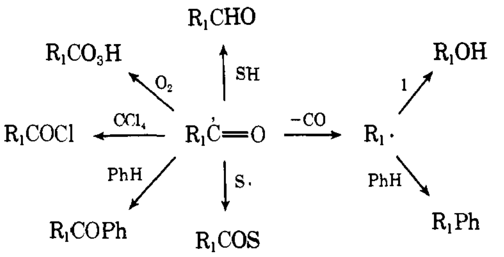
Scheme III. (SH = solvent or additive)



photolysis in toluene afforded bibenzyl and 1,1,2-triphenylethanol.[22] Benzil from **1d** is probably formed by the scission of benzyl radical from **2**, a competitive reaction with eq 6.[4] On the other hand, the photolysis of the parent ketone of **1a** gave benzil by dimerization of benzoyl radical,[12]

Experimental Section

Materials. α-Hydroperoxy ketones, **1a–d,f,g,** have been described previously.[3] Peroxide **1e** was synthesized in 100% yield by method I in the previous report;[3] mp 18–20 °C (petroleum ether); ir (neat) 3380 (OOH) and 1690 cm^{-1} (C=O); NMR (CCl$_4$) δ 1.45 (s, 6 H, 2 Me), 2.13 (s, 6 H, 2 *o*-Me), 2.21 (s, 3 H, *p*-Me), 6.80 (s, 2 H, 2 *m*-H), and 7.87 (broad s, 1 H, OOH).

Photolysis and Product Analysis. The photolysis of **1** in a Pyrex test tube was carried out mostly with a Halos 300-W high-pressure Hg lamp through a Pyrex filter. Mostly 0.01 M benzene solutions of **1** were irradiated under nitrogen after three to five freeze–pump–thaw cycles.

Quantum yields were determined by iodometry[3] and the ferrioxalate actinometry[23] using a Corning CS 7-51 filter and a Pyrex filter, and the results are listed in Table II. For comparison, the yield for the photoreduction of benzophenone in *i*-PrOH was determined and shown to agree well with the literature value (Table II).

All products were determined by GLC in comparison with an authentic sample using three different columns (1 m; temperature, 80–250 °C): (i) Apiezon grease L, 15% on Celite 545; (ii) PEG 20M, 2% on Chamelite CK; (iii) PEG succinate, 13% on Chromosorb. Propiophenone or benzophenone were used as an internal standard.

The peroxidic mixture of **1** and peracid was determined as follows: An aliquot (2 ml) was added into a mixture of MeOH (5 ml) and Me$_2$SO (0.5 ml), and peracid was completely consumed by 5 min of standing; the remaining **1** was titrated iodometrically as reported previously.[3] The total peroxide (**1** + peracid) may be determined without the preliminary treatment with Me$_2$SO. The difference between the two titrations corresponds to the amount of peracid.

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(15) We treaed here the carbonyl and the peroxy groups as separate chromophores, since the two groups are linked by a saturated carbon. In fact, the absorption maximum of **1** is very close (within 2 nm) to that of the corresponding parent ketones; e.g., the maxima for **1a** are at 249 and 327 nm in EtOH, while those for PhCOCHPh$_2$ are at 247 and 325 nm.
(16) In fact, the absorption maximum of **1f** is at 282 nm in water, which is very close to 284 nm for the parent ketone *i*-Pr$_2$C=O. For the tabulation of triplet energies of various ketones, see S. L. Murov, "Handbook of Photochemistry", Marcel Dekker, New York, N.Y., 1973, p 27.
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α-Diazobenzylphosphonate Dianions

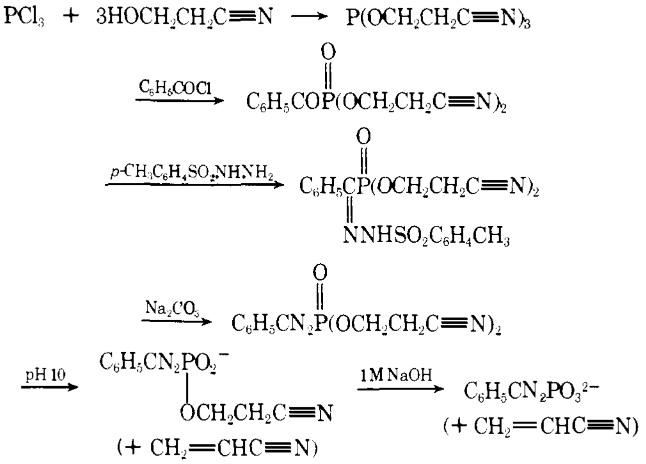
Jorge A. Goldstein,* Charles McKenna, and F. H. Westheimer

Contribution from the James Bryant Conant Laboratories, Harvard University, Cambridge, Massachusetts 02138. *Received February 17, 1976*

Abstract: Aqueous solutions of disodium α-diazobenzylphosphonate can be prepared by the action of alkali on the dicyanoethyl ester of α-diazobenzylphosphonic acid. The salt is somewhat unstable at room temperature even at neutrality; the decomposition is general acid catalyzed. The properties of the dianion are compared with those of the corresponding mono- and dicyanoethyl esters and of other diazo compounds that can serve as reagents for photoaffinity labeling.

