Tetrahedron Vol. 41, No. 8, pp. 1471 to 1478, 1985
Printed in Great Britain.

0040–4020/85 \$3.00 + .00
Pergamon Press Ltd.

THE REACTIONS OF FLUORENYLIDENE WITH HETEROATOMIC NUCLEOPHILES

Joseph J. Zupancic, Peter B. Grasse, Stephen C. Lapin and Gary B. Schuster*
Department of Chemistry, Roger Adams Laboratory, University of Illinois, Urbana, IL 61801, U.S.A.

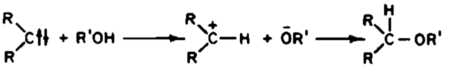
(*Received in USA* 2 *November* 1983)

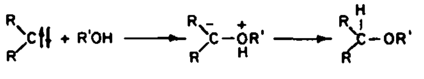
Abstract—The reaction of fluorenylidene with heteroatomic nucleophiles gives good yields of addition products. The rates of these reactions were determined using laser spectrophotometric techniques. Kinetic and product isotope effects were measured. The results of this investigation show that the reaction of the carbene with the nucleophile is not a one-step process. The implication of these conclusions to the spin selective reactions of carbenes and their utility as photoaffinity labels is discussed.

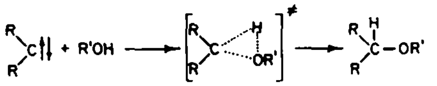
The reaction of carbenes with alcohols occupies a central and critical position in the network of observations that define the chemical and physical properties of these reactive intermediates. It has long been held that ether formation is characteristic of carbenes in their singlet state.[1–7] In contrast, triplet carbenes are believed generally to abstract H atoms from alcohols to give radical pairs that eventually go on to products associated with the combination and disproportionation of these radicals.[8–11] This simple description of spin-selective carbene chemistry has recently been challenged by the suggestion that triplet diphenyl carbene (the prototypical aromatic carbene, if one exists) reacts in one step with MeOH to give diphenylmethyl methyl ether.[12] The experimental support for this proposal comes from an analysis of the kinetics and temperature dependence of this reaction. However, the interpretation of these results requires a detailed understanding of the reaction mechanism.

There have been several attempts to expose the mechanistic details of the reaction of carbenes with alcohols, and with heteroatomic nucleophiles in general. Part of the motivation for this research is that these reactions form the basis of important photoaffinity labeling procedures where carbenes bind irreversibly to the nucleophilic residues of proteins.[13]

Historically, three broad mechanistic sequences have been considered likely for the reaction of carbenes with heteroatomic nucleophiles. The first sequence is initiated by proton transfer to give a carbocation and the conjugate base of the nucleophile. In a subsequent step this ion pair (or pair of ions) combines to give the observed product. This path is illustrated for an alcoholic nucleophile in Eq. (1). The second possibility is that reaction of the carbene and the nucleophile generates an intermediate ylide which in turn undergoes a proton shift to give the ether, this is illustrated in Eq. (2). Finally, the third possibility is a concerted three center interaction leading to "direct insertion" of the carbene into the heteroatom–H bond and is shown in Eq. (3).

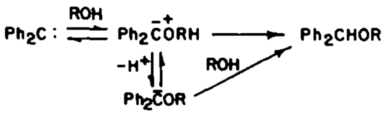

(1)


(2)

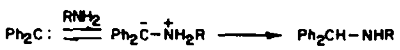

(3)

One of the most revealing investigations of the mechanism of the reaction of carbenes with alcohols was carried out by Bethell *et al.*[4] They measured the product isotope effect on ether formation from thermolysis of diphenyldiazomethane in acetonitrile solutions containing MeOH, t-BuOH and mixtures of methyl and t-BuOH. Competition reactions revealed that MeOH is *ca* 8 times more reactive than t-BuOH is toward the carbene. The investigation of the isotope effect on these reactions used alcohols tritiated specifically at the O—H bond. Analysis of the level of radioactivity in the isolated ethers showed a product isotope effect for MeOH (P_H/P_D, converted from P_H/P_T by Swain's equation[14]) of 1.54, and for t-butyl alcohol of 3.22. Critically, addition of 0.47 M MeOH to a solution containing 1.03 M t-BuOH caused a reduction in the t-BuOH isotope effect to 2.38. These findings, among others, led Bethell to conclude that the mechanism of ether formation in this case featured reversible ylide formation, Scheme 1. Indeed, it was claimed in this report that dissociation of the ylide to regenerate the carbene must occur at a rate comparable to the reactions leading irreversibly from the ylide to ether. It should be noted that reversible ylide formation will complicate interpretation of activation parameters derived by observations of the rate of reaction of the triplet carbene.

In a subsequent study, Bethell *et al.*[15] investigated the mechanism for reaction of diphenyl carbene with n- and t-butylamines. Among the significant observations made during this investigation is that there is no observable *inter*molecular product isotope effect. However, an *intra*molecular product isotope effect (P_H/P_D) of 2.6 is observed for both amines. Also, it was concluded that the rate of reaction of triplet diphenyl carbene with these amines is faster than the reformation of the singlet carbene, thus forcing intersystem crossing



Scheme 1.



Scheme 2.

by the initially formed singlet to be irreversible. Using the radioactivity of tritium as a tracer, Bethell concluded that triplet diphenyl carbene reacts in part by abstracting a H from the N—H bond of the amine. The major product, however, is argued to result from ylide formation followed by a prototropic shift to generate the secondary amine Scheme 2. Formation of this ylide is considered to be reversible, but, in this case, it is not required to be so by the data.

