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p-Toluenesulfonyldiazoacetates as Photoaffinity Labeling Reagents

Sir:

Photoaffinity labeling has become an important method for the identification of target sites in biological systems, as well as for marking the amino acid residues in the vicinity of the active sites of enzymes.¹ Many new reagents and some new procedures have been invented since the method was introduced² in 1962. The diazo functionality has been incorporated into many of the reagents for photoaffinity labeling and is advantageous insofar as the carbenes produced on photolysis are highly reactive and insert promptly into any nearby bond, including C-H bonds.³ Nevertheless, most of the diazo esters previously employed have suffered from two defects: instability toward heat and acid, and loss of reagent during photolysis because of Wolff rearrangement. To achieve effective labeling, the carbene produced on photolytic decomposition of diazo compounds must insert into surrounding molecules or amino acid residues; when, however, photolysis of a diazo ester leads to Wolff rearrangement, it produces a ketene which generally⁴ reacts with solvent; if it does so, the product provides no new information concerning the enzyme or other biological target. The Wolff rearrangement consumes (and therefore wastes) an important fraction of the photoproduct from ordinary diazo ester and all of it from most diazo esters of thiols.⁵

New configurations for the diazo functionality are therefore needed where the compounds are thermally stable, insensitive to acid, and not subject to Wolff rearrangement. This communication reports the preparation of compounds which, to a large extent, meet these requirements. We have examined the photolysis of ethyl 2-diazo-2-*p*-toluenesulfonylacetate, **A**, of ethyl 2-diazo-2-*p*-toluenesulfonylthioacetate, **B**, and of related *p*-toluenesulfonyldiazoacetates. These serve as models for proteins derivatized with the sulfonyldiazoacetyl group. Further, we have prepared and characterized two reagents that will be of use in derivatizing thiol or activated hydroxyl groups: 2-diazo-2-*p*-toluenesulfonyl acetyl chloride, **C**, and *p*-nitrophenyl 2-diazo-2-*p*-toluenesulfonyl acetate, **D**. The reagents themselves are apparently indefinitely stable on storage at room temperature; the ester, **A**, is unaffected by 1 M acid. The *O*-ethyl ester, **A**, undergoes photolysis at long wavelengths in methanol with at least 95% insertion into the -OH bond of the solvent; no product of Wolff rearrangement was detected. Furthermore, the extinction coefficient of ethyl 2-diazo-2-*p*-toluenesulfonylacetate is 140 at 370 nm, whereas that for ethyl diazoacetate⁷ is only 14. The greater extinction coefficient leads to rapid photolysis and may allow photolysis in reasonable times in those cases where the biological molecules to which the reagent is attached are destroyed by short wavelength ultraviolet. See Figure 1.

The principal product (90-95% yield) of the photolysis at 2537 or 3500 Å of ethyl 2-diazo-2-*p*-toluenesulfonylacetate in methanol is **E** (R = CH₃), of insertion of the carbene, produced by photolysis, into the -OH bond of methanol. The principal minor products are shown below.

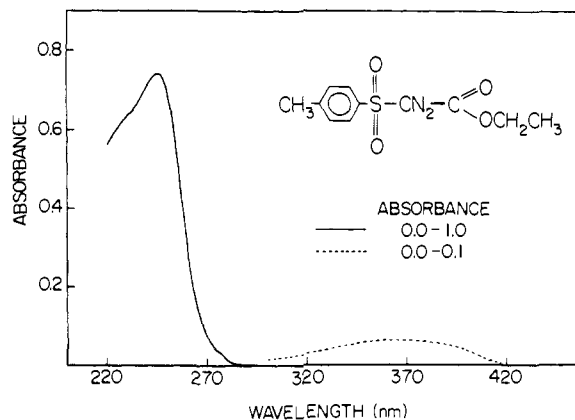
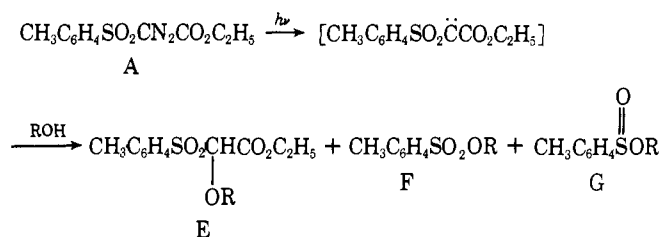
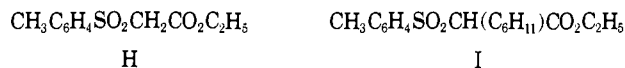


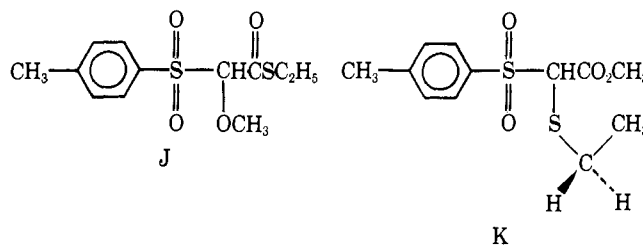
Figure 1. Ultraviolet spectrum of a 5.04×10^{-5} M solution of ethyl 2-diazo-2-*p*-toluenesulfonylacetate. Note change of absorbance scale at 300 nm.



Photolysis of **A** in ethanol leads, in addition to **E**, **F**, and **G** (R = C₂H₅), to the reduced compound, **H**. The photolysis of **A** in cyclohexane solution, either at 2537 or at 3500 Å, led to the formation of **I** (90% yield).