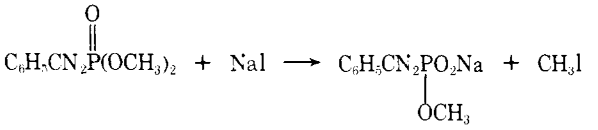
Diazo esters have proved useful for the thermal,[1] metal ion catalyzed,[2] and photoaffinity[3,4] labeling of enzymes and other important biological materials. While diazophosphonates might then be expected to prove useful in similar reactions at binding sites for phosphates on enzymes and membranes, no dianion of a diazophosphonate has previously been reported. This paper records the successful preparation of disodium α-diazobenzylphosphonate in aqueous solution. Although the

Scheme I



compound is somewhat unstable even in alkaline solution, it may nevertheless prove useful.

Seyferth[5,6] has previously prepared dimethyl α-diazobenzylphosphonate. We have found that the former reacts with sodium iodide to yield sodium methyl α-diazobenzylphosphonate (eq 1). Attempts to remove the second methyl group,



$$C_6H_5CN_2\overset{O}{\overset{\|}{P}}(OCH_3)_2 + NaI \longrightarrow C_6H_5CN_2\underset{OCH_3}{\underset{|}{P}}O_2Na + CH_3I \qquad (1)$$

however, failed, and were perhaps doomed to failure, since neither attack of hydroxide on phosphorus or of iodide on the methyl group could succeed in the presence of the negative charge of the monosodium salt and attempts to neutralize the charge on the anion by adding acid naturally caused prompt decomposition of the diazo functionality.

In order to overcome these difficulties, we investigated the synthetic route shown in Scheme I.

The β-cyanoethyl group has previously been successfully utilized in the preparations of various phosphate esters by Cherbuliez,[7] Khorana,[8] and Tener.[9] Central to this application of the method is the presumption that the site of attack of alkali on the protons α to the cyano group is sufficiently remote from the negative charge on the phosphate residue of the monoester monoanion to permit the deprotection reaction to proceed under the mild conditions essential to the preparation of diazo compounds. The scheme proved successful and was applied to the benzyl compound shown in Scheme I above and to the corresponding o-chlorobenzyl derivative.

Tris-β-cyanoethyl phosphite has been reported previously,[10,11] although details of its preparation and properties are not available and one of the earlier publications[10] reports in contrast with our findings[12] that it will not undergo the Arbuzov reaction with CH_3I. Although we have not obtained the phosphite in high purity, it served for the subsequent reactions, where the products were properly purified, analyzed, and identified. The details of the preparations outlined in eq 1 and Scheme I are presented in the Experimental Section and the properties of the compounds are reported in the Results section of this paper.

Experimental Section

General. Uv-visible spectra were determined with a Gilford 240 or a Cary 15 spectrophotometer and ir spectra with a Perkin-Elmer 137 spectrophotometer. ^1H NMR measurements were made with Varian A-60, T-60, or HA-100 instruments; ^{31}P spectra were recorded by Dr. Arnold Satterthwait and Mr. David Standring with a Varian XL-100 spectrometer in the Fourier transform mode. High voltage paper electrophoresis was run on a Savant Instruments flat plate system. p-Nitrophenol was used as reference standard and the results (R_f's) are expressed as mobility of spot/mobility of p-nitrophenol. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, Tenn.

Syntheses. Tris-β-cyanoethyl Phosphite (TCEP). Freshly distilled phosphorus trichloride (27.5 g, 0.2 mol) in 350 ml of dry benzene was introduced into a dry 1000-ml three-neck flask. An equimolar mixture of β-cyanoethyl alcohol (Aldrich) (42.6 g, 0.6 mol) and pyridine (47.4 g, 0.6 mol) in 50 ml of benzene was added drop by drop with stirring under nitrogen at 0 °C. The resulting suspension was stirred for an additional 4 h at 0 °C and then filtered at room temperature. The filter cake of pyridine hydrochloride was washed with benzene and the filtrate and washings rotoevaporated to yield an oil that was freed of most impurities by subjecting it to vacuum (1 mm) for 1 h. This crude phosphite could be used in further preparations, although it could not be stored successfully even under nitrogen at 25 °C for more than a few days. The phosphite showed a strong nitrile band at 2200 cm^{-1}, but only a weak absorption at 2400 cm^{-1} that presumably arose from the H-P stretch[13] of the small quantity of bis-β-cyanoethyl hydrogen phosphonate formed on hydrolysis of the triester with adventitious moisture. ^1H NMR spectrum in $CDCl_3$: δ 2.74 (t, J = 6.5 Hz), 4.2 (d of t, J = 6.5, 7.0 Hz).