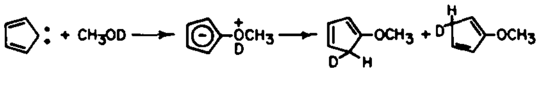
More recently, Kirmse *et al.*[16] have used deuterium labeling studies to examine the mechanism for addition of MeOH to cyclopentadienylidene and cycloheptatrienylidene. They report that the reaction of deuterated MeOH at −78° with cyclopentadienylidene gives directly 1- and 2-methoxy-5-deuteriocyclopentadiene, a result that excludes direct insertion, but which is consistent with ylide formation, Scheme 3. These authors do not comment on the possibility that the ylide can revert to carbene, but this path is not excluded by their observations.

In contrast, Kirmse *et al.*[16] conclude from an analogous investigation of the reaction of cycloheptatrienylidene that ether formation proceeds in this case not through the ylide, but by initial proton transfer to the carbene, Scheme 4. Of course, it is quite reasonable to expect that the relative stabilities of the intermediate ions will influence the selection of reaction path. And the change in mechanism accompanying the change from cyclopentadienylidene to cycloheptatrienylidene appears to be dictated by just such considerations.

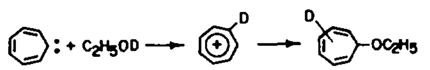
Closs and Rabinow[17] used conventional flash photolysis to examine the kinetics of the reaction of diphenyl carbene with MeOH in C_6H_6 and in acetonitrile solution. They assumed that the reaction of the singlet carbene with MeOH was no faster than diffusion controlled, and then employed the observed rate parameters to support the concept of equilibration of singlet and triplet diphenyl carbene previously suggested by Bethell. With the additional assumption that the observed ether is formed only from the singlet carbene, these authors estimated the energy difference between the ground-state triplet and the singlet (ΔE_{ST}) of diphenyl carbene to be *ca* 5 kcal/mol.

Similarly, Eisenthal *et al.*[18] used picosecond laser spectrophotometric techniques to study the reaction of diphenyl carbene with MeOH. Their data, again relying on the implied assumption that the ether is formed exclusively from the singlet carbene, permit them to conclude that MeOH reacts with the singlet carbene at a rate very close to the diffusion-controlled limit.

Bethell's observation of a primary product isotope effect on the reaction of diphenylcarbene with methanol appears to undermine the suggestions that the rate of this reaction is controlled by diffusion. However, we will present evidence below that singlet fluorenylidene reacts with MeOH in acetonitrile to form the appropriate ether at a rate quite close to the diffusion limit.



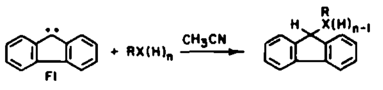
Scheme 3.



Scheme 4.

A study of the reaction of singlet phenylchloro carbene by Griller *et al.*[19] has added another complexity to the analysis of the mechanism of ether formation from carbenes and alcohols. They report that the observed *rate constant* for reaction of this carbene with MeOH or t-BuOH (k_{nuc}^{obs}) in isooctane or acetonitrile solution depends on the concentration of the alcohol. They attribute this to different rate constants for reaction of the carbene with alcohols associated to different degrees. It is well known that the extent of dimerization, and higher complex formation, of alcohols is concentration dependent in hydrocarbon solvents.

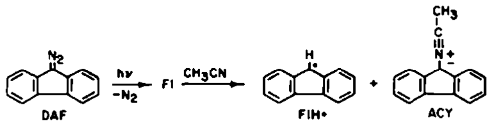
We have carried out an investigation of the kinetic isotope effect on the reaction of fluorenylidene with heteroatomic nucleophiles, Eq. (4), where X = O, N and S, using nanosecond pulsed laser spectrophotometric techniques. Comparison of these findings with conventional measurements of the product isotope effects illuminates additional details of the reaction mechanism. These details may prove useful in defining the spin-selectivity of carbene reactions, and in using these reactions in photoaffinity labeling experiments.



(4)

RESULTS

Irradiation of diazofluorene (DAF) in acetonitrile solution generates three observable transient products.[20–22] These have been identified as the triplet carbene, the 9-fluorenyl radical (FlH·) and the ylide (ACY) formed by reaction of the carbene with the solvent, Eq. (5). Triplet fluorenylidene is presumed to be formed from the unobserved singlet carbene, and appears with a rise time of *ca* 400 ps following picosecond flash photolysis of the diazo compound. Once formed, triplet fluorenylidene has a half-life of *ca* 17 ns, and is ultimately converted primarily to the ylide. The ylide has a lifetime of many microseconds in the absence of dipolar trapping reagents.



(5)

The optical absorption spectra of the three transient products formed from photolysis of DAF in acetonitrile are different. This permits independent monitoring of each of these products. The triplet carbene has a strong absorption at *ca* 470 nm, the ylide absorbs at 400 nm, and the radical has a maximum in its spectrum at *ca* 500 nm.

Irradiation of DAF *in* CH_3CN *containing nucleophilic reagents*

It is well known that irradiation of DAF in acetonitrile solutions containing MeOH gives high yields of 9-fluorenyl methyl ether.[20–22] This product is formed both by direct irradiation of DAF and by triplet sensitization, which presumably bypasses initial singlet carbene formation. This result, among others, has been employed to support the conclusion that intersystem crossing from ^3Fl to ^1Fl is rapid compared with most bimolecular reactions of the triplet.[20–22] In this the classical model, ^1Fl is the exclusive precursor to ether, and to nucleophilic trapping products in general.

Irradiation of DAF in acetonitrile solution containing t-BuOH similarly gives 9-fluorenyl t-butyl ether in 87% yield. When t-butylamine is used as the nucleophilic trapping reagent, 9-fluorenyl-t-butylamine is formed in *ca* 60% isolated yield. The lower yield in this case probably reflects competitive radical formation—just as Bethell[15] observed for diphenyl carbene. With t-butylthiol as the trapping reagent, 9-fluorenyl t-butylsulfide is formed in *ca* 80% yield.

