"Troika Acids": Synthesis, Structure, and Fragmentation Pathways of Novel α-(Hydroxyimino)phosphonoacetic Acids

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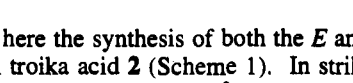
The classical investigations of the Westheimer school and others have demonstrated the chemical plausibility and biological importance of monomeric metaphosphate (or equivalent solvate complexes thereof) as a highly reactive, electrophilic phosphorylation intermediate.[1] Recent investigations have emphasized the desirability of designing new precursors that can function as phosphorylating agents under physiological conditions.[2]

In this communication we introduce a novel molecular system combining phosphonate, oxime, and carboxylate moieties anchored to a common carbon atom: α-(hydroxyimino)phosphonoacetic acids, or "troika acids".[3] By virtue of its unique central location in these compounds, the oxime hydroxy can interact via hydrogen bonding with either of its two neighboring groups, depending on whether it assumes *E* or *Z* orientation, and thereby should influence, if not direct, the course of reaction taken by the molecule under particular conditions. In addition, the α-carboxyl group embodies a new concept for modulation of the hydroxyimino phosphonate moiety's activity as a phosphorylating agent: chemical (or possibly enzymatic) unmasking of a neutral troika acid carboxyl derivative such as a C-ester to generate the free carboxylic acid (or carboxylate anion), resulting in a significantly modified interaction with the oxime hydroxy (and possibly phosphonate) groups. In principle, such a C-group-dependent P-activation process might be mediated by a reagent or catalyst that is highly specific for the C moiety.

Prototypic Troika Acids

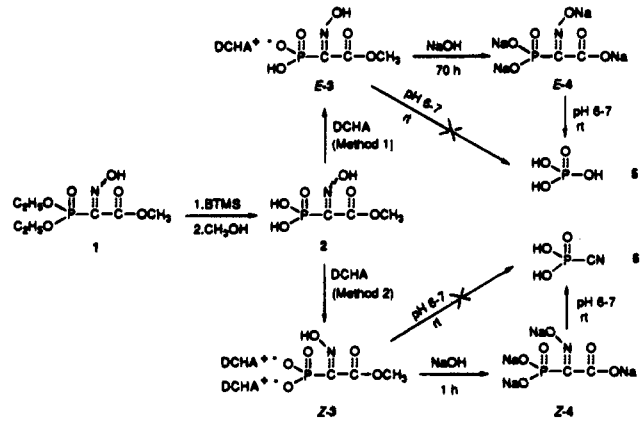


Z-isomer *E*-isomer

We report here the synthesis of both the *E* and *Z* isomers of the C-methyl troika acid **2** (Scheme 1). In striking difference from benzoyl oxime phosphonates,[2a] the *E* isomer of **2** is stable in H_2O at physiological pH, but on aqueous alkaline cleavage of the carboxylate ester group generates the *E* troika acid, which proves to be an active phosphorylating agent at pH 6−7.

Methyl α-(hydroxyimino)phosphonoacetic acid (**2**), obtained on regioselective didealkylation of the corresponding P,P-diethyl C-methyl ester **1**[4] (*E*:*Z*, 4:1; ^{31}P NMR) with 3 equiv of Me_3-

Scheme 1



$SiBr$[5,6] (BTMS) in refluxing CH_2Cl_2 (3 h) followed by methanolysis, gave on treatment with 2 equiv of dicyclohexylamine (DCHA) a 4:1 mixture of isomers (*E*)-**3** and (*Z*)-**3** (Scheme 1). Recrystallization from methanol/ether ("method 1") gave (*E*)-**3** as a mono-dicyclohexylammonium ($DCHA^+$) salt (66%).[7] Intriguingly, a different workup [slow evaporation of solvent to crystallize **2** (*E*:*Z* ratio of 5:95), addition of 2 equiv of DCHA and recrystallization from 1-propanol/acetone; "method 2"] gave pure (*Z*)-**3**, the first example of an isolated *Z* hydroxyimino phosphonic acid, as a bis-$DCHA^+$ salt (62%).[8]