The yellowish oil can be partially purified by loading it in 5-g batches dissolved in 5 ml of ethyl acetate on a 4 × 75 cm column containing about 350 g of Woelm silica gel. The ester was obtained by rotoevaporating the second 500 ml of solution obtained with ethyl acetate as eluent. The product shows only a single spot (R_f 0.65–0.70) on thin layer chromatography with ethyl acetate on silica gel, but may still contain some β-cyanoethyl alcohol. This partially purified material is satisfactory for further reactions.

Bis-β-cyanoethyl Benzoylphosphonate. The product named in the title was prepared by a procedure that parallels that of Berlin and Taylor[14] for the reaction of acyl halides with other phosphites. Freshly prepared tris-β-cyanoethyl phosphite (13.62 g) was added with stirring at 0 °C under nitrogen without solvent to freshly distilled benzoyl chloride (6.97 g). After 19 h, the ^1H NMR spectrum of the slightly viscous red solution was consistent with that expected for a mixture of the benzoyl phosphonate, β-chloropropionitrile and some residual starting material. A small sample of the crude product mixture was added to 20 ml of a stock solution of 2,4-dinitrophenylhydrazine in ethanol-sulfuric acid. The yellow solid that precipitated after 3 min was filtered, dissolved in methylene chloride, and the filtered solution diluted with an equal volume of hot absolute ethanol. When this solution was slowly cooled, yellow crystals, mp 178–179 °C (corr), were obtained. The ir spectrum showed a $-C{\equiv}N$ stretch at 2210 cm^{-1}, $C{=}N$ at 1620 cm^{-1}, and $P{=}O$ at 1280 cm^{-1}. A sample was dried for analysis at 100 °C under vacuum for 24 h. Anal. Calcd for $C_{19}H_{17}N_6PO_7$: C, 48.31; H, 3.63; N, 17.79; P, 6.56. Found: C, 48.16; H, 3.57; N, 17.67; P, 6.57.

Bis-β-cyanoethyl Benzoylphosphonate Tosylhydrazone. Tosylhydrazine[15] (10.11 g) and 5.2 ml of concentrated hydrochloric acid solution were mixed with stirring in 50 ml of tetrahydrofuran and added to a solution of the crude mixture containing the benzoylphosphonate diester in 50 ml of THF. After 2.5 h under nitrogen at room temperature, 800 ml of methylene chloride was added to the reaction mixture. A white solid (excess tosylhydrazine hydrochloride) was removed by filtration and the mother liquors rotoevaporated to a yellow, viscous residue, which crystallized from ethyl acetate. A series of crops were obtained over several days and the product recrystallized from methanol-water after clarification of the hot solution with charcoal. The white crystals, after drying over P_2O_5, melted at 133–134 °C (corr). The ir spectrum showed a strong $-C{\equiv}N$ band at 2210 cm^{-1}, a $C{=}N$ band at 1590, cm^{-1}, and a $P{=}O$ band at 1300 cm^{-1}; ^1H NMR spectrum in CD_3cn, δ 2.47 (s, 3 H), 2.75 (t, J = 7 Hz, 4 H, $(-OCH_2CH_2CN)_2$), 4.2 (q, J = 7 Hz, $J_{H\text{-}P}$ = 8 Hz, 4 H, $RP(O)(OCH_2CH_2CN)_2$), 7.46 (m, aromatic, 9 H). Anal. Calcd for $C_{20}H_{21}N_4PSO_5$: C, 52.17; H, 4.59; N, 12.16; S, 6.96; P, 6.72. Found: C, 52.31; H, 4.57; N, 12.20; S, 6.80; P, 6.71.

Bis-β-cyanoethyl α-Diazobenzylphosphonate. The tosylhydrazone (3.00 g) was stirred in a solution of 0.69 g of sodium carbonate in 50 ml of water at room temperature. After 13 h at room temperature the hydrazone had dissolved and a yellow solid (1.45 g; 73% of theory) had separated; it was dissolved in methylene chloride and, after a small amount of insoluble material had been removed by filtration, the product separated, on addition of 20 ml of n-pentane at 0 °C, as a yellow oil that crystallized when scratched. After it had been washed with pentane and dried over P_2O_5, it melted at 73–75 °C (corr). The

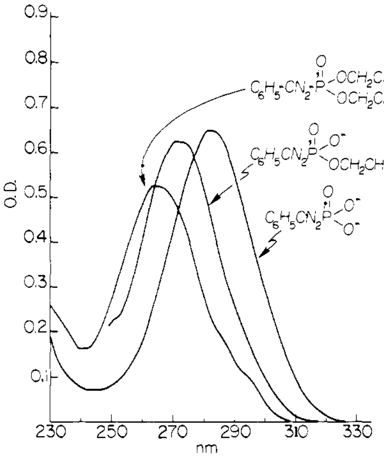


Figure 1. Ultraviolet absorption spectra of 4 × 10^{-5} M solutions of the diester, monoester monoanion, and dianion of α-diazobenzylphosphonate.

