Dephosphorylation by Amidoximate

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Acetamidoxime in its neutral form is a good nucleophile toward *p*-nitrophenyl diethyl phosphate and *p*-nitrophenyl diphenyl phosphate, and at pH >11 the amidoximate ion is an effective reagent. Benzamidoxime is less reactive than acetamidoxime by a factor of approximately 10.

Attack upon phosphoryl groups by nucleophilic anions is well established, but whereas nonionic nucleophiles are often very effective deacylating agents, they seem to be relatively ineffective in dephosphorylation.[2] There has been considerable work on the kinetics of dephosphorylation by oximate anions, which are effective reagents,[3,4] and these reactions are models for detoxification of nerve gases, which are phosphorylating agents.

We have examined amidoximes as potentially useful dephosphorylating agents.

$$\underset{\mathbf{1}}{\mathrm{RC(NH_2){=}NOH}} + \mathrm{(R'O)_2PO{\cdot}OC_6H_4\text{-}\mathit{p}\text{-}NO_2}$$

$$\rightarrow \mathrm{RC(NH_2){=}NOPO(OR')_2} + {}^{-}\mathrm{OC_6H_4\text{-}\mathit{p}\text{-}NO_2}$$

$$\mathrm{R = Me, Ph;\ R' = Et, Ph}$$

The pK_a values of amidoximes, e.g., **1a** (R = Me), are in the range 12 to 13, and in deacylation the neutral amidoxime is an effective reagent and reaction of the anion is observed only at pH > 10.[5,6]

Experimental Section

Materials. Acetamidoxime (**1a**) and benzamidoxime (**1b,** R = Ph) were prepared from the nitrile and hydroxylamine in 1-butanol by the method of Lenaers, Eloy, and Moussebois,[7] and **1a** was isolated as the free base and **1b** as the hydrochloride. Preparation of the phosphates has been described.[8]

Kinetics. Reactions at 25.0 °C were followed spectrophotometrically at 410 nm using $\sim 2 \times 10^{-5}$ M substrate and 10^{-2} M borate buffer, except for reactions at high pH. The pH of the solution was measured in the presence of the amidoxime. The substrates were added as a dioxane solution, and the reaction mixture contained 0.2 vol % dioxane.

The solubility of *p*-nitrophenyl diethyl phosphate (PNPDEP) is such that its reactions could be followed in water, but we used H_2O–MeCN for the reactions of *p*-nitrophenyl diphenyl phosphate (PNPDPP) and estimated the rate constants by extrapolation to water. For reaction with 0.01 M acetamidoxime, the first-order rate constants, $10^4 k_\Psi$ (s^{-1}), at pH 10 in the presence of the following volume percentages of MeCN were as follows: 11%, 1.56; 9.1%, 1.84; 7%, 1.98 (3.27); 4.8%, 2.05; 2.4% (3.58). The values in parentheses were at pH 10.5.

The reactions were overall second order over a concentration range of 0.03 to 0.1 M amidoxime, and allowance was made for the concomitant reactions of water and hydroxide ion.[8] The contribution of these reactions was relatively small, except at high pH. Examples of k_Ψ for the reaction of PNPDEP are in Table I.

Results

We generally used PNPDEP rather than PNPDPP because of the low solubility of the latter in water. The second-order rate constants, k_2, for reaction of the amidoximes are independent of pH up to ~10, but then they increase because of reaction involving the amidoximate ion (Table II). This behavior is very similar to that found for deacylation, where acetamidoxime is a more effective nucleophile than benzamidoxime.[5] (There is a linear free-energy relationship between deacylation rate and basicity of the amidoxime with α = 0.85.[5a])

Table I. Reaction of PNPDEP with Acetamidoxime [a]

[amidoxime], M	pH 8.0	pH 9.0
0.03	0.86 (0.80)	1.01 (0.84)
0.04	1.15 (1.10)	1.23 (1.06)
0.08	2.23 (2.18)	2.29 (2.12)
0.10	2.90 (2.85)	2.90 (2.73)

[a] Values of $10^5 k_\Psi$ (s^{-1}). Rate constants in parentheses are corrected for the spontaneous reaction.[8]

Table II. Second-Order Rate Constants for Reaction of Amidoximes with PNPDEP [a]

pH	$10^4 k_2$, $M^{-1}\ s^{-1}$
8.0	2.76 (0.29[b])
9.0	2.71 (0.30[b])
10.0	2.63
10.8	3.15
11.0	3.45 (2.4[b])
11.25	4.28
11.5	4.90 (8.3[b])
12.0	9.25 (22.0[b])
12.5	37.5
13.0	125
13.4	480

[a] Reaction of acetamidoxime unless specified. [b] Reaction of benzamidoxime.

