Heteroatom Chemistry
Volume 14, Number 1, 2003

Intramolecular Monodealkylation During the Attempted Synthesis of Diethylphosphonoacetohydroxamic Acid

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Received 2 February 2002; revised 15 April 2002

ABSTRACT: *The reaction of diethyl methyl phosphonoacetate (***1***) with hydroxylamine in NaOH solution resulted in the loss of one of the phosphorus ethyl groups, and yielded monoethylphosphonoacetohydroxamic acid (***2***) as the major product (79%) and diethylphosphonoacetic acid (***3***) as the minor product (21%). A series of control experiments were carried out to elucidate the sequence of the reactions leading to* **2**. *When the reaction of* **1** *with* NH_2OH *was carried out in* $NaHCO_3$ *solution, a transient product* **4** *was also observed, which slowly transformed to* **2**. *Compound* **4** *was assigned the structure diethylphosphonoacetohydroxamic acid. There was no dealkylation observed at the phosphorus when* **1** *was reacted with methoxylamine or when O-methyl diethylphosphonoacetohydroxamate (***7***) was placed in alkaline solution. The dealkylation at phosphorus was interpreted in terms of intramolecular nucleophilic catalysis by the hydroxamic OH group attacking the phosphorus in* **4**, *involving cyclic 1,2,5-oxazaphospholidine intermediates.* © 2003 Wiley Periodicals, Inc. Heteroatom Chem 14:67–71, 2003; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.10082

This work was presented at the 13th International Conference on Phosphorus Chemistry in 1995 in Jerusalem; also published in *Phosphorus, Sulfur and Silicon*, 1996, **111**, 797.

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Contract grant sponsor: German–Israeli Foundation for Scientific Research.

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INTRODUCTION

Hydroxamic derivatives have been attracting considerable interest in recent years due to the inhibitory effect they possess on several enzymes of considerable medical significance such as aminopeptidases [1], lipoxygenases [2], angiotensin converting enzyme [3], endothelin converting enzyme [4], and metalloproteinases [5]. In addition, there exist reports on hydroxamic acids having antimalarial [6] and anticancer [7] activity as well as synergism with some anti-AIDS drugs [8]. In contrast to the multitude of existing hydroxamic acids of various structures, there are only a few reports on molecules containing hydroxamic groups along with phosphorus containing functions [9].

In the course of our explorations of the chemical and biological properties of phosphorus containing hydroxylamine derivatives, such as oxyiminophosphonates [10] and phosphonohydroxamates, [11], which may serve as biological chelating agents, we turned our attention to phosphonoacetohydroxamic acid derivatives. Since phosphonoacetate esters are easily available via Arbuzov reactions of the corresponding α-bromoacetates with trialkyl phosphites, we considered it reasonable that reactions of the phosphonoacetates with hydroxylamine, followed by TMSBr-mediated dealkylation would constitute a convenient approach to these compounds.

RESULTS AND DISCUSSION

As a model experiment, we reacted diethyl methyl phosphonoacetate (**1**) with hydroxylamine in

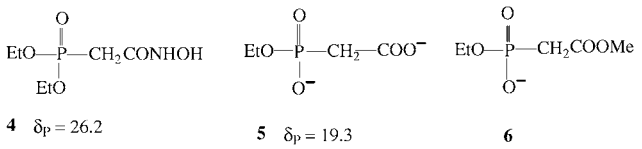
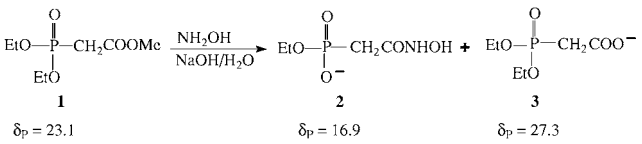


FIGURE 1 Structures of compounds **4, 5** and **6**.

sodium hydroxide solution. Examination of this reaction mixture by ^{31}P NMR spectroscopy revealed two products (Scheme 1). The major product (δ_P = 16.9 (triplet of triplets, 79%)) was identified as monoethylphosphonoacetohydroxamic acid (**2**) and the minor product (δ_P = 27.3 (triplet of quintets, 21%)) was identified as diethylphosphonoacetic acid (**3**). The isolation of **2** indicated that in addition to the expected carboxylate ester to hydroxamic acid transformation, unexpected monodealkylation had taken place at the phosphonic site, while carboxylic acid **3** clearly resulted from the hydrolysis of the carboxylate ester. Since, in **3**, both *P*-ethoxy groups remained intact, it seems that the loss of one P-OEt group in the course of the formation of **2** cannot be explained by a simple base-catalyzed phosphonate ester hydrolysis. This article describes a series of simple experiments that were carried out to elucidate the sequence of events leading to product **2**.

