92 vs 0.0018 $sec^{-1}M^{-1}$) under nearly the same experimental conditions. On this basis one would expect the rate of formation of the spiro Meisenheimer complex of the naphthalene derivative to be larger than the rate of formation of the purine analog. Experimental data shows this to be the case (1.28 vs 0.00021 $sec^{-1}M^{-1}$, respectively).

The entropy of activation, $\Delta S^{\ddagger}_{act}$, for the formation of the spiro Meisenheimer complex is very similar for the naphthalene and purine systems (-20 $\pm$ 2 vs - 12.44 $\pm$ 0.62 e.u., respectively) as one would expect, because in its formation both involve the cyclization of the alkoxide ion, which is an intramolecular process.

During the course of this research the question of whether the observed change in the ultraviolet spectrum of 6-(2-hydroxyethoxy)-9-methoxymethylpurine upon the addition of potassium t-butoxide in t-butyl alcohol, instead of being that of the formation of the spiro Meisenheimer complex could have been simply the formation of the alkoxide ion without further cyclization. In order to clarify the situation a simple experiment was devised in which the u.v. spectrum of 2-phenoxyethanol was observed first in pure t-butyl alcohol and then in the presence of potassium t-butoxide.

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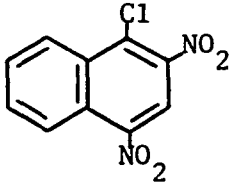
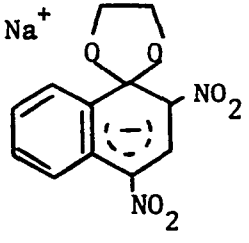
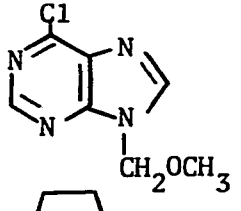
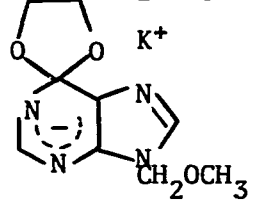
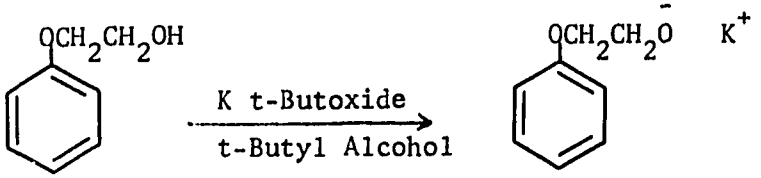
Table 34. Kinetic and Thermodynamic Parameters for the Formation of Meisenheimer Complexes.

Compound	k_{obs} ($sec^{-1}\underline{M}^{-1}$)	ΔE_{act} (kcal/mole)	$\Delta S^{\ddagger}_{act}$ (cal/deg/mole)	t, °C	Solvent
	0.93 (73)	4.39 ± 0.26	-45.53 ± 0.85	20.0	Ethanol
	1.28 (49)	11.8 ± 0.80	-20.0 ± 2	25.0	Methanol
	0.0018	9.98 ± 0.49	-34.93 ± 1.74	25.0	Methanol
	0.00021	19.35 ± 0.96	-12.44 ± 0.62	25.0	t-Butanol



2-Phenoxyethanol has a λ_{max} at 270.5 millimicrons (ε = 309) in t-butyl alcohol, at 25.0°C. The u.v. spectrum of it remained unchanged upon the addition of potassium t-butoxide. This can be rationalized as follows: the reaction of phenoxyethanol with potassium t-butoxide results simply in the formation of the phenoxyethoxide ion which is not capable of cyclization to form a spiro Meisenheimer complex, because the phenyl group does not have electron withdrawing groups attached to it to stabilize the negative charge. The formation of the ethoxide ion does not change the ultraviolet spectrum of the aromatic ring because the negative charge is three atoms away (held together by single bonds) and does not interact with the phenyl group. Therefore, the observed change in the u.v. spectrum of 6-(2-hydroxyethoxy)-9-methoxymethylpurine is indeed the formation of the spiro Meisenheimer complex. Also, the u.v. spectrum of the purine spiro Meisenheimer complex solution observed in the n.m.r. was identical with that of the reaction observed in the ultraviolet.