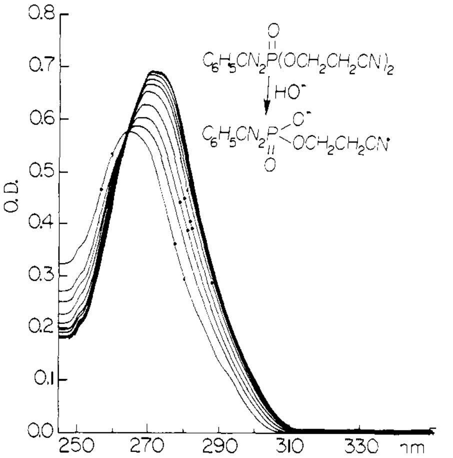


Figure 2. Repeated scans during the conversion of the diester to the monoester monoanion of α-diazobenzylphosphonate at 25 °C.

ir showed a sharp band at 2200 cm^{-1} (diazo function); ^1H NMR spectrum in deuterioacetone, δ 2.96 (t, J = 6.5 Hz, 4 H, $(OCH_2CH_2CN)_2$), 4.43 (d of t, J_1 = 6.5, J_2 = 8 Hz, 4 H, RP(=O)-$(OCH_2CH_2CN)_2$), 7.35 (s, aromatic, 5 H); ^{31}P NMR spectrum in CD_3CN/H_2O, δ −19.7 ppm from external 85% H_3PO_4 (m, J = 7-7.5 Hz); uv in absolute ethanol, 213 (log ε 4.11), 264 (log ε = 4.10), 410 nm (log ε = 2.48). The uv absorption, including the peak at 264 nm, can be destroyed by acid or irradiation. Anal. Calcd for $C_{13}H_{13}N_4PO_3$: C, 51.32; H, 4.30; N, 18.41; P, 10.18. Found: C, 51.12; H, 4.30; N, 17.92; P, 10.22.

Sodium Methyl α-Diazobenzylphosphonate. A solution of 2.25 g of sodium iodide in 100 ml of acetone was added with stirring over 10 min to 2.26 g of dimethyl α-diazobenzylphosphonate[5] in 20 ml of acetone and the mixture was refluxed for 30 min. The yellow solid that precipitated when the solution was cooled was collected by filtration and dried at 1 mm over Drierite; yield, 2.20 g (94% of theory). High voltage paper electrophoresis (0.1 M NaOH, Whatman 3MM, 1500 V, 30 min) resulted in a single spot, R_f 0.70;[16] ir (Nujol mull) 2083 cm^{-1} (diazo); ^1H NMR in D_2O, δ 3.58 (d, 12 Hz, 3 H), 6.90-7.70 (m, 5 H); ^{31}P NMR spectrum in 1 M NaOH in D_2O, δ −13.0 ppm from external 85% H_3PO_4 (q, J = 11.8 Hz). Anal. Calcd for $C_8H_8N_2O_3PNa$: C, 41.04; H, 3.44; N, 11.97; P, 13.24. Found: C, 40.92; H, 3.31; N, 11.26, 11.21; P, 12.52, 12.53.

Mono-β-cyanoethyl α-Diazobenzylphosphonate Monoanion. Solutions of the monoester monoanion were prepared by allowing a 4 × 10^{-5} M solution of bis-β-cyanoethyl α-diazobenzylphosphonate to stand in pH 10 buffer for 50 min at 25 °C. The compound was identified by its uv spectrum.

α-Diazobenzylphosphonate Dianion. Bis-β-cyanoethyl α-diazobenzylphosphonate (50 mg) was added to 4 ml of 2 M NaOH and stirred under nitrogen for 10 min to form a yellow homogeneous solution. The resulting dianion is too unstable to permit its proper isolation; even in 1 M alkali, its half-life at 25 °C is only about 3.5 h, and it is even less stable at lower pH (see Results). Some impure solid was obtained by titrating the basic solution to pH 11.1 with 56% aqueous hydroiodic acid and then adding 12 vol of ice-cold acetone. The resulting yellow gum was separated by centrifugation; it solidified on trituration with acetone. After the solid had been dried over P_2O_5 in vacuum, its ir spectrum (KBr pellet) showed a diazo band of medium intensity at 2050 cm^{-1}; it also showed the bands at 1450 and 880 cm^{-1} corresponding to sodium carbonate. High voltage paper electrophoresis (0.1 M NaOH, Whatman 3MM, 1500 V, 3 h): major spot R_f 1.41.

The ^{31}P spectrum of the dianion in 1 M NaOH in D_2O shows only a sharp singlet at δ −5.34 ppm from external 85% H_3PO_4. The uv spectrum is discussed under Results.