First, we repeated the reaction of **1** with hydroxylamine in a weaker base, namely sodium bicarbonate solution (control experiment no. 1 in the Experimental Section). Monitoring of this reaction by means of ^{31}P NMR spectroscopy revealed a transient product (δ_P = 26.2, Table 1), in addition to **2** and **3** described previously. The structure of this product was assigned as diethylphosphonoacetohydroxamic acid (**4**, Fig. 1) on the basis of its chemical shift in the ^{31}P NMR spectrum resembling that of the corresponding *O*-methylhydroxamate diester (**7**). This assignment is also supported by the subsequent mono de-ethylation of **4** to **2**, as it is apparent from the increase in the percentage of the latter at the expense of the former at 48 and 72 h (Table 1).

We carried out a series of further experiments to determine the roles of the base and of hydroxylamine in the phosphonate de-ethylation reaction.



SCHEME 1 The reaction of diethyl methyl phosphonoacetate with hydroxylamine.

TABLE 1 Composition of the Reaction Mixture of **1** + $NH_2OH/NaHCO_3/H_2O$ as Function of Time

	Composition/Time		
Compound	*18 h*	*48 h*	*72 h*
1 (δ_P = 23.1)	7%	0%	0%
4 (δ_P = 26.2)	22%	7%	0%
2 (δ_P = 16.9)	37%	59%	65%
3 (δ_P = 27.3)	34%	33%	35%

To clarify the role of the base in the reactions of **1**, we compared the effects of two alkaline conditions on **1**, without NH_2OH (control experiment no. 2 in the Experimental Section). The results, listed in Table 2, show that under weak basic conditions (5% $NaHCO_3$), the hydrolysis occurs only at the carboxylate ester with the formation of **3**. We have not observed monoethylphosphonoacetic acid (**5**, Fig. 1). The latter was formed only at higher pH (1N NaOH). Compound **5** was identified by comparison with an authentic sample that we synthesized. We have not observed at any point the third possible product, namely methyl monoethylphosphonoacetate (**6**, Fig. 1), which therefore, cannot be considered as an intermediate in the formation of **2**. *These results show that the phosphorus monodealkylation is not observed, under comparable conditions, without the simultaneous formation of the hydroxamic function.*

We examined the effect of added hydroxylamine in basic solutions of diethylphosphonoacetic acid (**3**) by monitoring the solutions by ^{31}P NMR spectroscopy, as described in the Experimental Section in control experiments no. 3. There was a slow formation (~70% in 24 h, as in the previous experiment) of monoethylphosphonoacetic acid (**5**) as the sole product in NaOH solution, whether accompanied or not by hydroxylamine. *In $NaHCO_3$ solution, there was no formation of* **5** *observed after 240 h, whether in the presence or in the absence of hydroxylamine.*

To test the assumption that the hydroxy group of the hydroxamic function plays a role in the

TABLE 2 The Decomposition of Diethyl Methyl Phosphonoacetate **(1)** in the Presence of Base as Function of Time

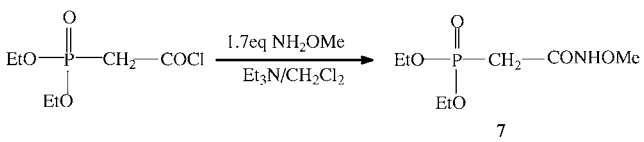
Base	*Time (h)*	*Compound **3**[a] (%)*	*Compound **5**[b] (%)*
5% $NaHCO_3$	18	30	0
	48	50	0
	72	70	0
1N NaOH	1	100	0
	24	26	74
	72	0	100

[a] δ_P = 27.3.
[b] δ_P = 19.3.

monodealkylation, we synthesized *O*-methyl diethylphosphonoacetohydroxamate (**7**) by reacting diethylphosphonoacetyl chloride with methoxylamine (Scheme 2). This compound was obtained as a stable oil, showing two signals $\delta_P = 22.03$ and 20.2 (~70 and ~30%, respectively), presumably because of the presence of two rotamers, observable as a result of the high barrier of rotation around the amide C—N bond. *O-methyl hydroxamate (**7**) underwent no visible dealkylation at the phosphorus*. In addition to the synthesis of **7**, we also carried out an experiment to react **1** with methoxylamine (control experiment no. 4 in the Experimental Section). In this experiment (data not shown), there was initial formation of **3** and **7**, in the approximate ratio of 3:1. After about 2 months at ambient temperature, all of *O*-methyl hydroxamate (**7**) was eventually converted to **3** as the sole product.