The spiro Meisenheimer complex of 6-(2-hydroxyethoxy)-9-methoxymethylpurine that precipitated out of the solution in the n.m.r. tube was soluble enough in isooctane to make a dilute solution that could

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be studied in the ultraviolet. This solution gave rise to a peak with λ_{max} = 260 millimicrons and a peak height of 0.1 absorbance units (this is about 1 x 10^{-5} M).

Upon addition of 0.001 M acetic acid, in isooctane, to the reaction cell containing the spiro Meisenheimer complex, the u.v. spectrum changed instantaneously to that of the starting compound, 6-(2-hydroxyethoxy)-9-methoxymethylpurine, λ_{max} = 248 millimicrons.

Addition of 0.0001 M acetic acid also reverts the u.v. spectrum to that of the purine glycol.

Other compounds containing an active hydrogen were tried, such as 0.01 M trifluoroethanol and 0.02 M methanol. Even 0.01 M piperidine affected the spiro Meisenheimer complex, reverting it to the purine glycol.

Addition of 0.01 M triethylamine (which does not have an active hydrogen) failed to affect the spiro Meisenheimer complex.

Thus, any compound containing an active hydrogen will react with the spiro Meisenheimer complex to give the starting purine glycol.

It was not possible to follow the rate of ring opening in the spiro Meisenheimer complex because the process occurred too rapidly.

Meisenheimer Complex Formation from the Reaction of 6-Methoxy-9-methoxymethylpurine with Potassium Methoxide in t-Butyl Alcohol

The formation of the Meisenheimer complex from the reaction of 6-methoxy-9-methoxymethylpurine with potassium methoxide in t-butyl alcohol was followed using n.m.r. spectroscopy. Figure 25 shows the n.m.r. spectrum of 6-methoxy-9-methoxymethylpurine in 0.48 M potassium methoxide

