

PREBIOTIC POTENTIAL OF REDUCED OXIDATION STATE PHOSPHORUS. THE H-PHOSPHINATE-PYRUVATE SYSTEM. D. E. Bryant,¹ K. E. R. Marriott,¹ S. A. Macgregor,² C. W. G. Fishwick,¹ C. Kilner,¹ E. K. Bullough,¹ M. A. Pasek,³ and T. P. Kee^{1*} ¹School of Chemistry, University of Leeds, Leeds LS2 9JT, UK. E-mail: t.p.kee@leeds.ac.uk, ²School of Engineering & Physical Sciences, Perkin Building Heriot-Watt University, Edinburgh EH14 4AS, UK. E-mail: S.A.Macgregor@hw.ac.uk, Department of Geology, University of South Florida, 4202 E. Fowler Avenue SCA 528 Tampa, Florida, 33620-8100 USA. E-mail: mpasek@cas.usf.edu.

Introduction: Contemporary organisms use, almost exclusively, orthophosphate derivatives (PO_4^{3-}) in their cell biochemistry,¹ yet thorny questions remain as to how Nature was able to accumulate, activate and exploit the orthophosphate group from geological sources with both poorly solubility and low chemical activity.² Consideration of this phosphate problem prompted Gulick to argue³ a central role for reduced oxidation state phosphorus (P) oxyacids such as H-phosphonates (H_2PO_3^-) and especially H-phosphinates (H_2PO_2^-) in prebiotic chemistry on account of the greater water solubility of their metal salts and, with the presence of P-H bonds, a different reactivity profile to that expected of orthophosphate. The recent demonstration that hydrothermal corrosion of P-rich mineral phases such as schreibersite ($(\text{Fe,Ni})_3\text{P}$) within iron meteorites leads to production of various P-oxyacids including H-phosphonic (H_3PO_3)⁴ and H-phosphinic⁵ acids as well as orthophosphate has reignited interest in reduced oxidation state P chemistry in prebiotic environments. We have recently embarked on a broad-based exploration of the prebiotic potential of reduced oxidation state P-chemistry and one of the first such systems we are examining involves reactions of H-phosphinates with carbonyl substrates. Several carbonyl compounds have either reasonable prebiotic provenance or have been proposed as being of considerable value to prebiotic chemistry should they have been available. Examples include formaldehyde & glycolaldehyde, both intimately involved in the formose reaction for sugar synthesis⁶ and pyruvic acid,⁷ a product of glycolysis and feedstock for the citric acid cycle, a fundamental cellular metabolic process whose heritage is considered an ancient one. In this contribution we present some of our preliminary results on the H-phosphinate-pyruvate system.

The H-Phosphinate-Pyruvate System: H-Phosphinic acid reacts cleanly with pyruvic acid in unbuffered water (pH *ca.* 3) under an atmosphere of dinitrogen as outlined in Figure 1. Room temperature reaction affords H-phosphinate **1** as the principal initial product, resulting from insertion of the P-H bond into the carbonyl function of pyruvic acid, along with trace amounts of H-phosphonic acid (H_3PO_3) which are presumed to form *via* oxidation of H-phosphinate with trace dioxygen in the system. Similar reaction takes place with

place with the sodium salt of H-phosphinic acid at pH *ca.* 4 albeit somewhat slower.

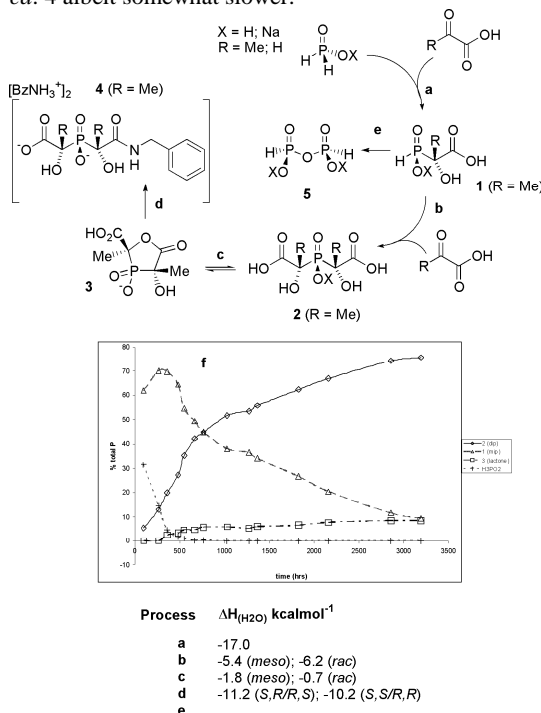
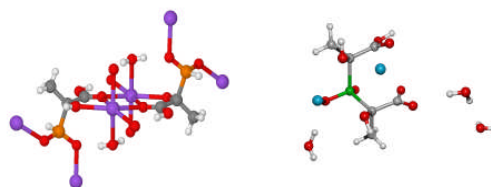


Figure 1. a: 25°C; pH 3; H_2O affords **1**. b: 70°C; H_2O affords **2** which exists in equilibrium (c) with lactone **3** ([**2**]/[**3**] of *ca.* 8 (pH 3; 25°C). d: Lactone **3** reacts with benzylamine to afford **4**. e: Thermolysis at 140°C of **1** affords **5**. f: Time evolution of the H-phosphinate-pyruvate system (25°C; pH 3; H_2O ; N_2) with H_3PO_2 (75 mM) and pyruvic acid (2.8 M). Percentage (%) total phosphorus estimated from integration of ^{31}P NMR spectra.