At low pH acetamidoxime (**1a**) is more reactive than benzamidoxime (**1b**), presumably because the −I effect of the phenyl group reduces nucleophilicity. At higher pH the situation is reversed, probably because benzamidoxime, as the stronger acid, provides more of the nucleophilic amidoximate ion.

The value of pK_a for acetamidoxime is 12.9,[5a] and from this value and that of k_2 at pH 11.5–12 (Table II) we estimate a value of $\sim 60\ M^{-1}\ s^{-1}$ for the second-order rate constant for reaction of the acetamidoximate ion with PNPDEP. There is considerable uncertainty in this value because of the difficulty of estimating these high pH values and the dependence of the calculated rate constant on pK_a.

For the reaction of 0.01 M acetamidoxime (**1a**) with PNPDPP the extrapolated overall first-order rate constants are ca. $\sim 3.6 \times 10^{-4}$ and $2.1 \times 10^{-4}\ s^{-1}$ at pH 10.5 and 10, respectively (Experimental Section). At these pH values the contributions of the reactions with OH^- and H_2O are ca. 2.6×10^{-4} and $1.1 \times 10^{-4}\ s^{-1}$, respectively,[8] so that the contribution of the acetamidoxime reaction is $\sim 1 \times 10^{-4}\ s^{-1}$ and the corresponding second-order rate constant is $\sim 10^{-2}\ M^{-1}\ s^{-1}$. (The diphenyl ester is more reactive toward nucleophiles than the diethyl ester.[8])

Aubort and Hudson have drawn attention to the high nucleophilicity of the neutral amidoximes in deacylation,[5] and they showed that they were much more reactive than the corresponding aldoximes, especially with reactive acylating agents. This difference of nucleophilicities is not unexpected because amidoximes are more basic than oximes. (For dissociation of protonated benzamidoxime, pK_a = 5.[9]) However, the limited evidence cited suggests that there is no striking difference between oximate and amidoximate ions in deacylation.[5]

Although we cannot make similar quantitative comparisons for dephosphorylation, the pattern seems to be similar to that found for deacylation. For reactions of aldoximes with phosphoryl compounds it is the anionic oximate ion which is highly reactive.[3,4] For example, the second-order rate constants for several aldoximate ions with PNPDPP are in the range of 10^2 to $10^3\ M^{-1}\ s^{-1}$ [4] and are much larger than the second-order

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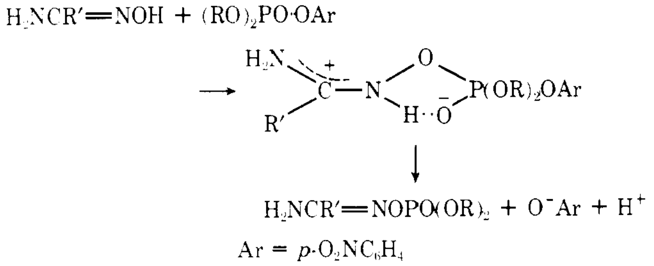
Scheme I



rate constant of ~60 M^{-1} s^{-1} estimated for reaction of the acetamidoximate ion with PNPDEP. To some extent these differences reflect the greater reactivity of PNPDPP over PNPDEP, but they suggest that amidoximate and aldoximate ions have similar reactivities toward phosphoryl groups.

The high nucleophilicity of amidoximes toward phosphoryl groups is similar to that found in deacylation, where intramolecular catalysis has been postulated.[2,5] One such reaction is shown in Scheme I. Intramolecular participation of this type has also been discussed for attack of the catechol monoanion on a phosphoryl group and in deacylation.[2,10]

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Registry No.—**1a**, 22059-22-9; **1b** HCl, 19655-67-5; acetonitrile, 75-05-8; benzonitrile, 100-47-0; hydroxylamine, 7803-49-8; *p*-nitrophenyl diethyl phosphate, 311-45-5; *p*-nitrophenyl diphenyl phosphate, 10359-36-1.