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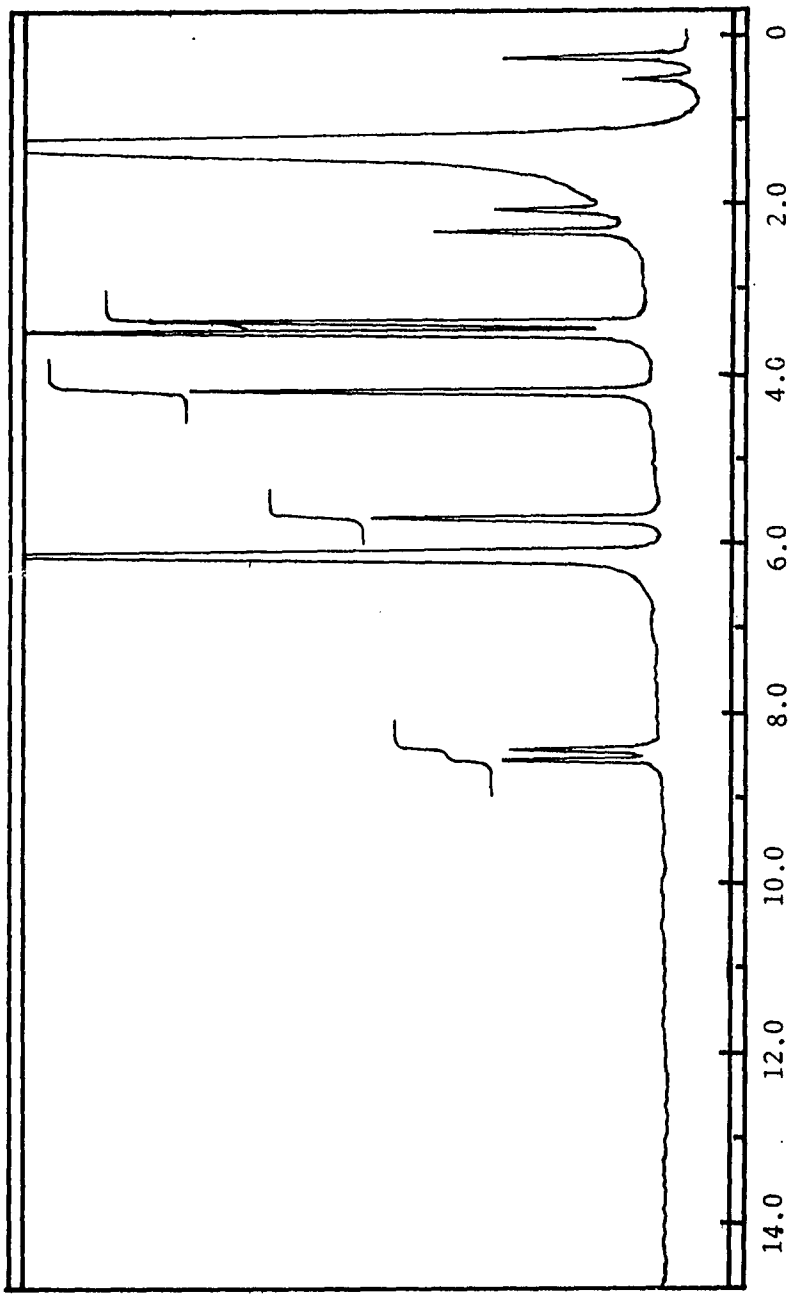

Figure 25. N.M.R. of 6-Methoxy-9-methoxymethylpurine in 0.48 M Potassium Methoxide in t-Butyl Alcohol (one minute after mixing), at 40°C.

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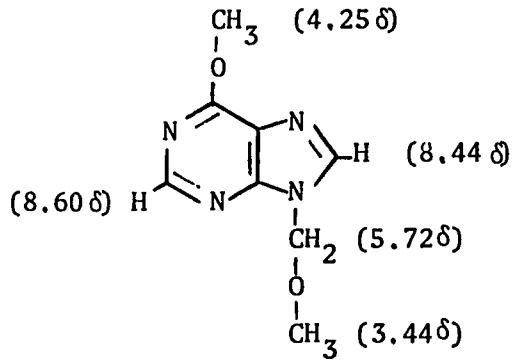
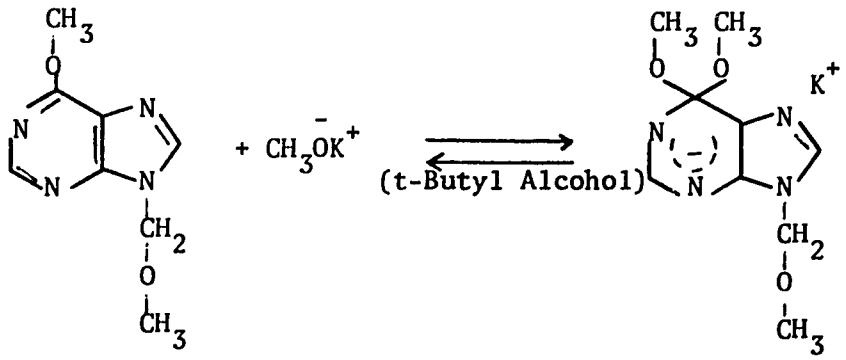
in t-butyl alcohol, one minute after mixing. No detectable change is observed at this point. The peak assignments are shown below in δ(ppm), external tetramethylsilâne = 0δ.



The remaining peaks are due to t-butyl alcohol and methanol. The signal for the latter is at 3.60 δ.