X-ray crystallographic analysis[9] (Figures 1 and 2) confirmed the structures of both (*E*)-**3** and (*Z*)-**3** and provided insight into the role of the iminohydroxy stereochemistry in making both compounds isolatable, but with differing salt stoichiometries, under the rather specific conditions of our methods 1 and 2. In the *Z* isomer, the PO anion is stabilized by an intramolecular hydrogen bond involving the oxime proton (expected to be weakly acidic: the pK_a of dialkyl benzoylphosphonate oximes is estimated to be about 6[12]). This interaction should decrease pK_{a2} for the phosphonate conjugate acid,[13] facilitating its protonation of a second molecule of DCHA. In the monobasic *E* isomer salt, the oxime proton forms instead a hydrogen bond to the phosphoryl group of a neighboring molecule. Another major difference between the two structures is manifested in their the P−C_α−N bond angles, which are 117.7° in (*E*)-**3** and 128.1° in (*Z*)-**3**. This association of *Z* isomerism with "obtuse canting" of the oxime group relative to the P−C_α−C_β reference

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(3) Evoking the Russian word for three steeds harnessed to a sleigh. The parent ketone, phosphonoglyoxylic acid, has been described: McKenna, C. E.; Levy, J. N. *J. Chem. Soc., Chem. Commun.* **1989**, 246−247.

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(7) Representative physical data for (*E*)-**3**: mp 143−144 °C; ^{1}H NMR (D_2O) δ (ppm) 1.0−2.1 (m, 20 H, CH_2), 3.19 (m, 2 H, CH), 3.84 (s, 3 H, OCH_3); ^{13}C NMR (D_2O) δ (ppm) 24.1, 24.7, 29.2, 53.4 (cyclohexyl), 53.1 (s, OCH_3), 152.5 (d, $^1J_{P-C}$ = 191.8 Hz, C=N), 165.1 (d, $^2J_{P-C}$ = 20.1 Hz, C=O); ^{31}P NMR (D_2O, pH = 4.3) δ (ppm) −1.13.

(8) Representative physical data for (*Z*)-**3**: mp 171−172 °C; ^{1}H NMR (D_2O) δ (ppm) 0.9−2.0 (m, 40 H, CH_2), 3.06 (m, 4 H, CH), 3.66 (s, 3 H, OCH_3); ^{13}C NMR (D_2O) δ (ppm) 23.8, 24.4, 28.9, 53.1 (cyclohexyl), 52.5 (s, OCH_3), 154.7 (d, $^1J_{P-C}$ = 130.8 Hz, C=N), 165.9 (d, $^2J_{P-C}$ = 18.6 Hz, C=O); ^{31}P NMR (D_2O, pH = 7.1) δ (ppm) 2.38.

(9) Crystal data for the $DCHA^+$ monoanion salt (*E*)-**3** ($C_{15}H_{29}O_6N_2P$): triclinic, space group *P*1, a = 11.682(12) Å, b = 17.222(32) Å, c = 10.657(9) Å, α = 103.03(12)°, β = 109.23(14)°, γ = 95.36(12)°, and V = 2009(5) Å^3. The unit cell contains two independent (*E*)-methyl α-(hydroxyimino)-phosphonoacetate anions and two independent DCHA cations. The structure was solved by direct methods[10] and refined to a final R factor of 0.068 for 3492 reflections [$I > 3\sigma(I)$].[11] Crystal data for the bis-$DCHA^+$ dianion salt (*Z*)-**3** ($C_{27}H_{52}O_6N_3P$): triclinic, space group *P*1, a = 12.562(14) Å, b = 21.472(37) Å, c = 12.130(20) Å, α = 98.33(14)°, β = 92.42(11)°, γ = 99.09(12)°, and V = 3189(9) Å^3. There are two independent (*Z*)-methyl α-(hydroxyimino)phosphonoacetate anions and four independent DCHA cations in the unit cell. Full-matrix least-squares refinement yielded a final R factor of 0.083 for 4705 reflections [$I > 3\sigma(I)$].[11]