References and Notes

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Phenyl Azide from the Reaction of Phenylhydrazine with Thionyl Chloride. Preparation of *N*-Thionylbenzylhydrazine and of *N*-Thionyl-β-phenethylhydrazine

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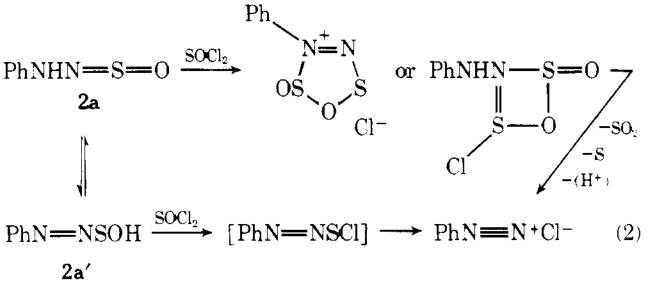
Despite the pioneering investigations of Michaelis and his group[1,2] at the turn of the century, *N*-thionylhydrazines, >NN═S═O, remain a relatively little studied class of compounds. *N*-Thionylphenylhydrazine (**2a**), first prepared (no yield given) in 1889 by Michaelis,[1a] was reported to be formed in ~82% yield from the reaction of phenylhydrazine with thionyl chloride by Klamann and his group[3,4] more than 70 years later. Our repeated failures to duplicate the reported yield of **2a** prompted a closer examination of this reaction.

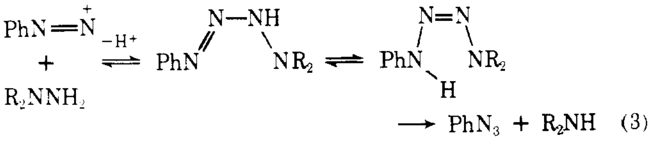
$$\underset{\mathbf{1}}{\mathrm{RNHNH_2}} + \mathrm{SOCl_2} \xrightarrow[\text{pyridine}]{\mathrm{CHCl_3}} \underset{\mathbf{2}}{\mathrm{RNHN{=}S{=}O}} + [2\mathrm{HCl}] \quad (1)$$

$\mathbf{a}$, R = Ph; $\mathbf{b}$, R = $PhCH_2$; $\mathbf{c}$, R = $PhCH_2CH_2$

The best yield of crystallized **2a** obtained by Klamann's procedure was 34%. Distillation or extraction of the reddish brown residue left from the isolation of **2a** gave a 16% yield of phenyl azide. Klamann and co-workers[3] reported without experimental details the isolation of an azo dye during the preparation of **2a.** Michaelis and Ruhl[1c] had demonstrated the formation of phenyldiazonium chloride from the reaction of **2a** with thionyl chloride. This early report suggested a possible rationalization for the formation of phenyl azide during the preparation of **2a.** As shown in eq 2, phenyldiazonium chloride could arise by reaction of thionyl chloride with **2a** directly or via the tautomeric phenylazosulfenic acid **2a′.**[1] The subsequent conversion of aryldiazonium salts to aryl azides with hydrazines is an old and well-known reaction (eq 3).[5,6] This note describes experiments which establish the validity of this hypothesis and the preparation and characterization of the novel *monosubstituted N*-thionylalkylhydrazines (**2b** and **2c**).

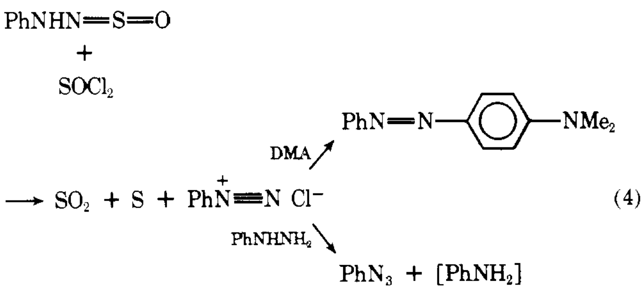


(2)



(3)

Treatment of **2a** with thionyl chloride in pyridine at 0 °C resulted in the evolution of sulfur dioxide and the precipitation of elemental sulfur. The addition of *N,N*-dimethylaniline (DMA) to the solution gave *p*-(dimethylamino)azobenzene. In a separate experiment, when the solution obtained above was treated with 1 equiv of phenylhydrazine instead of DMA, a 25% yield of phenyl azide was obtained (eq 4).



(4)

On the other hand, when thionyl chloride was added to a twofold excess of phenylhydrazine in chloroform at 0 °C in the presence of pyridine, very little phenyl azide was formed and the yield of **2a** increased to 40%. *p*-(Dimethylamino)azobenzene was detected from the reaction of phenylhydrazine with thionyl chloride in chloroform[3] using DMA as the base instead of pyridine. Additional support for the mechanism of the generation of phenyl azide was the preparation of **2a** in 89%

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