certain reactions, the product yield was increased by the addition of fluorotrimethylsilane (but not chlorotrimethylsilane). Cf. C. K. Ingold, "Structure and Mechanism in Organic Chemistry", 2nd ed, Cornell University Press, Ithaca and London, 1969, Chapter 13.

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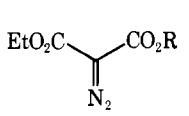
A New Class of Potential Photoaffinity Labels. α-Diazophosphonic Acids: Synthesis and Stability

Sir:

The technique of photoaffinity labeling has rapidly become a popular method for the covalent attachment of substrate analogues to protein binding sites.[1] Much of this popularity stems from an increasing demand for a versatile, specific labeling technique which is applicable to the study of heterogeneous systems, such as ribosomal and membrane-bound proteins.[2] Various problems are associated with the most commonly used photoactivated functional groups, the diazomalonyl esters (**1**)[3] and the aryl azides (**2**).[4] One of these problems arises from their "irrelevance" to most biological systems of interest, and requires that they be appended to a noninterfering region of a larger molecule which binds to the protein in question. This stricture precludes their use for small or highly selective binding sites, and no doubt in some cases forces them to project away from the protein, into solution. Recently, other functional groups, such as aryldiazirines,[5] aryl ketones,[6] phosphoryl azides,[7] 2-diazo-3,3,3-trifluoropropionates,[8] and α-diazobenzylphosphonate dianion,[9] have been suggested for photoaffinity labeling, but for the most part they do not address this problem.



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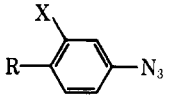

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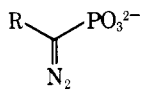
We are interested in α-diazophosphonic acids (**3**) as possible photoaffinity labeling reagents which may surmount these limitations. Our intention is that they may mimic the analogous phosphates (**4**) and make available a wide range of potential labeling reagents. In this communication we describe the synthesis and characterization of three diazophosphonic acids: the diethylsulfamoyl, diisopropylphosphono, and dimethylcarbamoyl derivatives of diazomethylphosphonic acid, **3a–3c**.



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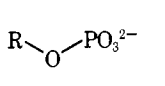

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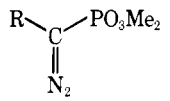

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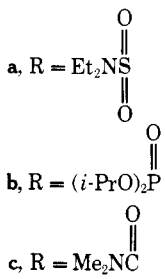
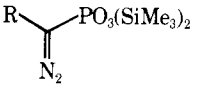


Except for a recent report[9] on the synthesis, in undisclosed yield, of the relatively unstable α-diazobenzylphosphonic dianion (**3**, R = Ph), no other α-diazophosphonic acid has been described, although the synthesis of their dialkyl esters is well-established.[10] In the present instance, condensation of dimethyl phosphorochloridate with the anions derived from *N,N*-diethylmethanesulfonamide and diisopropyl methylphosphonate afforded the methylene derivatives **5a**[11] and **5b**[11] in yields of 66 and 71%, respectively. The corresponding diazo compounds (**6**)[12] were prepared in more than 80% yield by treatment of these materials, and the dimethylcarbamoyl derivative (**5c**),[11,13] with potassium hydride and *p*-toluenesulfonyl azide in THF at 0–25 °C.[10] Transesterification of the methyl esters with bromotrimethylsilane[14] was rapid at 0–25 °C, cleanly furnishing the extremely moisture-sensitive bis(trimethylsilyl)esters (**7**), which were hydrolyzed under very mild



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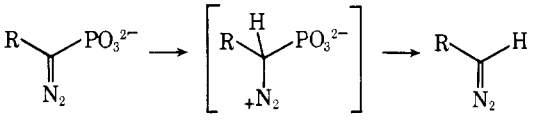
(pH 8–9) aqueous conditions without further purification. This two-step hydrolysis sequence is essentially quantitative and allows one to take advantage of the stability and convenience of phosphonate methyl esters.

Our major concern at the outset was the lifetime of the diazophosphonic acids at biochemically useful pH, when the stabilization of the diazo moiety by the phosphono group is reduced by ionization. For instance, while ethyl diazoacetate and diethyl diazomalonate are reasonably stable at neutral pH, diazoacetate ion and diazomalonate dianion have half-lives on the order of only 2 min and 3 h, respectively, under these conditions.[15] The half-life for decomposition of α-diazobenzylphosphonate dianion is also very short in aqueous solution (e.g., <1 min at pH 8.0 in 0.1 M Tris/KCl at 25 °C), although it can be prolonged by judicious choice of buffer.[9] However, the ^{13}C NMR spectra (Table I) of the materials produced on hydrolysis of **7a–7c** at pH > 9 clearly indicate that the desired functionality survives the ester hydrolysis procedure and that the diazophosphonic acids **3a–3c** can in fact be obtained by this route.

The most striking change in the ^{13}C NMR spectrum on going from the diesters to the dianions is the approximately 60-Hz decrease in the coupling constant of the diazo carbon to the phosphorus in question.[16] This reduction in coupling constant most likely reflects the expected decrease in interaction between the electron-rich α-carbon and the phosphorus as the electronegativity of the phosphorus moiety declines on ionization.