The rate constants for reaction of fluorenylidene with the nucleophilic trapping reagents were measured using a time-resolved Stern–Volmer technique. Laser flash photolysis of DAF in acetonitrile gives the ylide ACY. Formation of this ylide is complete *ca* 100 ns after the flash. The amount of ylide present is proportional to the change in absorbance at 400 nm ($\phi^{\circ}_{\text{yld}}$). When a nucleophilic trapping reagent is included in the photolysis solution, some carbene is diverted away from formation of ACY, and the change in absorbance at 400 nm is reduced (ϕ_{yld}). These considerations and the additional assumption that at low nucleophile concentration a negligible amount of Fl is captured before equilibrium is established, lead directly to Eq. (6) where k_{CH_3CN} is the known rate constant for reaction of fluorenylidene with acetonitrile,[22] and $k^{\text{obs}}_{\text{nuc}}$ is the apparent rate constant for reaction of the nucleophilic trapping reagent with the carbene. Thus, measurement of the change in absorbance, typically 2 μs after the flash when formation of ACY is complete, but before it reacts, as the concentration of

$$\left(\frac{\phi^{\circ}_{\text{yld}}}{\phi_{\text{yld}}}-1\right)=\frac{k^{\text{obs}}_{\text{nuc}}}{k_{CH_3CN}[CH_3CN]}[\text{nucleophile}] \quad (6)$$

nucleophile is varied gives a measure of $k^{\text{obs}}_{\text{nuc}}$.

We have applied this technique to the measurement of the rate constants for reaction of the heteroatomic nucleophiles listed in Table 1. In addition, we have obtained the rate constants for reaction of the nucleophiles having either H or D bound to the heteroatom. Comparison of the rate constants in these cases ($k^{H}_{\text{nuc}}/k^{D}_{\text{nuc}}$) gives the kinetic isotope effect for the trapping of fluorenylidene with the nucleophile. These data are shown in Fig. 1 for MeOH which exhibits a kinetic isotope effect, and in Fig. 2 for t-butylamine which does not. The calculated rate constants and kinetic isotope effects for the series of nucleophiles examined are summarized in Table 1.

The rate constants for reaction are a measure of the rate of irreversible consumption of fluorenylidene. The derived kinetic isotope effects indicate the effect of isotopic substitution on this process. If there is an intermediate between the carbene and the eventual trap product, then the kinetic isotope effect need not be identical to the product isotope effect. To explore this possibility we examined the products resulting from the competition between protonated and deuterated nucleophiles.

Irradiation of DAF in acetonitrile solution containing both CH_3OH and CH_3OD leads to 9-fluorenyl methyl ether containing either an H or D atom bound to C-9 of the fluorene moiety. Analysis of the product mixture by ^{1}H-NMR or mass spectroscopy permits ready calculation of the ratio of proton to D-containing ether. From these data, and the known concentrations of the isotopically substituted alcohol, the product isotope effect (P_H/P_D) is easily estimated. This result, and those similarly obtained for the other nucleophilic trapping reagents are summarized in Table 1.

Table 1. Data for reaction of fluorenylidene with heteroatom nucleophiles

Nucleophile	Concentration† (M)	Trap‡ yield %	$k^{\text{obs}}_{\text{nuc}}$ ($M^{-1}\,s^{-1}$)	$k^{H}_{\text{nuc}}/k^{D}_{\text{nuc}}$	P_H/P_D
CH_3OH	0.05 (0.5)	90	8.6×10^8	1.8 ± 0.04	2.4 ± 0.1 (1.4)
CH_3OD	0.1 (1.0)		4.7×10^8		
$(CH_3)_3COH$	0.4	87	1.6×10^8	3.8 ± 0.5	3.5 ± 0.3
$(CH_3)_3COD$	1.6		0.42×10^8		
$(CH_3)_3CNH_2$	3.5	60	6.3×10^7	0.94 ± 0.2	2.5 ± 0.3
$(CH_3)_3CND_2$	3.8		7.0×10^7		
$(CH_3)_3CSH$	0.56	78	1.1×10^9	0.92 ± 0.13	2.3 ± 0.2
$(CH_3)_3CSD$	0.55		1.2×10^9		
$CH_3(CH_2)_3SH$	—	55	1.4×10^9	—	—
CH_3OH	0.1	—	—	—	2.4 ± 0.2
CH_3OD	0.4				
$(CH_3)_3COH$	0.4				
$(CH_3)_3COD$	1.6				

† Concentration of reagents used in the determination of the product isotope effects.

‡ Traps are identified as the ether, sulfide, and secondary amine formed from the alcohol, thiol and primary amine respectively.

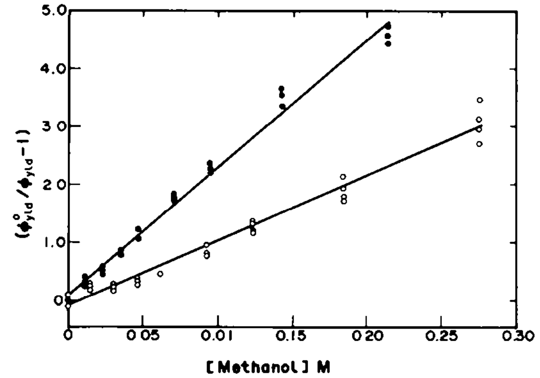


Fig. 1.

For methyl alcohol the product isotope effect shows a dependence on the nucleophile concentration that is not apparent but in the determination of the kinetic isotope effect. For t-butylamine and for t-butylthiol there is no kinetic isotope effect, but there is an easily measured product isotope effect. The observation of a product isotope effect in the absence of a kinetic isotope effect is typically taken to mean that there is a product-determining intermediate formed after the rate-determining step in a reaction sequence.

