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A Formal CH Insertion Reaction of An Aryl Nitrene into an Alkyl CH Bond. Implications for Photoaffinity Labelling.

Mary Jennifer Torres, José Zayas and Matthew S. Platz[1]

Department of Chemistry, The Ohio State University, Columbus, OH 43210

Abstract

Triplet m-nitrophenyl nitrene reacts with frozen polycrystalline toluene at the benzylic position to give appreciable yields of a formal CH insertion adduct. This reaction does not occur on photolysis of m-nitrophenyl azide in toluene solution.

Photoaffinity labelling (PAL) is a widely used technique in biochemistry and aryl azides are undoubtedly the most popular of all labelling reagents.[2] In the PAL experiment one appends an azide to a natural ligand of a biological receptor. Photolysis of the receptor-ligand complex should produce a reactive intermediate capable of forming a covalent bond to the biomolecular host. In an ideal case the reactive intermediate will undergo a formal insertion into an alkyl CH bond. Unfortunately, this is an extremely rare reaction for aryl nitrenes.[3] Photolysis of phenyl azide in alkanes or in toluene leads mainly to tar accompanied by small amounts of azobenzene, aniline, and virtually zero insertion product.[3] A few examples of formal nitrene insertion into aryl CH bonds have been reported.[4] The only known example of aryl nitrene insertion into an alkyl CH bond was reported by Lindley who performed triplet sensitized photolyses of phenyl azides at high (>100°) temperature.[5] Although very interesting the conditions of high temperatures and photosensitization are inappropriate for PAL. In this letter we report a set of experimental conditions which do lead to formal nitrene CH insertion that may be compatible with PAL.

Moss,[6] Tomioka,[7] and Platz[8] have found that carbenes experience markedly enhanced yields of formal CH insertion products when they are generated in low temperature solids. Accordingly the photochemistry of phenyl azide was studied as a function of temperature and phase.[9] Leyva found that photolysis of phenyl azide at low temperature gave high yields of the triplet phenyl nitrene derived products aniline and azobenzene, in marked contrast to the near exclusive formation of singlet derived products in solution at room temperature. No products of formal triplet phenyl nitrene CH insertion with toluene or cyclohexane were observed at low temperature, however. Reiser and Leyshon have shown that electron withdrawing groups increase the reactivity of triplet aryl nitrenes.[10] Accordingly we repeated our analysis of the temperature and phase dependent photochemistry of aryl azides with m-nitrophenyl azide 1.

Photolysis of **1** in toluene at 25° gives considerable tar, 24.1% azo compound **4** and only 4.7% m-nitro aniline **5** (Table 1).[11] At -76° the yield of **4** has increased to 55.4%. The polymeric tar formed on photolysis on phenyl azide has been attributed to singlet state intermediates (such as singlet phenyl nitrene or azacycloheptatetraene) because triplet sensitized photolysis gives zero tar and good yields of azo benzene.[3] By analogy we attribute tar formation from **1** to singlet intermediates. Sensitization was not practical with **1** because it absorbs very strongly above 300 nm (ε_{350} = 1300 $M^{-1}cm^{-1}$). Azo compound **4** is most likely formed by dimerization of nitrene **2t**, or by reaction of **2t** with azide **1**.[3]

Photolysis of 1 in frozen toluene between -125° and -196° gave somewhat different results than photolysis in fluid solution. Azo Compound **4** and tar are observed as before but formal CH insertion adduct 3 is now present. The yield of **3** is quite poor at -125° and -150° but at -196° a respectable 27-30% yield of adduct can be realized. It should also be noted that the material balance of 82.6% achieved in frozen toluene at -150° is almost 3 times larger than the material balance of 28.8% in solution at room temperature. EPR studies (vide infra) demonstrated that **2t** does not react at -196° but reacts only upon thawing the sample to -150° or still higher temperatures.

Table 1. The distribution of products formed on photolysis of **1** in toluene.[a]

T(±2°C)	Reaction Products (Absolute % Yield)		
	5	**3**	**4**
25[b]	4.7	-	24.1
-76[b]	<0.1	-	55.4
-125[b]	3.9	10.5	59.5
-150[b]	-	4.9	77.7
-150[c]	-	3.8	65.2
-150[d,g]	-	7.8	54.6
-196[e]	-	29.5	23.7
-196[f]	-	26.8	4.0

[a] λ = 350 nm Rayonet Photochemical Reactor, 4h, all products analyzed by HPLC

[b] 1.07 x 10^{-2}M 1 and nitrobenzene (internal standard) in toluene

[c] 2.13 x 10^{-3}M 1 " " " " " "

[d] 1.12 x 10^{-1}M 1 " " " " " "

[e] The frozen solution was thawed directly after photolysis.