Figure 26 shows that 60 minutes after mixing, some Meisenheimer complex has been formed, as shown in the following equation:



The spectrum is a mixture (about 62:38) of 6-methoxy-9-methoxymethylpurine and of its Meisenheimer complex formed by the attack of methoxide on the purine, at the C-6 position.

Assignment of the new peaks is as follows:

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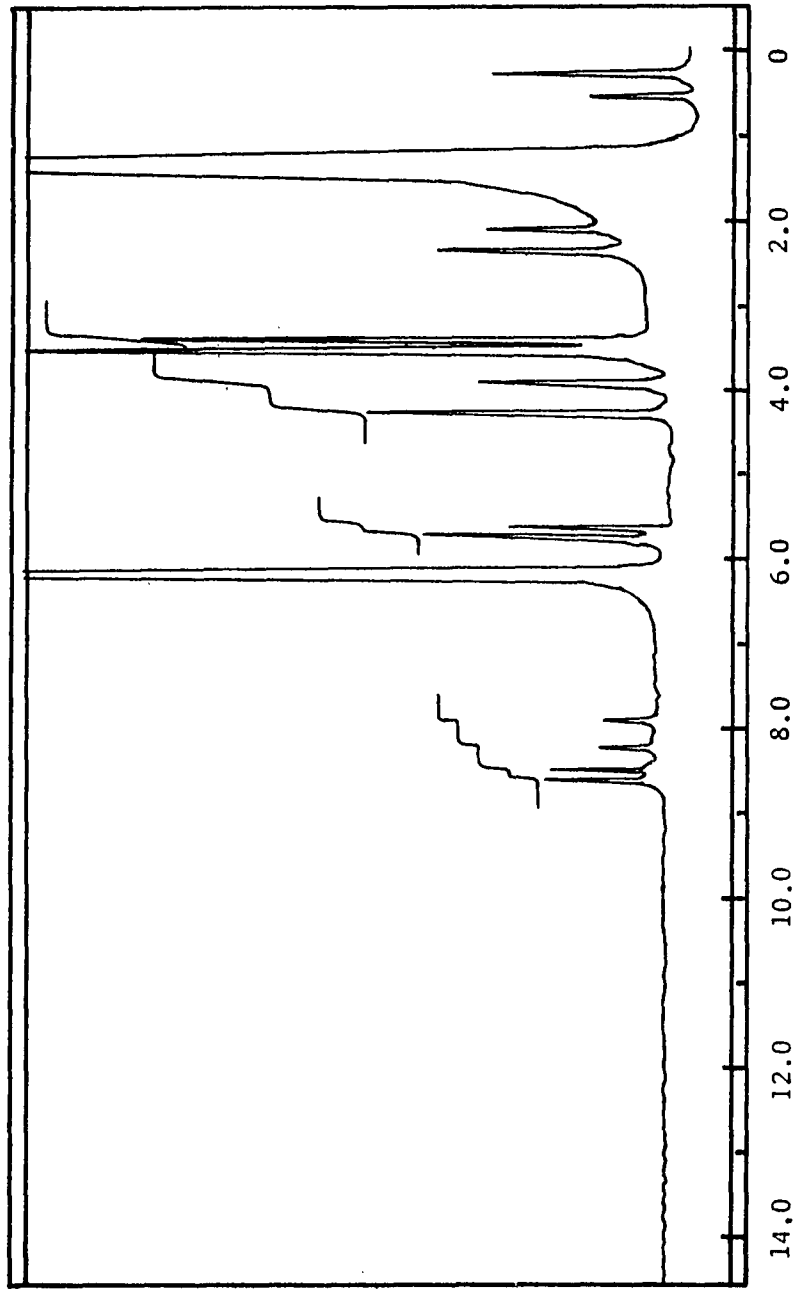

Figure 26. N.M.R. of 6-Methoxy-9-methoxymethylpurine in 0.48 M Potassium Methoxide in t-Butyl Alcohol (60 minutes after mixing), at 40°C.

