SYNTHESIS OF [17O]- AND [18O]-LABELLED HYPOPHOSPHATE

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SUMMARY

The preparation of ^{17}O- and ^{18}O-labelled hypophosphate by oxidation of diphosphite (P^2-P^4 acid) with iodine-water is described. The 162 MHz NMR $^{31}P(^{18}O)$ isotope shift of ^{18}O-labelled hypophosphate is 2.42 Hz (0.0149 ppm), the relatively large value compared to ^{18}O-pyrophosphate, 1.73 Hz (0.0107 ppm), being suggestive of an as yet unobserved $^{31}P(^{18}O)$ β-isotope shift of 1.38 Hz (0.009 ppm)

Key words: Hypophosphate   isotope shift ^{31}P NMR ^{17}O, ^{18}O

INTRODUCTION

The discovery that ^{18}O directly bonded to phosphorus gives rise to a bond order-dependent isotope shift in the ^{31}P NMR spectrum of labelled phosphates [1] has led to its extensive exploitation in investigation of the enzymology of phosphoryl transfer reactions [2]. ^{17}O labelling of phosphorus has also been shown to generate unusual effects [3,4]. It has therefore been of interest to develop methods for the synthesis of oxygen-labelled biophosphates [2,5]. This has resulted in a range of methods for synthesizing, for example, oxygen-labelled pyrophosphate in the bridging and non-bridging positions [6,7]. Although interest in the labelling of biophosphates has been extensive, this methodology could be usefully applied to mechanistic studies in phosphorus chemistry in general and such trends are now observable [8]. In particular it

seems that the approach could be of application in investigating the wealth of relatively unexplored mechanistic chemistry offered by the lower oxyacids of phosphorus and their transformations into phosphates [9]. We have initiated this approach with a positional isotope study of the oxidation of hypophosphate to pyrophosphate [7] and present here a simple procedure for the preparation of labelled hypophosphate.

RESULTS AND DISCUSSION

P^2-P^4 acid (1) was oxidized to [^{18}O]-hypophosphate (4) using an iodine-pyridine-$H_2^{18}O$ mixture (Scheme 1). The oxidation of diphosphite to hypophosphate (2) has been known for some time [10]. The incorporation of one ^{18}O isotope was confirmed by NMR spectroscopy. When the ^{31}P NMR spectrum of (4) was recorded a $^{31}P(^{18}O)$ isotope shift of 2.42 Hz (0.0149 ppm) was observable (Figure 1). The peak at lower field, assignable to unlabelled
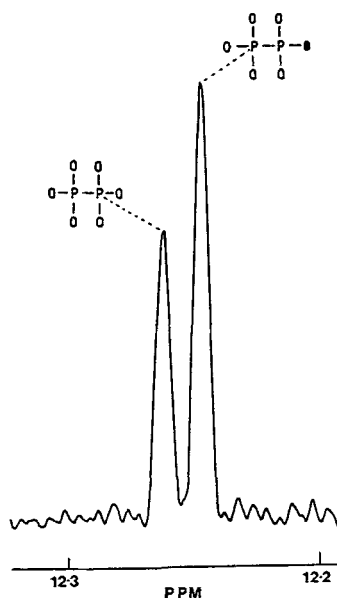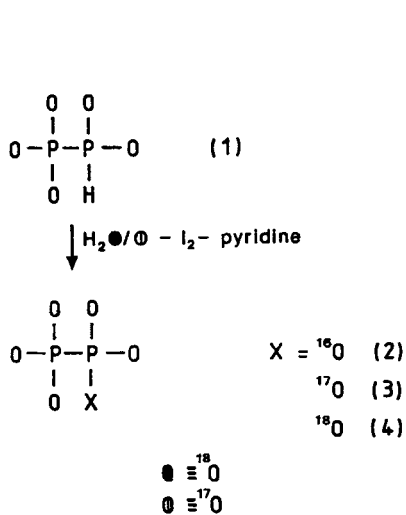
Scheme1:
Oxidation of diphosphite to
labelled hypophosphate

Figure 1:
162 MHz ^{31}P NMR parameters:
SW, 527 Hz; PW, 12 μs; AQ, 7.7 s;
8K points; NS, 112; LB, -0.4 Hz.