Examination of the effects of added MeOH on the product isotope effect for ether formation from fluorenylidene and t-BuOH reveals that there must be several steps in this reaction. Irradiation of 0.31 mmol of DAF in a solution made by mixing 12.5 mmol of deuterated MeOH, 12.5 mmol of t-BuOH, and 37.6 mmol of deuterated t-BuOH diluted to 25 ml with acetonitrile gives the expected ethers. The assumption of rapid and statistical distribution of the deuterium between the alcohols leads to calculated concentrations of protonated and deuterated t-BuOH of 0.4 and 1.6 M respectively. Comparison of the ratio of deuterated and protonated t-butyl fluorenyl ether formed from a solution containing 0.4 M protonated and 1.6 M deuterated t-BuOH but no MeOH shows that the product isotope effect for t-butylether formation is reduced to 2.4 by inclusion of the MeOH.

In a related experiment, the ratio of t-butylether to methyl ether products was determined at a constant ratio of methyl and t-BuOH, but over a range of total alcohol concentration. These data are summarized in Table 2. The product ratio changes with total alcohol concentration reaching a constant value when the concentration is greater than *ca* 0.5 M. These observations rule out a simple one-step mechanism and, in conjunction with the measured kinetic and product isotope effect, requires the reversible formation of an intermediate in the reaction sequence.

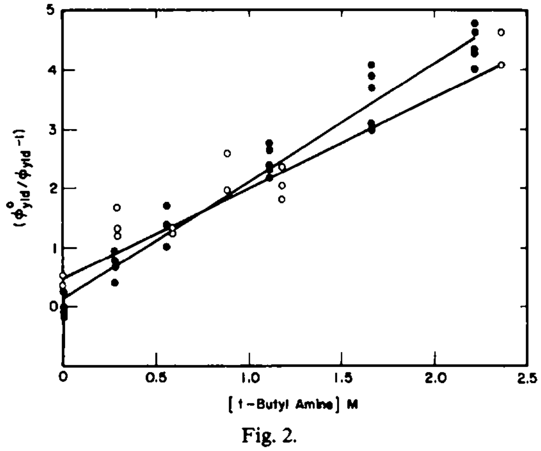
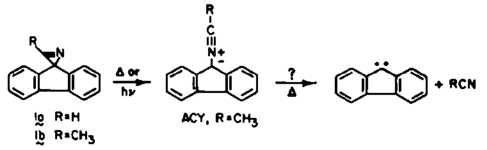


Fig. 2.

Reversibility of ylide formation

The indication is that an intermediate is involved in the reaction of fluorenylidene with the nucleophiles we have examined. A likely candidate for the structure of this intermediate is the ylide, Eq. (2), analogous to that considered by Bethell to be formed in the reactions of diphenylcarbene with alcohols and amines.[4,15] If such an ylide is formed in the reaction of fluorenylidene with methyl or t-BuOH, then our observations require that it be capable of reverting back to the carbene.

Bauer and Hafner[23] report that thermolysis of 2H-azirine **1a** leads eventually to products derived from fluorenylidene. One path from **1a** to the carbene would be through the nitrile ylide, Eq. (7). We examined the photochemistry of 2H-azirine **1b** in an attempt to detect formation of fluorenylidene from the acetonitrile ylide.



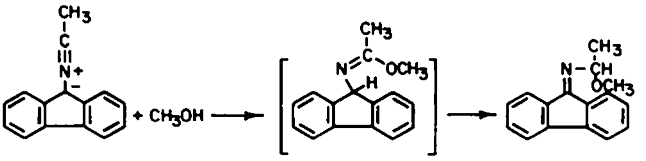
(7)

It is well-known that photolysis of 2H-azirines generate nitrile ylides.[24,25] We have shown that irradiation of **1b** does lead to the acetonitrile ylide.[22] To test for the thermal formation of the carbene from the ylide we irradiated a solution of azirine **1b** in Fluorolube at 77 K. Under these conditions the EPR absorptions of ^{3}Fl, generated by photolysis of DAF, are stable and easily observed. However, no EPR signal is detected from irradiation of **1b**. This indicates that ^{3}Fl is not formed from the ylide under these conditions.

Next, we attempted to trap fluorenylidene from photolysis of **1b** with MeOH. The carbene, as noted above, gives a high yield of 9-fluorenyl methyl ether. The detection of this product from photolysis of **1b** would be a strong indication that ACY can fragment to Fl. Irradiation of **1b** in MeOH gives 1-methoxyethane-fluorenylidene imine, presumably formed by tautomerization of the first formed enol ether, Eq. (8). None of the anticipated ether is found. The rate constant for reaction of the nitrile ylide with MeOH is 1.6×10^2 M^{-1} s^{-1}. Thus we can estimate an *upper limit* for the rate of

Table 2. Competition reaction between methyl and t-BuOH

(MeOH) M	(t-BuOH) M	$P_{MeOH}/P_{t\text{-}BuOH}$
0.08	0.08	5.6
0.1	0.1	5.3
0.2	0.2	4.2
0.5	0.5	2.8
1.0	1.0	2.5



(8)

fragmentation of ACY to Fl of *ca* $1 \times 10^3\ s^{-1}$. In order for fragmentation of the ylide formed from Fl and methanol to make a discernible impact on the mechanism for reaction of Fl with the alcohol, the rate constant for this process would have to be *ca* 1000 times larger than this limit.

DISCUSSION

There are four primary results to be considered. These are: (i) The observed rate constants for reaction of fluorenylidene with the nucleophiles. (ii) The measured kinetic isotope effects on this reaction. (iii) The corresponding product isotope effects and product ratios. (iv) The properties of ylides which have been detected. These results bear on several questions surrounding the mechanism of these nucleophile trapping reactions. The first of these centers on the existence and identity of intermediates in the nucleophile trapping reaction. The second concerns the properties of these intermediates. The third involves the notion of spin-selective reactivity. In particular, the suggestion that triplet carbenes might react with alcohols directly to give ethers.[12] In the discussion that follows we first adopt the classical conclusion that only singlet carbenes give ethers from alcohols, and then later examine the evidence that challenges this position.