The results hitherto presented indicate that the hydroxamic function has a catalytic role in the de-ethylation at phosphorus. To observe whether this can occur intermolecularly, an experiment was carried out in which *O*-methyl hydroxamate (**7**) was allowed to react with benzohydroxamic acid in $NaHCO_3$ solution. After 72 h, only the hydrolysis of the *O*-methyl hydroxamate to carboxylate **3** was observed to the extent of about 45%. *This experiment indicates that the hydroxamic group does not catalyze an intermolecular dealkylation reaction at a noticeable rate under comparable conditions.*

The results presented here are consistent with an intramolecular nucleophilic catalysis mechanism, in which the anion of the hydroxamic OH group attacks the phosphorus intramolecularly. This mechanism is not refuted by the negative result of the control experiment employing benzohydroxamic acid, in which catalysis can only be intermolecular and the molecules need to be brought together by random collisions in order to react. Intramolecular reactions, in contrast, are normally faster than their intermolecular analogs because the reacting groups are closer together from the start, provided that their interaction is not prevented by steric or strain factors. The operation of such intramolecular catalysis by aliphatic and phenolic OH groups has been demonstrated in phosphate diesters and triesters in recent years [12].



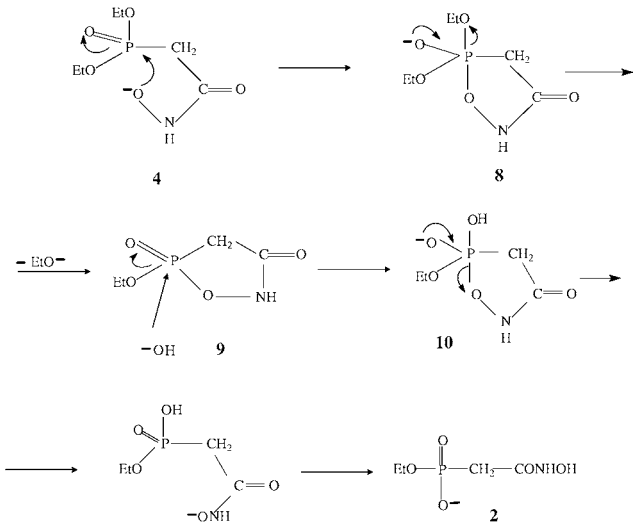
SCHEME 2 Synthesis of *O*-methyl diethylphosphonoacetohydroxamate.

As mentioned earlier, triester **1** can be converted to monoester **5** by NaOH but not by $NaHCO_3$. As the latter is sufficiently basic to ionize the COOH group, lack of dealkylation by it clearly means that the NaOH-catalyzed de-ethylation at the phosphorus does not result from intramolecular catalysis by the carboxylate anion. This is not surprising, considering that such a mechanism would require the formation of a four-membered cyclic, strained intermediate.

In summary, the de-ethylation leading to the formation of phosphonomonoester hydroxamic acid (**2**), can be accounted for by a mechanism, shown in Scheme 3, which consists of (a) the initial formation of diethylphosphonoacetohydroxamic acid (**4**), (b) followed by intramolecular attack of the anion of the hydroxamic OH on the phosphorus to yield an oxazaphospholidine phosphorane intermediate **8**, (c) departure of an ethoxy group to give the five-membered cyclic phosphonate **9**, and (d), which then undergoes ring-opening through the second heterocyclic phosphorane **10** to the final product.

EXPERIMENTAL

NMR spectra were recorded on a Varian VXR-300S instrument. The spectra were referenced to $SiMe_4$ as an internal standard (^1H) and to H_3PO_4 in D_2O as an external standard (^{31}P). Positive chemical shifts are downfield from that of the reference. All spectra were taken using a repetition time sufficiently long for complete relaxation. Broad band ^1H decoupling did not affect ^{31}P signal integration, as confirmed by



SCHEME 3 Mechanism proposed for the formation of compound **2**.

comparing the integration of decoupled to that of coupled signals.