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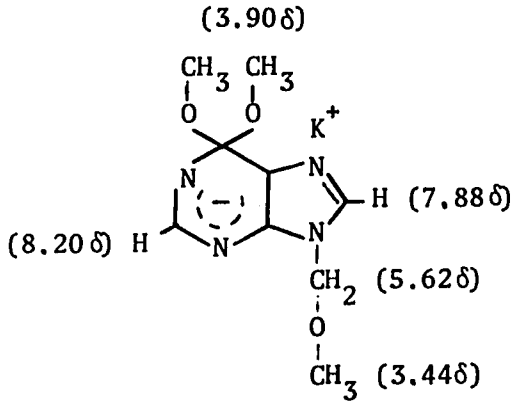

The attack of the methoxide ion on the purine system introduces a negative charge in the aromatic ring. This negative charge increases the electron density in the whole purine molecule. Thus, the proton attached to C-2 is shifted upfield from 8.60 to 8.20 δ. The proton at C-8 is shifted from 8.44 to 7.88 δ. The absorption of the methylene protons of the methoxymethyl group is moved upfield from 5.72 to 5.62 δ. The C-6 methoxy group in the starting purine (4.25 δ) becomes equivalent with the new methoxy group introduced at C-6 in the Meisenheimer complex. This new signal is centered at 3.90 δ. The methoxy protons of the methoxymethyl group are not affected by the introduction of the negative charge in the aromatic system. Their unchanged absorption occurs at 3.44 δ.

Ninety minutes after mixing, the n.m.r. spectrum of the mixture has now developed other absorptions, especially in the region where the aromatic protons absorb (see Figure 27). This absorptions are attributed to decomposition products of the starting purine. Based on the integration of the spectrum, about 50 percent of the starting purine has re-

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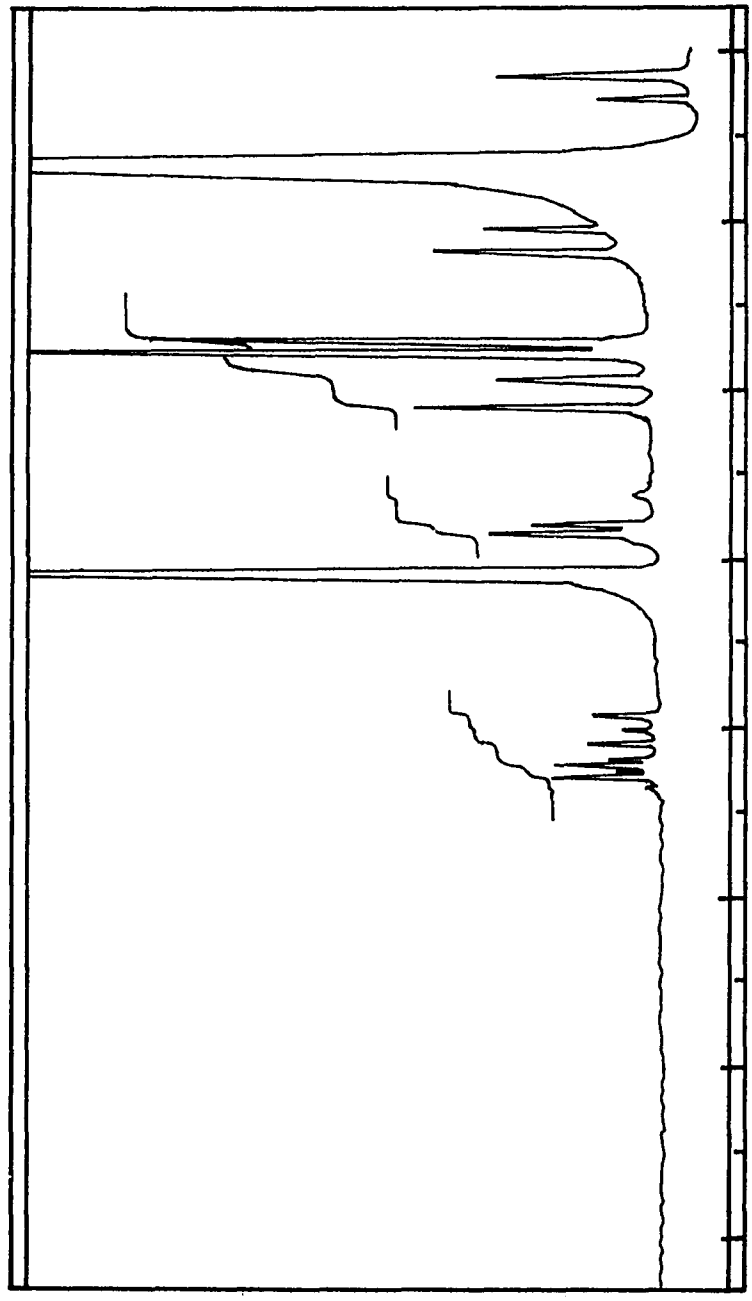