With the adoption stated above, we can separate k_{nuc}^{obs} for reaction of Fl with the alcohols into two parts. The first is the equilibrium constant that connects ^{1}Fl and ^{3}Fl (K_{eq}), and the second is the rate constant for reaction of ^{1}Fl with the alcohol (k_{nuc}^{sing}), Eq. (9).

$$k_{nuc}^{obs} = K_{eq} k_{nuc}^{sing} \quad (9)$$

In turn, k_{nuc}^{sing} may be a composite of other rate and equilibrium constants if ether formation is not a one-step process. One indicator of the presence of this additional complexity is the magnitude of k_{nuc}^{sing}. If this rate constant is at the diffusion-controlled limit, then reformation of the carbene from an intermediate, for example, cannot contribute significantly to the reaction mechanism.

It is clear from inspection of Eq. (9) that measurement of k_{nuc}^{obs} cannot independently provide an estimate of k_{nuc}^{sing}. However, comparison of k_{MeOH}^{obs} with k_{MeOD}^{obs} shows that the latter is less than the former. The isotope change in the alcohol will not affect K_{eq}, and thus at least $k_{CH_3OD}^{sing}$ must be less than the diffusion-controlled limit. Comparison of k_{MeOH}^{obs} with $k_{t\text{-}BuSH}^{obs}$ shows that reaction with the thiol is *ca* 30% faster than with the alcohol. Also, in contrast to the reactions of the alcohols studied, the thiols do not show a kinetic isotope effect, and there is no significant rate difference between n- and t-butylthiol. These observations, particularly in light of the product isotope effect shown by the thiol, suggest that these nucleophiles may be reacting with ^{1}Fl at the diffusion limited rate. In this circumstance, k_{MeOH}^{sing} is estimated to be close to, but not at, the diffusion limit in acetonitrile at room temperature. In any event, this analysis shows that the measured value for k_{MeOH}^{obs} cannot be used to exclude the possibility of reformation of fluorenylidene from an intermediate on the path to ether formation.

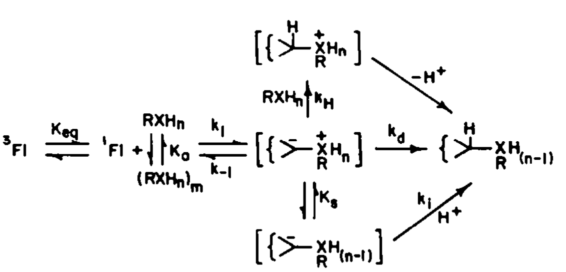
Consideration of the kinetic and product isotope effects on ether formation from fluorenylidene leads to a more definite conclusion about the mechanism of this reaction. There are three observations that must be considered in concert. These are the primary kinetic isotope effect, the product isotope effects, and the reduction by MeOH of the t-BuOH product isotope effect. Interpretation of these effects requires analysis of several "states" in the reaction sequence. Two of these are the starting state which consists presumably of ^{1}Fl and solvated or associated alcohol, and the transition state for formation of an intermediate or the ether.

It is possible that the observed kinetic isotope effects on ether formation might arise from changes in solvation or association equilibria of the alcohol. Indeed, it is known that amines and thiols are much less associated than are alcohols,[26,27] and this difference could account for the absence of a kinetic isotope effect with these nucleophiles. However, the magnitude of the isotope effect on alcohol association in hydrocarbon solution at 40°C is at most 4%. This value seems far too small to account for the observed kinetic and product isotope effects on ether formation.[28]

The results, particularly the reduction in the t-BuOH isotope effect by addition of methanol indicate that there must be some "communication" from one alcohol to another in the reaction sequence. This conclusion is supported by the results of the amine and thiol investigations. In these cases, different product and kinetic isotope effects demand formation of an intermediate after the rate limiting step.

The intermediate formed in the reaction of ^{1}Fl and nucleophiles might be associated alcohol, a carbocation, Eq. (1), or an ylide, Eq. (2). Of the latter two possibilities the ylide is far more likely for several reasons. First, by analogy to the findings of Kirmse *et al.*,[16] the formation of a fluorenyl cation (an anti-aromatic species) seems quite improbable. Next, the observation of a kinetic isotope effect requires that the intermediate revert to carbene, and this is not a known reaction of related cations.[4] Finally, ylide formation is becoming a widely recognized reaction of aromatic carbenes and heteroatomic functional groups.[29–34] For these reasons we suggest that ylide formation could be a step in the reaction of ^{1}Fl with these nucleophiles, Scheme 5.

If the intermediate is the ylide, then the results require that reversion to the carbene (rate constant k_{-1}) be comparable to the reactions that eventually result in irreversible consumption of the ylide (rate constants k_d, k_H or k_i). Our investigation of the properties of the acetonitrile ylide ACY, and the recent findings of others address the general question of carbene formation from ylides.



Scheme 5.

The photolysis of carbonyl ylides,[35,36] among other types, leads to formation of appropriate carbenes. The thermal fragmentation of an ylide to a carbene, though suggested several times, has only recently been documented experimentally. Bakhazi and Warkentin[37,38] report that thermolysis of some oxadiazolines gives first a carbonyl ylide which then fragments to a carbene. However, Wong *et al.*[33] claim that the carbonyl ylide formed from acetone and fluorenylidene does not reform the carbene.