H-Phosphinate **1** undergoes further reaction with pyruvic acid under ambient conditions to afford diinsertion product **2**. *In situ* identification by negative ion electrospray mass spectrometry confirms the atomic composition and ^{31}P -NMR spectroscopy reveals that **2** is present as an *ca.* 1:1 mixture of diastereoisomers (racemic *R,R/S,S* & *meso R,S*), each a clearly resolved and distinctive seven-line resonance at δ 36.8 and δ 37.3 ppm due to coupling [$^3J(\text{PH})=13$ Hz] between phospho-

Hz] between phosphorus and each of the six methyl hydrogen atoms. The **2-Na₂** form has been isolated as a mixture of diastereoisomers from which one specific form crystallises selectively from aqueous solution. Single crystal X-ray analysis identifies this as the *R,S* (*meso*) diastereoisomer. Thermodynamic calculations at the density functional theory level reveal that formation of **1** is enthalpically favoured by *ca.* 17 kcalmol⁻¹ whilst a second insertion reaction between pyruvate and **1** leading to **2** is also energetically favoured by between 5-6 kcalmol⁻¹ (Figure 1), there being little thermodynamic difference between the two diastereoisomers of **2**, consistent with ³¹P-NMR observations. A time-evolution plot (Figure 1) of the components in the room temperature HPP system reveals that **1** dominates in the earlier stages of reaction after which time (*ca.* 750h) **2** becomes the ultimate thermodynamic product when pyruvate is present in excess. A closer examination of the ³¹P-NMR spectrum leading to formation of **2** from **1** and pyruvic acid reveals two additional seven-line signals at δ 30.0 and δ 29.2 ppm [³J(PH)=12 Hz] which display interesting hydrothermal behaviour. Evaporating an aqueous solution in which the δ 30.0 and δ 29.2 components constitute *ca.* 9% of total solution phosphorus to dryness at 70°C for 3h results in augmentation of these signals to *ca.* 60% of total phosphorus upon re-dissolution in water. Subsequent warming of this latter aqueous solution at 70°C leads to attenuation of both δ 30.0 & 29.2 signals to *ca.* 22% of total solution phosphorus. Re-evaporation of the aqueous solution again results in the δ 30.0 & 29.2 signals dominating the spectrum. Our interpretation of these observations is that repeated evaporation-rehydration cycles result in reversible dehydration of **2** to afford a new compound **3** which exists, like **2**, as a mixture of two diastereoisomers. Negative ion electrospray mass spectrometric analysis confirms the presence of both hydrated and dehydrated compounds in the mixed system and identifies peak abundance ratio's [2]/[3] of 1.6 (measured ratio of 2.7 by ³¹P-NMR) and 0.3 (measured ratio of 0.4 by ³¹P-NMR) respectively. Subsequently, we have found that exhaustive dehydration at 140°C for 16 h of an aqueous solution of **2**-(NH₄⁺)₂ (initial pH 3) affords **3** as an *ca.* 1:1 mixture of diastereoisomers which constitute *ca.* 92% of total phosphorus. Crystallisation of this mixture from methanol in the presence of triethylamine affords diastereoisomerically pure **3** as a triethylammonium salt in *ca.* 20% yield (un-optimised). Of the two chemically plausible structures for **3**, a six-membered anhydride ring or a five-membered lactone ring, solution ¹³C{¹H} & ¹H-NMR spectroscopies are more consistent with the lower symmetry lactone form and this has been confirmed sub-

and this has been confirmed subsequently by single crystal X-ray analysis of the isolated triethylammonium salt. DFT calculations also confirm that the six-membered anhydrides are higher in energy than the 5-membered alternatives, by approximately 18 kcalmol⁻¹. Treatment of **2** with benzylamine (BzNH₂) at ambient temperature affords amide **4**. Amide bond formation between a carboxylic acid and amine under such mild conditions in aqueous solution without an external coupling agent would have been an extremely valuable reaction within a prebiotic chemistry context. Neither lactic acid itself nor **1** undergo amide formation under similarly mild, aqueous conditions, the obvious possibility was that lactone **3** facilitated amide bond formation specifically from **2**. DFT calculations (Figure 1) reveal that the conversion of **3** to **4** should be significantly favourable enthalpically. Subsequently, dry heating **2-Na₂** to 140°C for 1 h results in clean conversion to **3** which, upon treatment with pure BzNH₂ affords **4** directly. Compound **1**, on the other hand undergoes a rather different transformation upon heating to 140°C for several hours. Oxidative coupling results in formation of the condensed species hyperphosphite



The above transformations, along with computational and single-crystal X-ray diffraction analyses such as those shown above for **1-Na** (*left*) and **2-Na₂** (*right*) will be discussed further in the context of their potential for prebiotic chemistry and the feasibility of such chemistry being initiated under geologically plausible conditions.

References:

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