The stability and mode of decomposition of the phosphonates **3a–3c** were followed by ultraviolet spectroscopy at lower pH in 0.2 M potassium phosphate at 21–22 °C. In each case, the behavior was consistent with the two-step process of eq. 1,



3 **9** **10**

$$+ P_i \longrightarrow \text{loss of chromophore} \qquad (1)$$

with initial loss of phosphate followed in a slower step by decomposition of the neutral diazo compound (**10**). After completion, or near completion, of the first step in the decomposition of **3**, extraction of the aqueous solution with CH_2Cl_2 affords a neutral material, with the UV spectrum of the intermediate (Table II). In the case of the carbamoyl derivative, this substance was identical with an authentic sample of **10c**[17]

Table I. ^{13}C NMR Resonances of the Diazo Carbons of **3** and **6**

R	**3**, δ^a (J_{CP})	**6**, δ^b (J_{CP})
a, Et_2NSO_2	60.9 (152 Hz)	59.4 (216 Hz)
b, *i*-Pr_2O_3P	37.9 (147, 200 Hz)0137.6 (204 Hz)	
c, Me_2NCO	55.0 (165 Hz)	49.7 (220 Hz)

[a] In D_2O, pH > 9, dioxane as internal reference (δ 66.5). [b] In $CDCl_3$, referenced to $CDCl_3$ as δ 77.0.

Table II. λ_{max} (log ϵ) of Diazo Derivatives

R	**6**[a]	**3**[b]	**10**[b]
a, Et_2NSO_2	227 (3.85)	233 (3.85)	226 (3.93)
	373 (1.65)	393 (1.64)	390 (1.78)
b, $(i\text{-PrO})_2PO$	227 (3.83)	238 (3.85)	225 (4.09)[c]
	337 (1.08)	367 (1.71)	365 (1.14)[c]
c, Me_2NCO	244 (3.86)	263 (4.17)	254 (4.35)
	357 (1.58)	381 (1.81)	376 (1.36)

[a] Spectra run in H_2O. [b] Spectra run in 0.2 M phosphate buffer, pH 9. [c] Log ϵ for the dimethyl ester.

Table III. Half-Lives for the Decomposition of **3** → **9** at 21–22 °C

Derivative	pH[a]	$t_{1/2}$
a, R = Et_2NSO_2	6.0	5 h
	7.5	>5 days
b, R = $(i\text{-PrO})_2PO$	6.0	20 h
	7.5	>5 days
c, R = Me_2NCO	7.5	<5 min[b]
	9.0	40 min

[a] 0.2 M potassium phosphate. [b] $t_{1/2}$ = 3 min in 0.01 M potassium phosphate, pH 7.5.

by TLC, UV, and by its rate of decomposition at pH 6.0. The UV spectrum of the compound extracted from the decomposition of **3b** was very similar to that of authentic dimethyl diazomethylphosphonate.[10a] Although an authentic sample of *N,N*-diethyldiazomethanesulfonamide (**10a**) prepared by another method was not available for comparison, the IR and UV spectra of the material extracted from the decomposition of **3** were fully consistent with the assigned structure. Decomposition of the monomethyl ester of diazomalonic acid occurs similarly at pH > 3, with loss of CO_2 and formation of ethyl diazoacetate.[15b] In the decomposition of **3a–3c**, loss of metaphosphate from the diazonium zwitterion **9** is strictly analogous. We have not attempted to determine the structure of the final, UV-inactive products.

The half-lives for the decomposition of the diazophosphonic acids via loss of phosphate are indicated in Table III. Both the sulfamoyl- and phosphono-stabilized diazomethylphosphonic acids are moderately stable even at pH 6.0, and exceedingly stable at pH 7.5. In this respect they are the first examples of this class of compounds to show real promise as photoaffinity labeling groups. Their stability is in marked contrast to that of the carbamoyl derivative **3c** and the α-diazobenzylphosphonate dianion.[9] The latter compounds decompose within minutes at neutral pH in buffers commonly employed for biochemical work, and could be applied only to protein systems which have sufficient stability at much higher pH.

The relative instability of carbonyl- and phenyl-stabilized compounds would be a definite limitation for the design of possible photoaffinity labels incorporating the α-diazophosphonic acid moiety. Nonetheless, a variety of useful compounds based on sulfonyl- and phosphono-stabilized structures can still be envisaged. We are currently investigating the photochemistry of compounds **3a** and **3b** as further evaluation of their potential for photoaffinity labeling.

Acknowledgment. Support for this research was provided by the National Institutes of Health (through Grant No. GM-21612) and the National Science Foundation (through departmental Equipment Grant CHE-76-05512).

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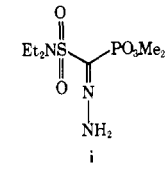

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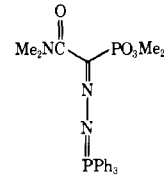

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Hypovalent Radicals.[1] 1. Electrochemical Generation of Diphenylcarbene Anion Radical

Sir:

Nine possible substituted carbon molecular fragments, either neutral or bearing unit charge, may be derived from tetracovalent carbon species, R_4C, by stepwise loss of $R:^-$, R·, or