Bis-β-cyanoethyl o-Chlorobenzoylphosphonate. This compound was prepared from 1.67 g of freshly distilled o-chlorobenzoyl chloride and 2.3 g of tris-β-cyanoethyl phosphite that had been purified chromatographically, following the procedure for the unsubstituted compound. A sample of its 2,4-dinitrophenylhydrazone, melting at 160.5-162 °C (corr), showed an ir band at 2240 cm^{-1} (C≡N). Anal. Calcd for $C_{19}H_{16}N_6ClPO_7$: C, 45.02; H, 3.18; N, 16.58; P, 6.11; Cl, 6.99. Found: C, 44.95; H, 3.20; N, 16.50; P, 6.26; Cl, 7.15.

Bis-β-cyanoethyl o-Chlorobenzylphosphonate Tosylhydrazone. The tosylhydrazone was prepared from 1.77 g of tosylhydrazine and an equivalent quantity of bis-β-cyanoethyl o-chlorobenzoylphosphonate following the procedures for the unsubstituted compound: yield, 0.5 g (15% of theory), mp 174-175 °C (corr). The ir spectrum showed the band at 2220 cm^{-1} for the C≡N group. Anal. Calcd for $C_{20}H_{20}N_4PSClO_5$: C, 48.54; H, 4.07; N, 11.32; P, 6.25; Cl, 7.16. Found: C, 48.73; H, 4.10; N, 11.39; P, 6.40; Cl, 7.29.

Bis-β-cyanoethyl α-Diazo-o-chlorobenzylphosphonate. The diazo ester was prepared from 0.179 g of the corresponding tosylhydrazone following the procedure for the unsubstituted compound. The product, however, was an oil which has not yet been completely purified: ir 2240 (C≡N), 2080 (diazo), 1260 cm^{-1} (P=O); uv 258 nm (log ε = 4.08 ± 0.08).

α-Diazo-o-chlorobenzylphosphonate Dianion. An aqueous solution of the dianion in alkali (prepared as for the unsubstituted compound) shows λ_{max} at 283 nm (log ε = 4.18). This absorption is destroyed by brief irradiation at 254 nm or by acid.

Determination of pK's. The pK's of glycine and Tris were measured at varying ionic strengths using a glass electrode and a Radiometer pH meter. Solutions of the base and the corresponding hydrochloride, in equal concentrations, were brought to the desired ionic strength with potassium chloride, and the measurement made in a thermostat at 25 °C. The pK of glycine proved relatively insensitive to ionic strength; the value used was 9.70 (lit.[17] for zero ionic strength 9.78). The pK of Tris was found to increase with increasing ionic strength; the value used was 8.35 (lit.[18] for zero ionic strength 7.76).

Kinetics. The kinetics were measured spectrophotometrically, following the ultraviolet absorptions illustrated in Figures 1 and 2. For slow reactions, the entire scans were obtained; fast reactions were followed at a single wavelength. The reactions were carried out at constant acidity and showed good first-order kinetics with the qualification, discussed under "Results", concerning the slight deviations in uv spectra observed in the initial phase of the conversion of the mono- to the dianion.

The buffers listed in Table I were made up to a total concentration of acid plus basic component listed in Table I and adjusted to the appropriate pH, measured at the ionic strength specified, by addition of HCl or KOH.

Reactions were initiated by adding 5-10 μl of a stock solution (4 × 10^{-2}-4 × 10^{-3} M) of the diester in absolute ethanol to an appropriate buffer or alkaline solution. When reactions of the dianion were studied, the appropriate solutions were prepared by mixing the stock solution of the diester with 5-10 μl of 1 N sodium hydroxide solution in a micro centrifuge tube; 6-8 μl of this solution was then mixed with 3.00 ml of the appropriate buffer solution for spectroscopic observation.

Results

The identification of the diazobenzylphosphonate dianion rests on its method of preparation, its ir and ^{31}P NMR spectra, and the results of paper electrophoresis. In particular, the ^{31}P

Table I. Rate Constants for the Formation and Decomposition of α-Diazobenzylphosphonates in Water at 25.00 °C