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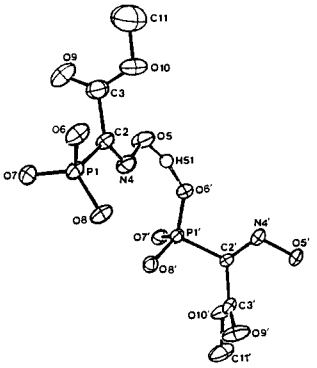


Figure 1. A perspective view of the anion in (*E*)-**3** showing intermolecular hydrogen bonding. Selected bond lengths (Å) and angles (deg): P1−C2, 1.814(6); C2−C3, 1.536(8); C2−N4, 1.264(8), O5−H51, 1.22(6), O6′−H51, 1.51(5); P1−C2−C3, 118.8(5); P1−C2−N4, 117.7(4); C3−C2−N4, 123.4(5), O5−H51−O6′, 146(3).

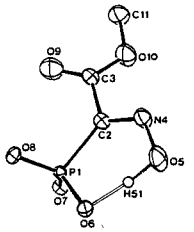
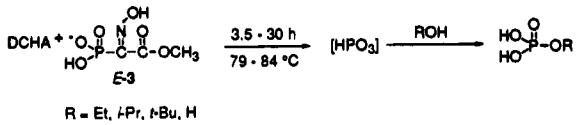


Figure 2. A perspective view of the anion in (*Z*)-**3** showing intramolecular hydrogen bonding. Selected bond lengths (Å) and angles (deg): P1−C2, 1.858(11); C2−C3, 1.462(13); C2−N4, 1.304(12), O5−H51, 1.11(6), O6−H51, 1.40(5); P1−C2−C3, 120.4(6); P1−C2−N4, 128.1(8); C3−C2−N4, 111.4(9), O5−H51−O6, 164(4).

Scheme 2



framework (and "acute canting" in the case of *E* isomerism) is also observed in phosphonoglyoxylate arylhydrazones.[14] *E/Z* isomerism in both groups of compounds can be predictively correlated with their ^{13}C NMR $^{1}J_{PC}$ values.[15]

In contrast to α-(hydroxyimino)benzylphosphonate, which has a $t_{1/2}$ of 0.2−2 h for fragmentation to benzonitrile and orthophosphate (via monomeric metaphosphate) over pH 1.5−9.2 at ambient temperature,[2b] both **3** isomers were *stable* at 22 °C (24 h by ^{31}P NMR) in D_2O (pH 2−8). Nevertheless, on heating of (*E*)-**3** in EtOH−*i*-PrOH, EtOH−*t*-BuOH, and H_2O, products (^{31}P NMR) strongly suggestive of monomeric metaphosphate-like fragmentation[1a] were seen (Scheme 2). (*E*)-**3** fragmented over several hours in refluxing acetonitrile to yield chiefly (81%) products with ^{31}P NMR $\delta = -10$ and -22, identified[2c] as polyphosphates, which are known self-condensation products of metaphosphate.[1a] (*E*)-**3** was unchanged after 5 h in 1:1 MeOH−*i*-PrOH at 72 °C, but after 30 h in 1:1 EtOH−*i*-PrOH at 79 °C, it gave ethyl phosphate and isopropyl phosphate (84%) in a 1.3:1 ratio. A similar experiment in 1:1 EtOH−*t*-BuOH gave a product ratio of ~2:1 ethyl phosphate−*tert*-butyl phosphate. Lack of selectivity in phosphorylation of a primary alcohol vs the sterically hindered *tert*-butyl alcohol is a well-known criterion for a dissociative (metaphosphate) mechanism and excludes an associative−elimination phosphorylation mechanism, where a much larger EtOH−*t*-BuOH selectivity ratio would be expected. Heating (*E*)-**3** in H_2O (3.5 h, 82−84 °C) gave 100% conversion to phosphate at pH 4.0, and 88% conversion at pH ~ 6.5. The *Z* isomer of **3** was stable for 4 h in 1:1 EtOH−*i*-PrOH at 79 °C and showed 46% conversion to a mixture of phosphate (28%), phosphonitrile **6** (13%; see below), and the *E* isomer (5%) in H_2O (5.7 h, 82−84 °C).