hypophosphate, was derived from isotope dilution as a result of the water of crystallisation of (1) and provided a convenient internal reference. Approximately 60% of the hypophosphate had been

isotopically labelled. This preparation was repeated with ^{17}O - enriched water (23.2% ^{16}O, 45.6% ^{17}O, 31.2% ^{18}O) to obtain predominantly [^{17}O]-hypophosphate (3). Phosphorus atoms directly bonded to ^{17}O are not observed in the ^{31}P NMR spectrum [3], thus the spectrum (Figure 2) only exhibited peaks due to unlabelled material (2) and ^{18}O-labelled material (4) in approximately the correct ratio. The observation of a broad resonance in the ^{17}O NMR spectrum attributable to (3) [δ 129.3 p.p.m.) directly confirmed the incorporation of ^{17}O isotope.
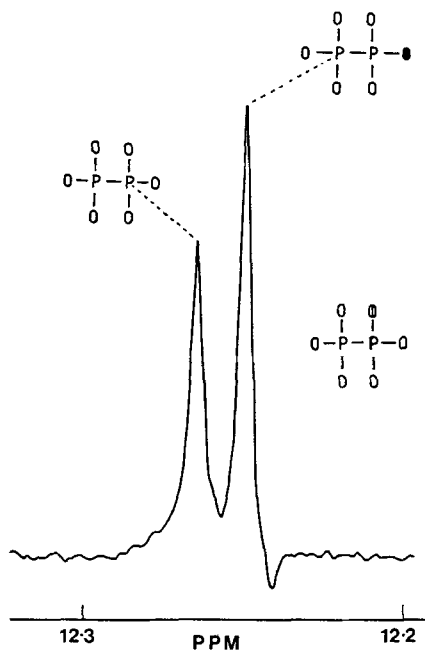

Figure 2:
162 MHz ^{31}P NMR parameters:
SW, 527 Hz; PW, 12 μs; AQ, 7.7 s
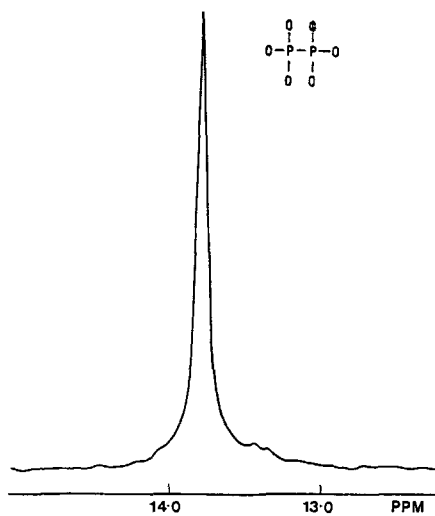8K points; NS, 3200; LB -1.0 Hz.

Figure 3:
162 MHz ^{31}P NMR parameters:
SW, 527, Hz; PW, 12μs; AQ, 7.7s;
8K points; NS, 2000; LB, -1.0 Hz.

^{17}O is a quadrupolar nucleus and thus causes extreme broadening of the ^{31}P resonance of a directly-bonded phosphorus [3] In a recent study of the effects of ^{17}O substitution in the non-bridging position of pyrophosphate (8) we demonstrated that the ^{17}O quadrupole does not decouple the neighbouring phosphorus even in a tightly coupled system and the adjacent phosphorus nucleus resonated as a

doublet, thus enabling the measurement of the ^{31}P-^{31}P geminal coupling constant for pyrophosphate to be measured [4]. It was of interest to examine the effects of such incorporation in hypophosphate since the bridging oxygen atom of pyrophosphate is absent and the P-P spin-spin coupling is expected to be very strong [11].

In the event, it was evident from the ^{31}P NMR spectrum of (3) (Figure 3) that no resonances due to the phosphorus atom in (3) not directly bonded to ^{17}O could be observed and only a singlet was seen for the isotopomers of this molecule at low resolution. It is likely therefore that the ^{17}O nucleus is not only broadening the resonance of the directly-bonded phosphorus but also that of its neighbour. Therefore, it was not possible to determine the ^{31}P-^{31}P spin-spin interaction in hypophosphate by this method.

To verify by ^{31}P NMR spectroscopy that (3) did indeed contain ^{17}O and that the above conclusions were valid, a sample of (3) was oxidized as described [7] (Scheme 2) in normal water to [^{17}O]-pyrophosphate. Since the bridging oxygen in pyrophosphate prepared in this way is known not to come from solvent water [7] one sixth of the ^{17}O should end up in the bridge and five sixths in non-bridging positions and the expected result should be a mixture of (5) (16.7%) and (6) (83.3%). Resonances from (5) would not be observable due to broadening of signals from both phosphorus nuclei, but at low resolution (6) should appear as described [4], namely a singlet flanked by a doublet. The ^{31}P NMR spectrum of (3) after oxidation is shown in Figure 4. The small sidebands with a splitting of ca. 19 Hz clearly demonstrate the presence of (6) and therefore confirm that the ^{17}O effect is unique to pyrophosphate and not observable in (3).