*Disodium Ethylphosphonoacetohydroxamate (**2**)*

To a mixture of a solution of methyl diethyl phosphonoacetate (**1**, 1.84 ml, 10 mmol) in doubly distilled water (10 ml) and a solution of hydroxylamine hydrochloride (0.85 g, 12 mmol) in doubly distilled water (10 ml) was added dropwise a 4 N NaOH solution until the pH remained constant at 12 (about 5 ml). The reaction mixture was stirred at ambient temperature for 24 h. ^{31}P NMR spectroscopy of the solution showed two signals: δ_P = 16.9 (triplet of triplets, 79%) and $\delta_P = 27.3$ (triplet of quintets, 21%). The solution was concentrated to about half of its volume, and the residue was treated with 2-PrOH (50 ml) causing precipitation of the main product, which was filtered off and dried, yielding 1.5 g, 66%. NMR (D_2O): ^{1}H: $\delta = 1.1$ (t, 3H, $^3J_{HH} = 6.9$ Hz), 2.5 (d, 2H, $^2J_{PH} = 19.8$ Hz), 3.7 (dq, 2H, $^3J_{HH} = 7.2$ Hz, $^3J_{PH} = 7.8$ Hz). ^{31}P: (δ): 16.5 (tt, $^2J_{PH} = 19.5$ Hz, $^3J_{PH} = 7.8$ Hz). IR (Nujol): $\nu = 3170$m, 2900s, 1660m, 1630m, 1560w, 1340w, 1190s, 1040s cm^{-1}. FAB-MS Found: $[M+H]^+ = 228.2$ $[M-Na+H]^+ = 206.4$, calcd $M_W = 227.06$. Anal calcd for $C_4H_8Na_2NO_5P$: C, 21.14; H, 3.55; N, 6.16. Found: C, 22.16; H, 4.13; N, 6.13.

*Diethylphosphonoacetic Acid (**3**) (Synthesized According to Malevannaya et al. [13])*

NMR (D_2O): ^{1}H: $\delta = 1.19$ (t, 6H, $^3J_{HH} = 6.9$ Hz), 3.0 (d, 2H, $^2J_{PH} = 21.6$ Hz), 4.06 (dq, 4H, $^3J_{HH} = 6.9$ Hz, $^3J_{PH} = 7.5$ Hz). ^{31}P: (δ): 27.3 (tqu, $^2J_{PH} = 20.5$ Hz, $^3J_{PH} = 7.8$ Hz). IR (Neat): $\nu = 2988$m, 2936m, 2633w, 2361w, 1729s, 1395w, 1241s, 1165w, 1120w, 1026s, 976s cm^{-1}. Anal calcd for $C_3H_{13}O_5P$: C, 36.74; H, 6.68. Found: C, 35.42; H, 6.69.

O-Methyl Diethylphosphonoacetohydroxamate

To a solution of methoxylamine hydrochloride (0.8 g, 9.5 mmol, freshly dried over P_2O_5) suspended in dry CH_2Cl_2 (45 ml) was added dropwise triethylamine (1.7 ml, 12 mmol) followed by slow addition of diethylphosphonoacetyl chloride (1.2 g, 5.6 mmol), causing the immediate appearance of precipitate. Examination of the reaction mixture by ^{31}P NMR spectroscopy, after it had been stirred at ambient temperature for 24 h, revealed the absence of the starting material's signal at δ14.75, and two new signals at δ_P 22.03 and 20.2 (~70 and ~30%, respectively), indicating the formation of the desired product as two rotamers. After removal of the solvent, the residue was treated with ethyl acetate (50 ml) in order to precipitate $Et_3N{\cdot}HCl$, which was filtered off. The evaporation of the solvent from the filtrate gave the product as an oil, 1.1 g, 90%.

NMR (D_2O): ^{1}H: $\delta = 1.2$ (t, 6H, $^3J_{HH} = 6.9$ Hz), 2.8 (d, 2H, $^2J_{PH} = 21.3$ Hz), 3.62 and 3.65 (2 singlets, ~80 and ~20%, respectively, 3H), 4.0 (dq, 2H, $^3J_{HH} = 7.1$ Hz, $^3J_{PH} = 8.1$ Hz). ^{31}P: (δ): 24.1 (m, ~25%); 23.3 (m, ~75%). IR (Neat): $\nu = 3470$w, 3183m, 2984m, 1674s, 1516m, 1467m, 1328w, 1244s, 1162m, 1040s, 974m cm^{-1}. MS-FAB Found: $[M]^+ = 225.3$, calcd. $M_W = 225.18$.