[f] The frozen solution was warmed to and kept at -125°C for 1h after photolysis before it was thawed to room temperature.

[g] Complete conversion of 1 was achieved in all cases except this one. In this case 36% of 1 remained after photolysis.

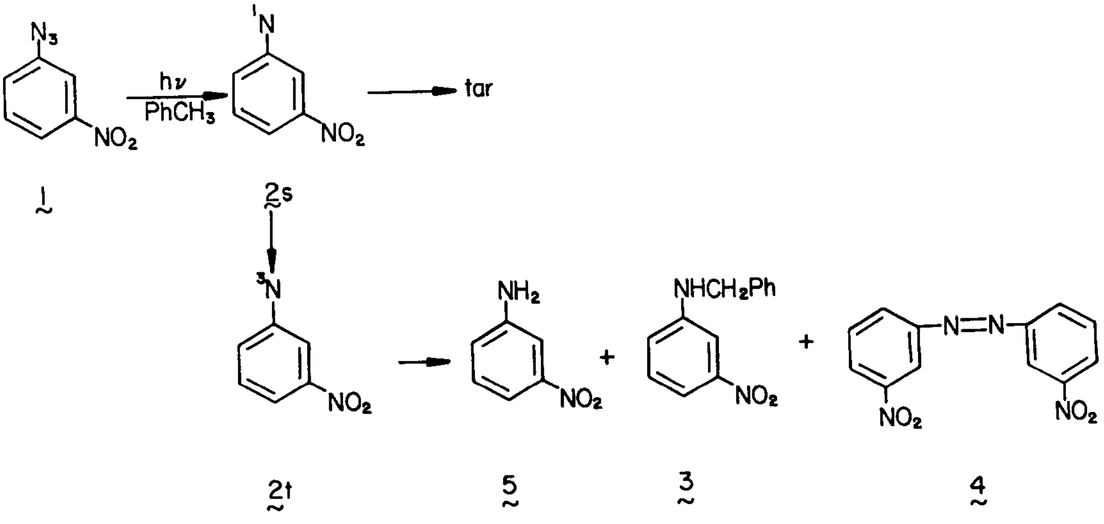
Solid state conditions enhance the yield of insertion product by retarding the rate of azo formation. In the polycrystalline solid, diffusion is quite limited and nitrene **2t** can react only with neighboring molecules. There must be some matrix sites in which **2t** is surrounded by toluene and can not react with azide **1**. This approach appears to be quite promising for PAL because an enzyme bound nitrene can not react with an azide precursor and the solid state conditions prevent diffusion and subsequent reaction of the nitrene outside of the binding site.

EPR analysis of this system demonstrates that reaction occurs in the solid state and not on annealing the sample. Brief photolysis of 0.1M **1** in toluene between -152^0 and -164^0 gives the meta stable EPR signal of **2t**. The initial decay kinetics were analyzed to give the data of Table 2. At temperatures above -150^0 signal decay was too rapid to measure, below -170^0 **2t** was very long lived. Product studies reveal that the major decay route of **2t** at -150^0 is by azo formation, only a small fraction of the overall EPR signal decay of **2t** can be associated with reaction with toluene. A small kinetic isotope effect of ≃ 2-3 was observed which is consistent with partial contribution of hydrogen atom abstraction to the overall matrix decay of **2t**. The Arrhenius parameters are strongly influenced by the matrix environment and have little relevance for solution phase work.

Studies of the low temperature matrix photolysis of enzyme bound aryl azides are in progress.

Table 2. Initial decay kinetics of **2T** in polycrystalline toluene determined by EPR[12]

Temp^0(C)	Toluene $k(s^{-1})$	Toluene-d8 $k(s^{-1})$
-152	$(2.5\pm0.2)x10^{-2}$	$(7.3\pm0.3)x10^{-3}$
-154	$(5.0\pm0.5)x10^{-3}$	$(1.4\pm0.2)x10^{-3}$
-157	$(1.4\pm0.2)x10^{-3}$	$(6.7\pm2.4)x10^{-4}$
-161	$(8.0\pm0.8)x10^{-4}$	$(2.8\pm0.3)x10^{-4}$
-164	$(4.0\pm0.3)x10^{-4}$	$(1.4\pm0.1)x10^{-4}$
E_a (Kcal/Mol)	7.39	7.02
log A (s^{-1})	11.3	10.2



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