Figure 27. N.M.R. of 6-Methoxy-9-methoxymethylpurine in 0.48 M Potassium Methoxide in t-Butyl Alcohol (90 minutes after mixing), at 40°C.

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acted to products. The n.m.r. spectrum shown on Figure 27 remained unchanged after three days. It looks like the reaction mixture has reached an equilibrium point.

Reaction of 6-methoxy-9-methoxymethylpurine with sodium methoxide-d_3 in DMSO-d_6 showed exchanged of methoxide ion at C-6. The signal due to the C-6 methoxide-h_3 (4.50δ) decreased very rapidly and at the same time the absorption for methanol-h_3 started to grow (at 3.60δ). Finally an equilibrium was reached containing a ratio of 9:1 methoxide-d_3 to methoxide-h_3 incorporated at the C-6 position of the purine molecule. This shows that there is readily exchange of methoxide at the C-6 purine position. It was also noticed that the signal for the C-8 proton disappeared while that of the C-2 proton remained unchanged (8.75 and 8.80 δ, respectively). This is attributed to the rapid exchange of the C-8 proton for deuterium. Other workers have reported this phenomenon.[74]

The signals of the n.m.r. spectrum in DMSO of 6-methoxy-9-methoxymethylpurine in the presence of methoxide ions became smaller as time went by (two to four hours after mixing) until almost no signal, corresponding to the purine, was detected. This must mean that the purine molecules were being destroyed by the action of the base. It is known[75] that purines are decomposed in strong basic or acidic media.

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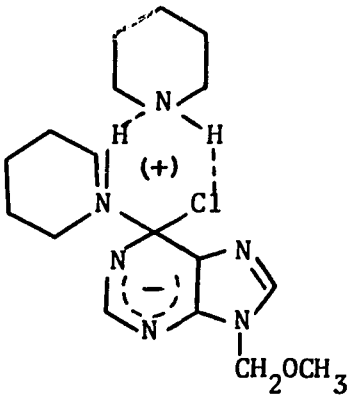
CHAPTER IV

CONCLUSIONS

The results obtained in this research indicate that the mechanism of the reaction of piperidine with 6-chloro-9-methoxymethylpurine in non-polar aprotic solvents, such as cyclohexane and benzene, goes through an intermediate complex whose breakdown to products may be either catalyzed or uncatalyzed. The catalyzed decomposition is accomplished by the excess amine. The catalysis appears to be bifunctional in nature and may be represented by the following structure



The uncatalyzed process may involve a four-centered transition state such as the following

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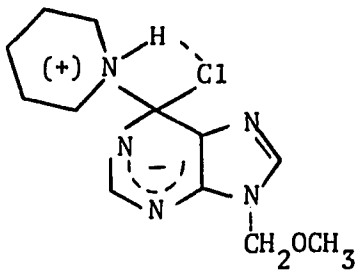

In solvents such as water, methanol, and dimethyl sulfoxide the rate of reaction is independent of the concentration of piperidine. In these cases the solvent helps decompose the intermediate complex by assisting in the removal of HCl from it. Also, the high dielectric of these media accelerate the rate of formation.