Our finding that ylide ACY does not fragment rapidly to Fl is consistent with the findings for the acetone ylide. However, Turro *et al.*[39] have recently reported that irradiation of some 3,3-diphenyl substituted aziridines does give a detectable yield of diphenyl carbene. Also, they report that diphenyl carbene does not react rapidly with acetonitrile to give the expected ylide. These observations indicate that the rates of ylide formation and dissociation depend strongly on the nature of the carbene, and similarly may depend on the properties of the nucleophile. In this regard, the required 1000-fold difference in reactivity between the nitrile and alcohol ylides of fluorenylidene may be achievable. For these reasons we feel that it is not appropriate to eliminate consideration of ylides as the intermediates involved in ether formation based on the requirement that formation of the intermediate be reversible.

The involvement of an intermediate ylide accommodates the isotope effects observed for t-butylamine and t-butylthiol. In these cases, the absence of a kinetic isotope effect indicates that reformation of the carbene from the ylide does not compete with the reactions that consume the ylide. For the amine, intramolecular discrimination between hydrogen and deuterium bound to nitrogen (the step characterized by k_d in Scheme 5) can account for the observed product isotope effect. For the thiol, isotopic discrimination could occur either by competitive exchange (K_s) followed by either intramolecular transfer (k_d), by direct transfer (k_H), or by intermolecular protonation of the anion (k_i).

It is not possible from our results to identify conclusively the reversible step in the reaction sequence leading from alcohol and carbene eventually to the observed ethers. One possibility is that association of the alcohols (K_a in Scheme 5) affects their rate constants for reaction.[19] In this circumstance the influence of MeOH on the t-BuOH isotope effect and the alcohol concentration dependence in the competition experiment could be due to formation and reaction of a mixed alcohol dimer. A second possibility is that the reversible step is ylide formation. In this case the mixed alcohol isotope effects and competition results are accommodated by changes in the rate of reactions associated with rate constants k_i and k_H in Scheme 5. In support of this proposal we note that intramolecular conversion of the ylide to the ether is a forbidden [1,2] shift. The barrier associated with such a process may encourage reversion to the carbene in competition with intermolecular protonation by alcohol (k_H) or irreversible ether formation from the anion (k_i). Regardless of which path actually operates, these findings do show clearly that ether formation is not a simple one-step reaction.

There is a remarkable parallel between our findings for fluorenylidene and those of Bethell[4] for diphenyl carbene. Apart from differences in observed rate constants (which we have attributed to a smaller fraction of singlet carbene in the equilibrium mixture of carbene spin states for diphenyl carbene)[22] the qualitative observations for both these carbenes are quite similar. It is therefore not surprising that the mechanism we suggest for Fl is closely related to that proposed earlier for diphenyl carbene. We will assume that this parallelism holds in the discussion of the results reported by Scaiano, Griller and Nazran[12] that are used in support of the notion that triplet diphenyl carbene can react directly (i.e. not by forming the singlet carbene first) with MeOH to give diphenylmethyl methyl ether in one step.

Two groups of experiments are suggested to indicate the intervention of the direct reaction of triplet diphenyl carbene with MeOH. The first group is an investigation of the temperature dependence of k^{obs}_{MeOH}, the second involves estimation of a limit for k^{sing}_{MeOH}.

Scaiano *et al.*[12] report that conventional analysis of the temperature dependence of k^{obs}_{MeOH} gives an apparent activation energy (E^{obs}_a) in acetonitrile solution of 1.7 kcal/mol. They argue that in the classical model, which requires exclusive reaction of the singlet carbene with MeOH, the magnitude of this activation energy should be the sum of (i) the energy gap separating ground-state triplet diphenyl carbene from the singlet (ΔE_{st}) and (ii) the activation energy for reaction of the singlet carbene with MeOH (E^{MeOH}_a). Assuming the classical model of carbene reactivity, ΔE_{st} is estimated to be 3–5 kcal/mol. With the implicit assumption that E^{MeOH}_a cannot be negative, Scaiano *et al.*[12] conclude from the observation that E^{obs}_a is smaller than the estimate of ΔE_{st} that the classical mechanism is incomplete, and that triplet diphenyl carbene can react directly with MeOH to give the ether.

Of course, if E^{obs}_a does not correspond to a simple one-step process, then it is certainly possible that its value can be negative. Our results, and the previous findings of Bethell,[4] show without a doubt that ether formation is *not* a one-step process. In this circumstance E^{MeOH}_a itself is the sum of at least two components. One of these can be thought of as the activation energy for the reaction leading to irreversible consumption of the carbene and the other is related to the free energy difference between the species in equilibrium. This latter value can certainly be negative, and thus the sum of these components to give E^{MeOH}_a can also be negative. It is therefore clear that measurement of E^{obs}_a smaller than ΔE_{st} does not necessarily invalidate the classical mechanism.

The second group of experiments used by Scaiano *et al.*[12] to support direct reaction of triplet carbene with MeOH is based on the requirement of the classical mechanism that the triplet carbene does not appear to react with MeOH more rapidly than it forms singlet carbene. They measured the apparent rate of reaction of triplet diphenyl carbene with MeOH at high MeOH concentrations. These admittedly are difficult experiments since the absorbance of the carbene declines as the concentration of MeOH increases. Nonetheless, they are able to extract a maximum rate constant for disappearance of triplet carbene of 2×10^7 s^{-1}. This is somewhat greater than the rate constant for reformation of the singlet carbene from the triplet (k_{ts}) estimated by Turro and Eisenthal[18] using picosecond spectrophotometric techniques. This finding is used by Scaiano *et al.* to support their proposal that reformation of the singlet carbene need not occur before reaction with MeOH.

However, all experimental measurements of k_{ts} are indirect, and even if the true value of this rate constant is the greater value required by the experiments of Scaiano *et al.*, then this finding does not require abandonment of the classical mechanism, but simply a downward revision of ΔE_{st} to *ca* 4 kcal/mol. Actually, this lower value is well within the experimental error associated with the measurements of ΔE_{st}. Thus, this experiment fails to require convincingly the direct reaction of the triplet carbene with MeOH.