The photolysis of the *p*-toluenesulfonyldiazoacetates of thiols is of interest because simple diazoacetates of thiols⁵ undergo complete Wolff rearrangement.⁸ When the photolysis of **B** was carried out at 3500 Å, the products consisted of 25% **J**, the product of insertion into the solvent, and 68% **K**, the product of Wolff rearrangement. The sulfonate and sulfinate, **F** and **G** (R = CH₃), occur as minor by-products.



The acid chloride, **C**, was prepared in 46% yield by mixing 710 mg of *p*-toluenesulfonyldiazomethane,⁹ 0.9 mL of phosgene, and 775 mg of Proton Sponge in 50 mL of methylene chloride in the dark at Dry Ice temperatures. The black reaction mixture was warmed to room temperature overnight, washed in ether solution with acid and water, dried, and evaporated. The resulting brown solid was chromatographed in toluene-hexane (3:1) over 35 g of Woelm grade II silica gel. The product, recrystallized from methylene chloride-hexane, melted at 107-108 °C.¹⁰ The *p*-nitrophenyl ester, **D**, was prepared in 83% yield by stirring 150 mg of **C** with 112 mg of dried sodium *p*-nitrophenylate for 8 h at room temperature under nitrogen in 50 mL of methylene chloride. Evaporation of the filtered solution yielded yellow crystals that, after re-

crystallization from methylene chloride-hexane, melted at 145.5–146.5 °C. The thioethyl ester, B, was prepared in 65% yield from the acid chloride, C, and ethyl mercaptan in ether solution with added 2,6-lutidine: mp 62.5–63.5 °C. The *p*-toluenesulfonyldiazoacetate, L, of *N*-acetylcysteine methyl ester was prepared in 83% yield from C and acetylcysteine methyl ester in methylene chloride solution in the presence of 2,6-lutidine: mp 123.5–124.5 °C.

The photolyses were carried out with 0.1–0.2-mL samples of 0.1–0.3 M solutions of diazo compounds in water-jacketed quartz or Pyrex tubes in Rayonet RPR 100 reactor at 25 °C. The products of the various photolyses were separated by gas phase chromatography on a 6 ft × 1/8 in. aluminum column packed with Dexil 400 GC/Chromosorb WHP 80/100, using a Hewlett-Packard Model 5750 chromatograph. The NMR spectrum of E (R = C₂H₅) is distinguished by an eight-line pattern from the diastereotopic hydrogen atoms of the methylene group of the ethyl ether. By contrast, the signals from the ethyl group of E (R = CH₃) appear as a simple quartet.

The identity of the reduction product, H, was determined by comparison with an authentic sample.¹¹ The mass spectrum of F is in agreement with that in the literature.¹² The NMR spectrum of the methyl ether thioethyl ester, J, shows a quartet for the methylene group of the ethyl residue, whereas the spectrum of the methyl ester thioethyl ether, K, shows a doublet of quartets, arising from the diastereotopic protons of the methylene residue of the thioethyl group in close proximity to the asymmetric center of the molecule.

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Photochemistry of Iron(II) Diimine Complexes in a Room Temperature Molten Salt

Sir:

Recently, several authors have reported that excited states of tris(2,2'-bipyridine)ruthenium(II), Ru(bipy)₃²⁺, and related metal complexes can be quenched very efficiently by electron transfer to suitable acceptors.^{1–5} In some cases, the quenching and the electron-transfer processes occur separately,⁶ and the quenching process occurs mainly by energy transfer.^{7,8} Some authors⁴ have reported examples in which both quenching processes occur in parallel when using quenchers of relatively low excited triplet state energy. The quenching of long-lived excited states by electron transfer yielding unstable products in the ground state can be an important energy conversion process.

We wish to report the first preliminary studies of the photochemical behavior of iron(II)-diimine complexes in a room temperature molten salt composed of aluminum chloride and ethylpyridinium bromide in a 2:1 molar ratio.⁹ We have found that, upon irradiation with low intensity visible light, most of the iron(II) complexes are converted into the corresponding iron(III) complexes. Two examples, shown in Figure 1, are

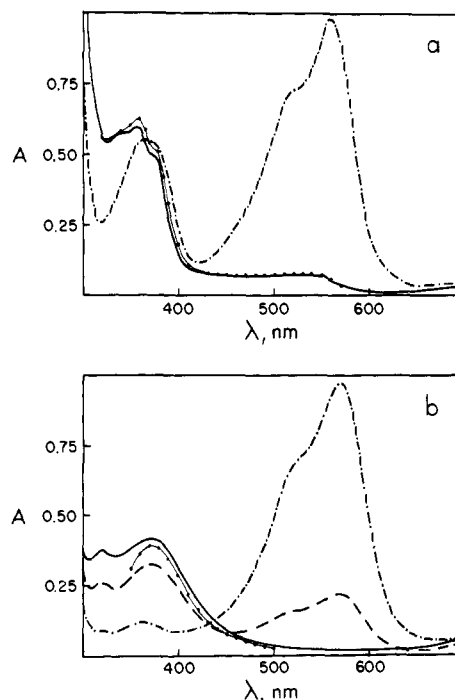


Figure 1. a: absorption spectra of 10^{-3} M Fe(PMM)₃²⁺ in the 2:1 aluminum chloride-ethylpyridinium bromide melt (---); after irradiation with visible light for 96 h (—); absorption spectra of 6×10^{-4} M Fe(PMM)₃³⁺ prepared by controlled potential coulometry (....). b: absorption spectra of 10^{-3} M Fe(BMI)₃²⁺ in the 2:1 aluminum chloride-ethylpyridinium bromide melt (---); after irradiation with visible light for 72 h (---); after irradiation for 96 h (—); absorption spectra of 10^{-3} M Fe(BMI)₃³⁺ prepared by controlled potential coulometry (....). All spectra at room temperature.