*Monoethylphosphonoacetic Acid (**5**)*

To a solution of methyl diethyl phosphonoacetate (1.0 ml, 5.4 mmol) in distilled water (10 ml) was added dropwise a 4 N NaOH solution until the pH remained constant at 12 (about 5 ml). The reaction mixture was stirred at ambient temperature for 24 h. Monitoring the reaction mixture by ^{31}P NMR spectroscopy indicated the decay of the signal at δ 23.1 ppm, belonging to the starting material, and the development of a transient signal: δ 27.3 ppm belonging to diethylphosphonoacetic acid. The solution was concentrated, and the residue was treated with MeOH (20 ml) causing precipitation of the product, which was filtered off and dried. NMR (D_2O): ^{1}H: $\delta = 1.15$ (t, 3H, $^3J_{HH} = 7.2$ Hz), 2.55 (d, 2H, $^2J_{PH} = 20.7$ Hz), 3.8 (dq, 2H, $^3J_{HH} = 7.2$ Hz, $^3J_{PH} = 7.3$ Hz). ^{31}P: (δ): 19.3 (tt, $^2J_{PH} = 20.6$ Hz, $^3J_{PH} = 7.3$ Hz).

Phosphonoacetic Acid

Methyl diethyl phosphonoacetate (5 ml, 23.8 mmol) was refluxed with 21% HCl (100 ml) for 24 h. After evaporation of the solvent to dryness the residue was recrystallized from diethyl ether, yielding 2 g, 60% yield of product, mp 140–143°C. NMR (D_2O): ^{1}H: δ = 2.6 (d, 2H, $^2J_{PH} = 19.5$ Hz). ^{31}P: (δ): 15.5 (t, $^2J_{PH}$ = 21.7 Hz).

Control Experiment No. 1

To a solution of methyl diethyl phosphonoacetate (0.2 ml, 1 mmol) in $NaHCO_3$ solution (5%, 5 ml) was added $NH_2OH{\cdot}HCl$ (69.5 mg, 1 mmol). The solution was kept at ambient temperature for 72 h and monitored by ^{31}P NMR spectroscopy.

Control Experiments No. 2

Two samples of methyl diethyl phosphonoacetate (0.2 ml, 1 mmol) were taken. One was dissolved in $NaHCO_3$ solution (5%, 5 ml) and the other in NaOH solution (1 N, 5 ml). The solutions were kept at ambient temperature by for 72 h and monitored by ^{31}P NMR spectroscopy. The results are listed in Table 2.

Control Experiments No. 3

(a) Two portions of diethylphosphonoacetic acid (0.2 g, 1 mmol) were dissolved in NaOH solution (1 N, 5 ml). To one of the solutions was added $NH_2OH{\cdot}HCl$ (69.5 mg, 1 mmol). Both solutions were kept at ambient temperature for 24 h and monitored by ^{31}P NMR spectroscopy.

(b) Two portions of diethylphosphonoacetic acid (0.2 g, 1 mmol) were dissolved in $NaHCO_3$ solution (5%, 5 ml). To one of the solutions was added $NH_2OH{\cdot}HCl$ (69.5 mg, 1 mmol). Both solutions were kept at ambient temperature by for 240 h and monitored by ^{31}P NMR spectroscopy.

Control Experiment No. 4

To a solution of methyl diethyl phosphonoacetate (**1**, 0.2 ml, 1 mmol) in $NaHCO_3$ solution (5%, 5 ml) was added $NH_2OMe{\cdot}HCl$ (83.5 mg, 1 mmol). The solution was kept at ambient temperature by for 60 days and monitored by ^{31}P NMR spectroscopy.

Control Experiment No. 5

O-methyl diethylphosphonoacetohydroxamate (**7**, 23 mg, 0.1 mmol) and benzohydroxamic acid (17 mg, 0.1 mmol) were dissolved in $NaHCO_3$ solution (5%, 2 ml). The solution was kept at ambient temperature by for 7 days and monitored by ^{31}P NMR spectroscopy.

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