CONCLUSIONS

It is clear from this work, and that of previous investigations, that the reaction of a heteroatomic nucleophile with a singlet carbene is usually not a one-step process. Our results indicate the formation of an intermediate. Based in part on the analogy to other systems, we favor identification of this intermediate as an ylide. However, the major findings of this work are independent of the specific structure of the intermediate. There is no compelling evidence for the direct reaction of a triplet carbene with an alcohol to give an ether. The conclusion that ether formation is a characteristic exclusively of singlet carbenes appears, for the time being, to be consistent with all of the known experimental facts.

The application of these findings to photoaffinity labelling experiments is clear. Fluorenylidene reacts rapidly with nucleophilic groups typically found in proteins. The reaction leads to eventual covalent bond formation, and therefore, should be a useful labelling reagent. However, care must be exercised if it is important to identify the site of covalent attachment of the carbene since their reaction with alcohols may be reversible while that with amines and thiols is not.

EXPERIMENTAL

General

PMR spectra were recorded on a Varian Associates HR220 spectrometer with TMS as internal standard. Mass spectra were obtained with Varian MAT CH-5 and 731 spectrometers. The laser spectrometer has been described previously.[40]

Materials

Acetonitrile (Aldrich Gold Label) was refluxed for 4 hr over CaH_2, distilled and then stored under dry N_2. Diazofluorene was prepared by the oxidation of the hydrazone using HgO, m.p. 96–98° (lit.[41] 94–95°). MeOH, t-BuOH, t-butylamine, t-butylthiol, MeOH-d and t-BuOH-d were purchased from Aldrich Chemical Company and used as received. t-Butylamine-d_2 and t-butylthiol-d were prepared by repetitive exchange of the proton(s) bound to the heteroatom with D_2O followed by distillation from Na and then from K for the amine, and from CaO for the thiol. ^{1}H-NMR spectroscopy showed the D incorporation of both compounds to be greater than 98%.

Identification of trap products

Methyl fluorenyl ether has been identified previously as the product formed from photolysis of DAF in acetonitrile containing MeOH.[22] In a similar way we isolated and identified the products resulting from irradiation of DAF in acetonitrile containing the other nucleophiles. For example, a 1.1×10^{-2} M soln of t-BuOH in acetonitrile was purged with dry N_2 and then irradiated with the N_2 laser for 45 min at 5 pulses/s. The acetonitrile was evaporated and the ^{1}H-NMR spectrum of the residue was recorded using hexamethylbenzene as an internal standard and showed the yield of t-butyl fluorenyl ether to be 87%. ^{1}H-NMR ($CDCl_3$) δ 1.56 (S, 9H), 5.42 (s, 1H), 7.2–8.0 (m, 8H); MS (70 eV) *m/e* (rel. abundance): 238 (17), 183 (14), 182 (100), 181 (94), 166 (10), 165 (94), 153 (19), 152 (30); molecular ion calc for $C_{17}H_{18}O$: *m/e* 238.1356. Found: 283.1350.

With t-butylamine photolysis give t-butylfluorenyl amine: ^{1}H-NMR ($CDCl_3$) δ 1.40 (s, 9H), 4.83 (S, 1H), 7.24–7.68 (M, 8H); MS (70 eV) *m/e* (rel. abundance): 237 (5), 222 (20), 220 (11), 180 (22), 165 (100), 164 (27); molecular ion calc for $C_{17}H_{19}N$: *m/e* 237.1518. Found: 237.1518.

With t-butylthiol photolysis give t-butylfluorenyl sulfide: ^{1}H-NMR ($CDCl_3$) δ 1.46 (S, 9H), 4.83 (S, 1H), 7.25–7.71 (M, 8H); MS (70 eV) *m/e* (rel. abundance): 254 (12), 198 (8), 166 (15), 165 (100): molecular ion calc for $C_{17}H_{18}S$: *m/e* 254.1124. Found: 254.1119.

Typical procedure for determining rate constants for reaction of Fl with nucleophilic trapping reagents (MeOH)

A soln of DAF in acetonitrile (7.14×10^{-3} M) was purged with N_2 and irradiated using the N_2 laser. The absorbance at 400 nm 1 μs after the pulse was measured (ϕ°_{yld}). Five measurements of this absorbance were made, the average value was 0.181 ± 0.004. Similar solns were prepared except that they also contained amounts of MeOH ranging from 0.011 to 0.27 M. The absorbance of these solns at 400 nm 1 μs after the laser pulse was also determined (ϕ_{yld}). These data are plotted according to Eq. (6) on Fig. 1.[22] Using 4.06×10^7 M^{-1} s^{-1} for k_{CH_3CN} gives the value of the k^{obs}_{nuc} listed in Table 1. The corresponding rate constants for the other nucleophilic reagents were similarly determined.

Typical procedure for determining the product isotope effects (t-BuOH)

A soln containing DAF (50 mg, 0.26 mmol), t-BuOH (1.26 g, 17 mmol) and t-BuOH-d (5.10 g, 69 mmol) in 25 ml of anhyd acetonitrile was prepared. The soln was purged with dry N_2 for 15 min and then irradiated in a Rayonet photoreactor (350 nm lamps) for 30 min. After the irradiation the solvent and unreacted alcohol were removed under vacuum and the remaining t-butylfluorenyl ether was analyzed by ^{1}H-NMR and mass spectroscopy using electron impact ionization. From the observed D content of the ether, the product isotope effects P_H/P_D reported in Table 1 were calculated. The product isotope effects for the other nucleophiles were obtained similarly except that field ionization was used in the mass spectrometric analysis of 9-fluorenyl methyl ether because electron impact ionization gives a significant M-1 peak.

Acknowledgement—This work was supported by grants from NSF and NIH. We particularly thank Dr. J. C. Cook of this department for his assistance with the mass spectroscopy experiments.