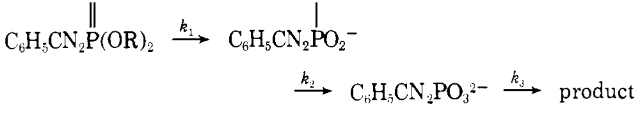
Conditions				k_1		k_2		k_3
Buffer	pH	μ	Diester, concn $\times 10^5$ M	$10^4 k_{obsd}$, s^{-1}	k_1, $M^{-1} s^{-1}$	$10^2 k_{obsd}$, s^{-1}	$10^2 k_2$, $M^{-1} s^{-1}$	$10^5 k_{obsd}$, s^{-1}
Borate 0.05 M[a]	10.00		4.10	12.1	12.1			
Borate 0.05 M	10.00		0.41	12.2	12.2			
Borate 0.01 M	10.18		4.62	16.5	10.9			
Borate 0.05 M	9.05		4.78	1.21	10.8			
0.1 M NaOH	13.26	0.18	4.10			1.23	6.77	
0.1 M NaOH	13.26	0.18	0.41			1.31	7.21	
0.051 M NaOH	12.60	0.11	4.10			0.33	8.29	
0.051 M NaOH	12.60	0.11	0.41			0.33	8.04	
1 M NaOH	14.00	1.00	3.42					5.63
1 M NaOH	14.00	1.00	3.42					70 ($h\nu$)[b]
0.1 M NaOH	13.26	0.18	4.78					5.78
0.1 M NaOH	13.26	0.18	0.48					5.77
Borate 0. 5 M	10.00		4.78					92.4
Borate 0.05 M	10.00		0.48					88.8
Borate 0.05 M	10.00		5.40					12 (0 °C)
Glycine/KCl 0.10 M	10.00	1.00	3-5					131
Glycine/KCl 0.05 M	10.00	1.00	3-5					76.4
Glycine/KCl 0.05 M	10.00	0.05	3-5					96.3
Glycine/KCl 0.01 M	10.00	1.00	3-5					15.8
Glycine/KCl 0.10 M	9.50	1.00	3-5					275
Glycine/KCl 0.05 M	9.50	1.00	3-5					136
Glycine/KCl 0.01 M	9.50	1.00	3-5					30.4
Tris/KCl 0.10 M	8.50	1.00	3-5					1245
Tris/KCl 0.08 M	8.50	1.00	3-5					1160
Tris/KCl 0.05 M	8.50	1.00	3-5					677
Tris/KCl 0.01 M	8.50	1.00	3-5					143
Tris/KCl 0.10 M	8.00	1.00	3-5					1860
Tris/KCl 0.08 M	8.00	1.00	3-5					1455
Tris/KCl 0.05 M	8.00	1.00	3-5					1021
Tris/KCl 0.01 M	8.00	1.00	3-5					248
Tris/KCl 0.01 M	8.00	1.00	3-5					213[c]
Tris/KCl 0.10 M	8.00	0.05	3-5					4200
Tris/KCl 0.1 M	7.50	1.00	3-5					2130
Tris/NaClO$_4$ 0.01 M	8.00	1.00	3-5					80.2
Collidine/NaClO$_4$ 0.01 M	7.95	1.00	3-5					13.3

[a] Commercial buffers of undefined ionic strength. [b] Illuminated with a short wavelength Mineralight UVS-12 lamp. [c] α-Diazo-o-chlorobenzylphosphonate.

NMR spectra of the dicyanoethyl diazobenzylphosphonate, the methyl diazobenzylphosphonate anion, and the diazobenzylphosphonate dianion accord with the postulated structures in chemical shift (δ −19.7, −13.0, and −5.34, respectively) and with respect to H–P coupling (the signal from the dianion is a singlet). The R_f on paper electrophoresis of the diester is zero, as expected, whereas that for the dianion is twice that for the monoanion. (The monoanion was that of the monomethyl, rather than the monocyanoethyl ester, since the former, but not the latter, is stable in 0.1 M alkali.)

The uv spectra of bis-β-cyanoethyl α-diazobenzylphosphonate, mono-β-cyanoethyl α-diazobenzylphosphonate monoanion, and α-diazobenzylphosphonate dianion are shown in Figure 1. The diester is stable at neutral pH; at pH 10, it is converted into the monoester, whereas in strong alkali it yields the dianion. When the diester is placed in pH 10 buffer, the uv maximum shifts from 264 to 272 nm with an accompanying increase in extinction coefficient from 800 to 15 300. A typical spectrophotometric experiment is shown in Figure 2. From such data, good first-order rate constants can be obtained, valid to ±5%. When the diester is placed in 1 M alkali, the spectrum of the dianion is rapidly obtained; the rate constants (Table I) indicate that the reaction is better than 99% complete in less than 2 min. Subsequently, the dianion slowly decomposes; the products of decomposition are currently under investigation. The rates of decomposition, whatever the products, can be measured by the loss of the uv absorption band for the diazo group.

Measurement of the rate of conversion of the monoanion to the dianion is complicated by the slow decomposition of the latter. When the diester is placed in 0.1 M NaOH solution, the monoanion is essentially completely formed in a few seconds. Subsequently, the uv absorption rises for 10-15 min as the maximum shifts from 272 to 282 nm and the extinction coefficient rises from 15 300 to 16 000; these spectral changes correspond to the conversion of the monoanion to the dianion. Thereafter, however, the absorption falls over a period of 24 h as the diazo compound undergoes decomposition with loss of the diazo absorption. The rate constants k_1, k_2, and k_3 are widely separated and can be measured individually by choosing appropriate experimental conditions.



$$C_6H_5CN_2\overset{\overset{\displaystyle O}{\|}}{P}(OR)_2 \xrightarrow{k_1} C_6H_5CN_2\overset{\overset{\displaystyle OR}{|}}{P}O_2^- \xrightarrow{k_2} C_6H_5CN_2PO_3^{2-} \xrightarrow{k_3} \text{product}$$

The conversion of the monoester monoanion to the dianion of α-diazobenzylphosphonate, however, is more complicated than might at first appear. The spectroscopic measurements generally showed a small but reproducible decrease in absorption at around 280 nm, a decrease that usually persisted

