α-Hydroxyiminophosphonic Acids. New Types of Phosphorylating Agents through Monomeric Metaphosphate

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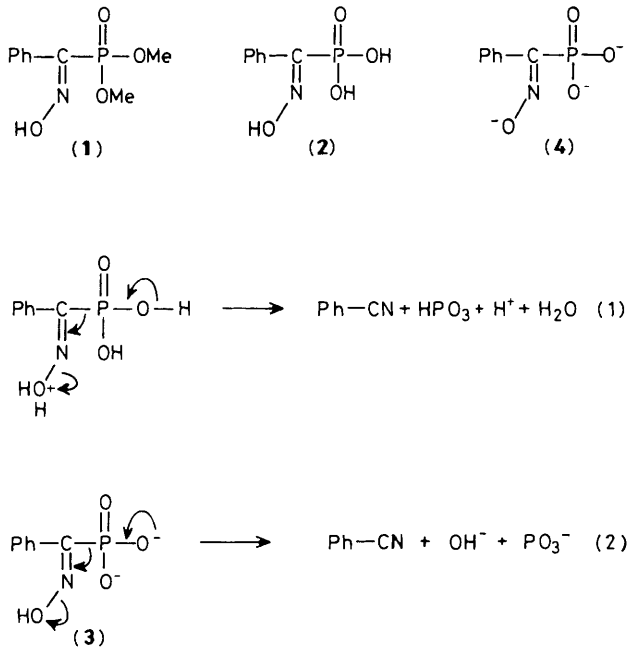
(*E*)-α-Hydroxyiminobenzylphosphonic acid decomposes in aqueous solution to benzonitrile and phosphoric acid in a pH dependent manner, which is consistent with a dissociative mechanism involving, in the first step, the formation of monomeric metaphosphate.

Phosphates are essential constituents of living organisms and phosphorylation is one of the most widespread chemical reactions of life.[1] Consequently, the study of phosphate transfer reactions continues to be in the focus of bio-organic chemistry. Despite some arguments about details, it is generally agreed that phosphoryl transfer reactions proceed by a dissociative mechanism either involving monomeric metaphosphate[2] as reactive intermediate, or through an open transition state of a dissociative nature, resembling monomeric metaphosphate.[3] Recent results regarding racemization at phosphorus in the alcoholysis of *p*-nitrophenyl phosphate[4] and positional isotope exchange in adenosine 5′-[α,β-^{18}O]diphosphate trianion in t-butyl alcohol[5] both indicate that monomeric metaphosphate may exist in protic solvents.

While it is undoubtedly of utmost importance to gain as much insight as possible into the mechanistic details of phosphate transfer reactions, it is no less important to design and prepare new molecules that are capable of generating monomeric metaphosphate under physiological conditions and which may serve as phosphorylating agents. In this communication we wish to describe a new functional group that fulfills such a role.

Dimethyl α-hydroxyiminobenzylphosphonate was obtained



as a mixture of (*E*) and (*Z*) isomers by reacting dimethyl benzoylphosphonate with hydroxylamine.[6] Treatment of this mixture of isomers with methanolic hydrogen chloride at room temperature resulted in its conversion into the (*E*) isomer (**1**) (90%), which was crystallized from benzene, m.p. 100—101 °C, ^{31}P n.m.r. ($CDCl_3$): δ 11.6 p.p.m. (septet), (all ^{31}P chemical shifts are reported relative to 85% H_3PO_4). The structure of this compound was determined by *X*-ray crystallography.[7] Treatment of (**1**) with trimethylsilyl bromide (2 equiv., acetonitrile, room temp., 3 h), followed by the addition of methanol, evaporation of the solvents and volatile byproducts (at 25 °C *in vacuo*), and washing with acetonitrile, left behind α-hydroxyiminobenzylphosphonic acid (**2**) as a pure solid, m.p. 98—99 °C, (decomp. to benzonitrile and orthophosphoric acid), ^{31}P n.m.r.: δ [dimethylsulphoxide (DMSO)] 5.85 p.p.m., δ(D_2O) 4.52 p.p.m. (s).† Dissolution of (**2**) in water (0.33 M) yielded a solution of pH 1.5. Monitoring of this solution by ^{31}P n.m.r. spectroscopy revealed slow decrease of the signal at δ 4.52 p.p.m., and a concomitant growth of a singlet at δ 0 p.p.m. which is attributed to orthophosphoric acid. Further, the pH dependence of the decomposition of (**2**) was examined and the results of this study are listed in Table 1.