The isotopic study involving piperidine-N-h and -N-d, in cyclohexane and benzene, showed a kinetic isotope effect (for the catalyzed reaction) of about 0.90 $\pm$ 0.03. This behavior may be interpreted as a delicate balance between bond breaking and bond formation in the bifunctional catalysis. The uncatalyzed reaction showed no kinetic isotope effect.

In the solvent 1,4-dioxane at 23.0°C, K_{1H}/K_{1D} (the ratio of the rate constant for the initial attack of piperidine on the purine) is equal to 0.92 $\pm$ 0.03. This difference is attributed to the slightly larger nucleophilicity of piperidine-N-d compared to piperidine-N-h. This is the first case in which this phenomenon is observed.

The direct observation of the addition (Meisenheimer) complex in the case of 6-(2-hydroxyethoxy)-9-methoxymethylpurine and 6-methoxy-

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9-methoxymethylpurine was accomplished by using n.m.r. and u.v. spectroscopy. The addition complex is taken, generally, as the prototype of the activated complex in nucleophilic aromatic substitution.

The significance of this observation is that these are the first instances in which Meisenheimer complexes have been prepared in the purine nucleus, and provides evidence for the intermediacy of this type of intermediate in the reported kinetic study.

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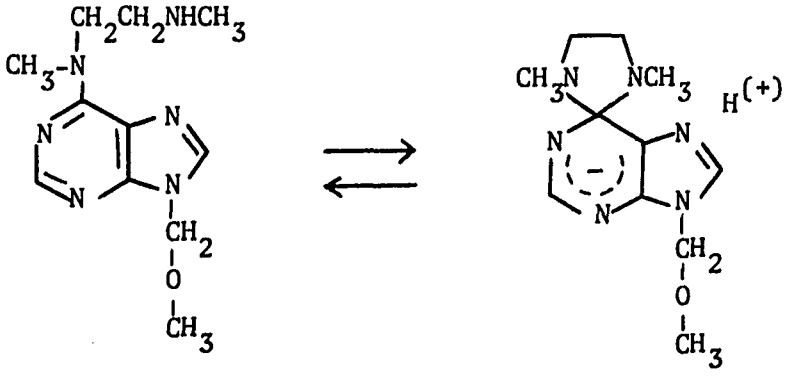
CHAPTER V

RECOMMENDATIONS

The reaction of piperidine with 6-fluoro-, 6-bromo- and 6-iodo-9-methoxymethylpurine in cyclohexane should be studied. Based on the results obtained in this thesis from the 6-chloro-9-methoxymethylpurine reactions, it is expected that the order of reactivity of the 6-halopurines would be F > Cl > Br > I, i.e., the catalytic effect of piperidine is expected to be the largest for the fluoro and the smallest for the iodopurine.

The formation of other spiro Meisenheimer complexes, such as the one derived from 6-(N-N'-dimethylethylenediamino)-9-methoxymethylpurine would be interesting.



In the case of 6-(2-hydroxyethoxy)-9-methoxymethylpurine, the hydroxy oxygen being a poor nucleophile had to be converted to its sodium salt in order to have the subsequent formation of the spiro Meisenheimer

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complex. On the other hand, 6-(N-N'-dimethylethylenediamino)-9-methoxymethylpurine contains a secondary amine (which is a better nucleophile than a hydroxy oxygen) properly positioned to effect an intramolecular nucleophilic attack on the purine. X-ray and n.m.r. analyses on this compound should be done to confirm its structure.

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*Periodical abbreviations follow those in Access, 1969.

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VITA

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