The observation that C-alkyl esters of **3** are stable under physiological conditions but reactive under more vigorous conditions prompted examination of the stability of the parent troika acids in H_2O. Alkaline hydrolysis of (*E*)-**3** and (*Z*)-**3** (aqueous NaOH, 25 °C, pH 13−14) gave the corresponding sodium salts (*E*)-**4**[16] and (*Z*)-**4**,[17] the *Z* ester reacting much more rapidly (1 h vs 70 h for (*E*)-**3**). Both salts **4** were quite stable at high pH and room temperature, but at pH 6−7 facile fragmentations were observed, which proceeded *stereospecifically*. (*E*)-**4** ($t_{1/2}$ < 10 min) produced exclusively orthophosphoric acid[18] (**5**, ^{31}P NMR, δ 1.0 ppm), consistent with phosphorylation of the solvent, whereas (*Z*)-**4** ($t_{1/2}$ ~ 15 min) gave a product with an intact P−C bond, identified as phosphorocyanidic acid (**6**)[20] (Scheme 1[21]). The transformation of *anti* α-keto acid oximes to nitriles in aqueous solution at 40−100 °C is well documented,[23] but the corresponding process giving a phosphonitrile from a phosphonate α-oxime, observed with (*Z*)-**4**, has no precedent. Most remarkable is the finding that simple removal of the carboxy alkyl group of (*E*)-**3** creates a reagent [(*E*)-**4**] able to phosphorylate under mild aqueous conditions.

In conclusion, the position of the oxime hydroxy group in troika acids mediates phosphorylation vs nitrile formation via alternative fragmentation pathways, with the *state of the carboxyl group controlling reactivity* in neutral aqueous solution at ambient temperature. While we utilized saponification of the inactive ester **3** to generate active form **4** in this preliminary study, it is apparent that suitable modification of the acyl moiety to render it susceptible to cleavage by a specific chemical or biochemical reagent offers the potential for convenient control over generation of an active phosphorylating agent under mild, aqueous conditions.

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Supporting Information Available: Synthesis and characterization details for **1**−**6**, experimental data from stability studies, and tables of crystallographic data, atomic coordinates, thermal parameters, bond distances and angles, and calculated hydrogen positions for (*E*)-**3** and (*Z*)-**3** (33 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(16) ^{13}C NMR (D_2O) δ (ppm) 161.8 (d, $^{1}J_{P-C}$ = 175.7 Hz, C=N), 175.4 (d, $^{2}J_{P-C}$ = 20.5 Hz, C=O); ^{31}P NMR (D_2O, pH = 13−14) δ (ppm) 2.8.

(17) ^{13}C NMR (D_2O) δ (ppm)161.1 (d, $^{1}J_{P-C}$ = 126.8 Hz, C=N), 171.8 (d, $^{2}J_{P-C}$ = 17.1 Hz, C=O); ^{31}P NMR (D_2O, pH = 13−14) δ (ppm) 2.0.

(18) The side product initially formed from this fragmentation is expected to be cyanocarboxylate, which should hydrolyze: $NCCO_2^- \rightarrow CO_2 + CN^-$. Gas evolution, attributed to CO_2, was observed during (*E*)-**4** fragmentation at pH 6−7, and the reaction medium gave a positive test for CN^- [19] that visually matched a positive control with NaCN. The test was negative when applied to (*E*)-**3**.

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(20) ^{31}P NMR (D_2O, pH = 6) δ (ppm) −14.8; ^{13}C NMR (D_2O) δ (ppm) 123.5, (d, $^{1}J_{P-C}$ = 145 Hz, P−CN). These data are consistent with those of the authentic compound prepared from dimethyl phosphorocyanidate[22] by silyldealkylation with BTMS (3 equiv, 25 °C, 4 days) and quenching with MeOH and evaporation. The acid was also isolated as a $DCHA^+$ salt.

(21) In the schemes, the monomeric metaphosphate intermediate and the **5**, **6**, and alkyl phosphate products are represented as fully protonated for convenience only.

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