These results show that the fragmentation of (**2**) is catalysed by either acid or base. The acid catalysis can best be rationalized by the same type of mechanism we proposed previously to explain the fragmentation of methyl hydrogen α-hydroxyiminobenzylphosphonate, which was postulated to lead to monomeric methyl metaphosphonate.[8] In the present instance, protonation of the oxime oxygen initiates fragmentation of (**2**) to give monomeric metaphosphoric acid and benzonitrile [equation (1)].‡

Table 1. pH Dependence of the decomposition of α-hydroxyiminobenzylphosphonic acid (**2**).[a]

	pH	Medium	$t_{\frac{1}{2}}$/min
1	1.5		20
2	2.1	Trace of NaOH	55
3	5.5	1 mM $NaHCO_3$	120
4	7.5	3 mM $NaHCO_3$	28
5	8.5	1 mM Na_2CO_3	12
6	9.2	Borate buffer	18
7	11.7	2.1 mM NaOH	4300[b]
8	14	3mM NaOH	∞[c]

[a] These results were obtained from the integrated ^{31}P n.m.r. signals of (**2**) (decreasing) and of orthophosphate (increasing) during the reactions, using solutions of 1 mM of (**2**) in 3 ml D_2O, and the added base indicated, at ambient temperature. [b] Calculated from 15% reaction observed in 9 h. [c] No decomposition was observed in 15 h.

Further examination of the results in Table 1 reveals that the fragmentation reaction is relatively slow at pH 5.5, which corresponds to the monoanion derived from (**2**). The reaction rate increases with increase in pH up to 8.5, at which point the phosphonic acid is presumably fully di-ionized. It seems unlikely that such a di-ionized species would be more electrophilic than the monoanion or the unionized diacid, therefore we envisage the fragmentation of the dianion (**3**) to take place by the dissociative mechanism outlined in equation (2), analogous to that proposed for the Conant–Swan fragmentation[9] and to the decomposition of aryl phosphates.[10] The gradual drop in the rate of fragmentation with additional increase in pH is probably caused by partial ionization of the oxime hydroxy group as its pK_a is approached. Finally, the total lack of fragmentation at pH 14 is attributed to the complete ionization of the oxime OH, with the formation of trianion (**4**), which no longer has a suitable leaving group to allow fragmentation.

Compound (**2**) behaved similarly when dissolved in methanol (0.33 M) in the presence of 1 and 2 equivalents of di-isopropylethylamine yielded, at ambient temperature, methyl dihydrogen phosphate [^{31}P n.m.r.: monoanion, δ 1.92 p.p.m. (q); dianion, δ 1.47 p.p.m. (q)] as the sole phosphorus containing product, with approximate $t_{\frac{1}{2}}$ values of 11 and 4 h, respectively.§

α-Hydroxyiminobenzylphosphonic acid (**2**) described above is merely a representative example of this functional group as a new type of phosphorylating agent. The ease of preparation of these compounds (from acyl halides *via* acylphosphonates and their oximes) facilitates the attachment of the oximinophosphonic functionality to virtually any kind of organic molecule possessing a carboxy group, such as fatty acids, peptides, steroids, *etc.*, and therefore makes it possible, in principle, to design site-specific phosphorylating agents by tethering the oximinophosphonic moiety to molecules with special transport or binding properties.

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† Satisfactory elemental analysis was obtained.

‡ While our results are consistent with dissociative, metaphosphate-like mechanisms for the fragmentation of oximinophosphonates under both acidic[8] and basic conditions, they do not provide evidence for monomeric metaphosphate as a discrete intermediate involved in these reactions.

§ One of the commonly accepted diagnostic tests for monomeric metaphosphate is the absence of steric effects in the phosphorylation of t-butyl alcohol as compared to a primary alcohol.[10,11] The performance of further experiments with (**2**) is hampered by its solubility properties. Compound (**2**) is insoluble in acetonitrile, while its bis(di-isopropylethylammonium) salt is insoluble in dioxane and t-butyl alcohol.

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