REFERENCES

[1] W. Kirmse, *Liebigs Ann. Chem.* **9**, 666 (1963).

[2] D. Bethell and R. D. Howard, *J. Chem. Soc. B* 745 (1969).

[3] D. Bethell, G. Stevens and P. Tickle, *Chem. Commun.* 792 (1970).

[4] D. Bethell, A. R. Newall and D. Whittaker, *J. Chem. Soc. B* 23 (1971).

[5] H. Tomioka, T. Miwa, S. Sazuki and Y. Izawa, *Bull. Chem. Soc. Jpn.* **53**, 753 (1980).

[6] I. Moritani and T. Nagai, *Nippon Kagaku Zasshi*, **86**, 157 (1965).

[7] H. Tomioka and Y. Izawa, *J. Am. Chem. Soc.* **99**, 6128 (1977).

[8] G. L. Closs and A. D. Trifunac, *J. Am. Chem. Soc.* **92**, 2186 (1970).

[9] H. D. Roth, *Accts. Chem. Res.* **10**, 85 (1977).

[10] J. E. Baldwin and A. H. Andrist, *Chem. Commun.* 1512 (1971).

[11] R. A. Moss and U.-H. Dolling, *J. Am. Chem. Soc.* **93**, 954 (1971).

[12] J. C. Scaiano, A. S. Nazran and D. Griller, Abstracts 186th ACS National Meeting, Washington, D.C., 28 August-2 September (1983); D. Griller, A. S. Nazran and J. C. Scaiano, *J. Am. Chem. Soc.* **106**, 198 (1984).

[13] H. Bayley and J. R. Knowles, *Methods Enzymol.* **46**, 69 (1977); and refs cited.

[14] C. G. Swain, E. C. Strivers, J. F. Renwer and L. J. Schaad, *J. Am. Chem. Soc.* **80**, 5885 (1955).

[15] D. Bethell, J. Hayes and A. R. Newall, *J. Chem. Soc. Perkin II* 1307 (1974).

[16] W. Kirmse, K. Loosen and H.-D. Sluma, *J. Am. Chem. Soc.* **103**, 5935 (1981).

[17] G. L. Closs and B. E. Rabinow, *J. Am. Chem. Soc.* **98**, 8190 (1976).

[18] K. B. Eisenthal, N. J. Turro, M. Aikawa, J. A. Butcher, Jr., C. Dupuy, G. Hefferon, W. Hetherington, G. M. Korenowski and M. J. McAuliffe, *J. Am. Chem. Soc.* **102**, 6563 (1980).

[19] D. Griller, M. T. H. Liu and J. C. Scaiano, *J. Am. Chem. Soc.* **104**, 5849 (1983).

[20] D. Griller, C. R. Montgomery, J. C. Scaiano, M. S. Platz and L. Hadel, *J. Am. Chem. Soc.* **104**, 6813 (1982).

[21] B.-E. Brauer, P. B. Grasse, K. J. Kaufmann and G. B. Schuster, *J. Am. Chem. Soc.* **104**, 6814 (1982).

[22] P. B. Grasse, B.-E. Brauer, J. J. Zupancic, K. J. Kaufmann and G. B. Schuster, *J. Am. Chem. Soc.* **105**, 6833 (1983).

[23] W. Bauer and K. Hafner, *Agnew. Chem. Int. Ed. Engl.* 772 (1969).

[24] A. Padwa and P. H. J. Carlesen, *Reactive Intermediates* (Edited by R. A. Abramovitch), Vol. 2. Plenum, New York (1982).

[25] P. Gilgen, H. Heimgartner, H. Schmid and H. J. Hansen, *Heterocycles* **6**, 143 (1977).

[26] H. Wolff, J. Szydlowski and L. Dill-Staffenberger, *J. Phys. Chem.* **85**, 1047 (1981).

[27] J. Valero, M. Garcia and C. Gutierrez-Losa, *J. Chim. Phys.* **76**, 445 (1979).

[28] H. Landeck, H. Wolff and R. Gotz, *J. Phys. Chem.* **8**, 718 (1977).

[29] W. H. Pirkle and G. F. Koser, *Tetrahedron Lett.* 3959 (1968).

[30] W. A. Sheppard and O. W. Webster, *J. Am. Chem. Soc.* **95**, 2695 (1973).

[31] W. Ando, *Int. J. Sulfur Chem. B* **7**, 189 (1972).

[32] P. deMarch and R. Huisgen, *J. Am. Chem. Soc.* **104**, 4953 (1982).

[33] P. C. Wong, D. Griller and J. C. Scaiano, *J. Am. Chem. Soc.* **104**, 6631 (1982).

[34] A. S. Kende, P. Hebeisen, P. J. Sanfilippo and B. H. Tader, *J. Am. Chem. Soc.* **104**, 4244 (1982).

[35] T. Do-Minh, A. M. Trozzolo and G. W. Griffin, *J. Am. Chem. Soc.* **92**, 1402 (1970).

[36] K. N. Houk, N. G. Rondan, C. Santiago, C. J. Gallo, R. W. Gandour and G. W. Griffin, *J. Am. Chem. Soc.* **102**, 1504 (1980).

[37] M. Bekhazi and J. Warkentin, *J. Am. Chem. Soc.* **103**, 2473 (1981).

[38] M. Bekhazi and J. Warkentin, *J. Org. Chem.* **47**, 4870 (1982).

[39] N. J. Turro, D. A. Hrovat, I. R. Gould, A. Padwa, W. Dent and R. J. Rosenthal, *Angew. Chem. Int. Ed. Engl.* **22**, 625 (1983).

[40] K. A. Horn and G. B. Schuster, *Tetrahedron* **38**, 1095 (1982).

[41] A. Schonberg, W. I. Awad and N. Latif, *J. Chem. Soc.* 1368 (1951).