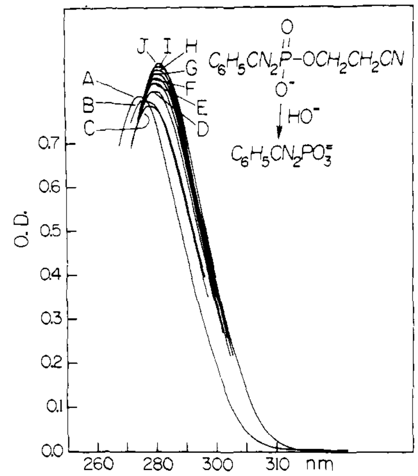


Figure 3. Repeated scans during the conversion of the monoester monoanion of α-diazobenzylphosphonate to the corresponding dianion. For the first 50 s (scans A, B, C) the absorption dropped; the rate constant for the reaction was taken over the next hour as the absorption rose regularly.

for about 50-60 s before the steady and regular increase in absorption that permitted the determination of the rate constants (Figure 3). The reason for this abnormality has not been determined; the small change could be accounted for if a few percent of an intermediate or side product were produced that does not absorb in this spectral region. The slight variation (Table I) in the second-order rate constant for the conversion of the monoanion to the dianion may arise from errors caused by this still unexplained, if minor, phenomenon.

The decomposition of the α-diazobenzylphosphonate dianion is general acid catalyzed, as is that of some other diazo compounds,[19,20] including the diazoacetate anion.[21] The data for general acid catalysis are presented in Table I and Figure 4. In the latter, the catalysis is shown for glycine and Tris buffers, where $k_{\text{obsd}} - k_0$ is plotted against the concentration of the acid component of the buffer system. The data apply to two different buffer ratios for both glycine and the Tris buffers; the points for each of the two systems lie on a satisfactory straight line, confirming general acid catalysis with no suggestion of general base catalysis. The values of k_0 for the systems are 4×10^{-5} s^{-1} for the glycine buffers and 5×10^{-4} s^{-1} for the Tris buffers; these rate constants, which represent catalysis by water and hydrogen ion, are so small as to be almost negligible in comparison with the general acid catalysis. All of these measurements were made at 25 °C and at an ionic strength of 1.00, maintained with potassium chloride. The concentrations of the buffer components were calculated from pK measurements in solutions of this ionic strength, as explained in the Experimental Section.

In some instances,[22] of which this is one, general acid catalysis is sensitive to steric hindrance. The rate of decomposition of the diazobenzylphosphonate dianion is only about a sixth as great in 0.01 M 2,4,6-collidine buffer as in 0.01 M Tris buffer, where ionic strength was maintained with sodium perchlorate in both cases.

Discussion

The loss of the cyanoethyl groups in the deprotection of phosphonate esters involves elimination rather than ester hydrolysis. The second-order rate constant at 25 °C for the first step in the deprotection of the diazobenzylphosphonate diester—forming the monoanion—is 11.4 M^{-1} s^{-1}, and the rate constant for the formation of the dianion is 7.6×10^{-2} M^{-1} s^{-1}. By way of comparison, Tener gives the half-life[9] for the deprotection of $N{\equiv}CCH_2CH_2OP(OR)O_2^-$, where R is alkyl, as 1 min at 50 °C in 0.1 N NaOH; this result, extrapolated to 25 °C, is comparable to ours. Cherbuliez gives the rate constant for the formation of inorganic phosphate from mono-β-cyanoethyl phosphate[7] as 1.20×10^{-4} M^{-1} s^{-1} at 21 °C. Assuming that this rate constant at 25 °C would be around 1.5×10^{-4} M^{-1} s^{-1}, then the ratio of the rates of deprotection for the second and third steps in β-cyanoethyl phosphate esters is about 300; the rate ratio for the first and second steps, in the deprotection of the α-diazobenzylphosphonate, determined in this work, is around 150. The rate ratios reflect the effect of the negative charge of the phosphonate esters on the attacking hydroxide ion; the relatively small ratios can be correlated with the rather large distance between the electric charge and the hydrogen atoms adjacent to the cyano group, which are attacked by the base. These data stand in sharp contrast to those for the saponification of simple phosphate and phosphonate esters, where hydroxide ion attacks at phosphorus. The first ester group is removed from phosphonate esters by alkali with rate constants[23] in the range of 10^{-2}-10^{-3} M^{-1} s^{-1} in water at 100 °C. Extrapolation to 25 °C, assuming an activation energy[23] of $\approx$15 kcal/mol, yields rate constants in the range of 10^{-4}-10^{-5} M^{-1} s^{-1}; these are smaller than that for the loss of the first cyanoethyl group from our phosphonate or Cherbuliez's phosphate by a factor of 10^5-10^6. Furthermore, the second ester group is lost on saponification no more than 10^{-5} times as fast as the first.[23] The much greater absolute rate of saponification of the cyanoethyl esters and the much smaller ratio of rates for the loss of the first and second ester groups supports the view that the cyanoethyl groups are removed by the accepted mechanism,[9] i.e., by enolization followed by ejection of acrylonitrile.

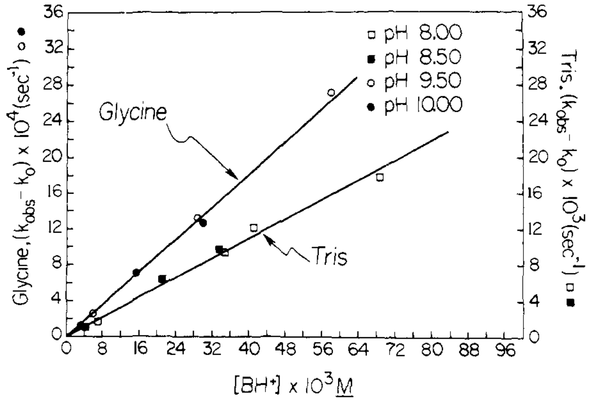


Figure 4. First-order rate constants at 25 °C for the loss of the diazo function from α-diazobenzylphosphonate dianion as a function of the concentration of the acidic component of glycine and Tris buffers at various pH's and at an ionic strength of 1.00, maintained with potassium chloride.

The electrically neutral diester, bis-β-cyanoethyl α-diazobenzylphosphonate, can be kept in aqueous solutions down to a pH around 1-2. The monoanions of methyl α-diazobenzylphosphonate and β-cyanoethyl α-diazobenzylphosphonate are reasonably stable at alkaline pH, but decompose rapidly when solutions are brought to pH 6.2-5.0. The rate constant for the loss of the diazo function from sodium methyl α-diazobenzylphosphonate in 0.1 M Tris buffer, ionic strength 0.05, is 2.22×10^{-5} s^{-1} at pH 8.0. The dianion of α-diazobenzylphosphonate, however, is sensitive to general acid catalysis and decomposes rapidly in buffer solutions at pH's from 8 to 10. Since, however, the decomposition is general acid, rather than specific hydrogen ion catalyzed, and since even this general acid catalysis is subject to steric hindrance, the dianion can survive for moderate periods near neutrality. The half time for the decomposition of α-diazobenzylphosphonate dianion at 25 °C and pH 8.0, in a 0.01 M buffer of 2,4,6-collidine-2,4,6-collidine perchlorate is about 1.5 h, and since the decomposition has a normal temperature coefficient, the half times at 0 °C are correspondingly longer. It follows that, despite the inherent sensitivity of the diazophosphonate dianion to acid, it should be possible to test it, and perhaps use it as a photoaffinity labeling reagent.

Presumably the decomposition of diazo compounds proceeds by way of the aliphatic diazonium cation:

$$R_2CN_2 + HA \rightarrow R_2CHN_2^+ \rightarrow \text{product} + N_2$$

or

$$R_2CN_2 + H^+ \rightleftharpoons R_2CHN_2$$

$$R_2CHN_2 + A^- \rightarrow \text{products} + N_2$$

where the diazonium cation may be formed in a rate-limiting general-acid-catalyzed step or in a prior equilibrium involving protons. In any event, the negative charge on the monoalkyl α-diazobenzylphosphonate salt and a fortiori the two negative charges on the α-diazobenzylphosphonate salt will favor protonation; this electrostatic effect accounts for the ready acid-catalyzed decomposition of the anions. The compounds here discussed differ fundamentally from trifluorodiazoethane,[24] which survives at −60 °C in fluorosulfuric acid, and 2-diazo-3,3,3-trifluoropropionate esters[25] that are stable in 1 M hydrochloric acid. In these derivatives, the electron-withdrawing trifluoromethyl group makes protonation at the carbon atom of the diazo group very difficult, and therefore allows the diazo compound to survive in acid solution, whereas in the new compounds here reported the negative charges of the anions have the opposite effect. Further, the enormous magnitudes of the differences in rate are reasonable. A negative charge decreases the acid strength of phosphoric acid by a factor of about 10^5; one could therefore anticipate that the diester of α-diazobenzylphosphonate might be stable in a solution 5 pH units more acid than for the monoester monoanion, and that the anion would be stable in turn in solutions 5 pH units more acid than those for the dianion. On the other hand, the trifluoromethyl group might well enhance the acidity of a proton on a neighboring carbon atom by more than 10^6-fold,[26] so that the corresponding diazo compounds would be expected to be acid stable. This line of reasoning suggests that the preparation of diazophosphonates with electron-withdrawing groups attached might lead to acid-stable reagents for photo affinity labeling. Unfortunately, our attempts to prepare 1-diazo-2,2,2-trifluorodiazoethylphosphonate and α-diazo-p-nitrobenzylphosphonate have not been successful, and the rate of decomposition of α-diazo-o-chlorobenzylphosphonate proved to be only slightly less than that of the unsubstituted derivative.

Tests of these phosphonates as enzyme inhibitors are in progress.

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