The $^{31}P(^{18}O)$ isotope shift observed for (4) is 2.42 Hz. This is significantly larger than the 1.73 Hz reported for pyrophosphate labelled with ^{18}O in the non-bridging position [6]. It is worthwhile considering why this should be the case. To do this it is instructive to consider the example of non-bridge ^{18}O-labelled pyrophosphate (8) (Figure 5a). The isotope shift for the latter at 162 MHz is 1.73 Hz, yet the shift for bridge-labelled pyrophosphate (not shown) would be larger at 3.1 Hz [6], although the bond order is effectively smaller in the latter case. This is because non-bridge-labelled pyrophosphate is effectively an AB system (outer lines not
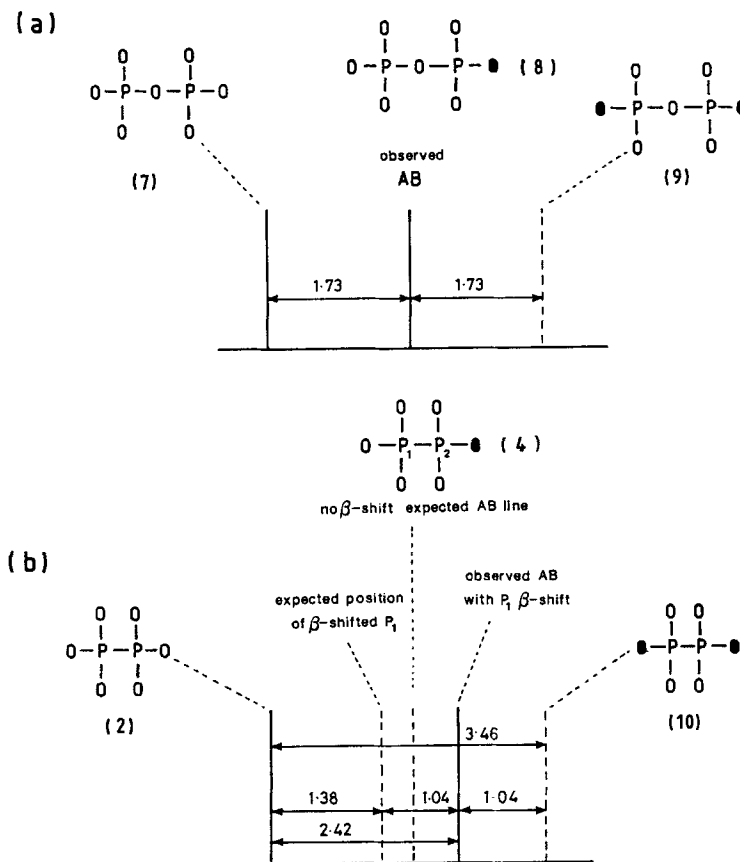
Scheme 2

Figure 4:
121.5 MHz ^{31}P parameters: SW,
2429 Hz; PW, 5 μs; 8K points; AQ,
1.69 s: NS, 4261; LB, -2.0 Hz; GB, 0.08 Hz.

observed) and relative to unlabelled pyrophosphate (7) resonates midway between unlabelled material and material labelled with ^{18}O on each phosphorus (9) (shift expected to be ca. 3.46 Hz).

In polyphosphate systems, where the chemical shifts of the various phosphates are well separated, no ^{18}O-induced shifts on phosphorus nucleii other than on the directly bonded nucleus have been observed. This contrasts with $^{13}C(^{18}O)$ shifts where several recent examples of β-isotope shifts have been reported [12] and also with $^{13}C(^2H)$ and $^{19}F(^2H)$ shifts [13].

We have already demonstrated that ^{17}O substitution can have a broadening effect on the β-phosphorus atom of hypophosphate and in view of the absence of a bridging oxygen atom in hypophosphate there should be a strong possibility of observing a $^{31}P(^{18}O)$ β-isotope shift in (4). In Figure 5(b) we infer the presence of such a shift to rationalize the larger shift observed for (4). Thus, the

Figure 5: (a) Position of isotope shifted resonances in the ^{31}P NMR spectrum of ^{18}O-labelled pyrophosphate, (b) Rationalisation of expected and observed resonances in the ^{31}P NMR spectrum of ^{18}O-labelled hypophosphate.

separation between unabelled hypophosphate and hypophosphate labelled on each phosphorus atom (10) would be expected to be 3.46 Hz at 162 MHz, similar to that of (9). The position of the observed central AB line of (4) occurs 2.42 Hz upfield from unlabelled hypophosphate, i.e. 1.04 Hz downfield from (10). Since this line will be symmetrically situated between the shifts for (10) and the true shift for the non-labelled phosphorus atom of (4), we can infer that the latter resonates 1.04 Hz downfield of the central AB line and therefore 1.38 Hz upfield of unlabelled hypophosphate (2). This value of 1.38 Hz (0.009 ppm) must therefore represent an upfield $^{31}P(^{18}O)$ β-isotope shift. Such a shift has not yet been

observed. Attempts are now under way to provide a direct observation of this effect in similar compounds.

EXPERIMENTAL

Materials and Methods
The trisodium salt of diphosphite (P^2-P^4 acid according to the nomenclature of Ohashi [9]) was prepared by the oxidation of phosphorus tribromide according to Blaser [10]. ^{31}PNMR (D_2O) δ, AB system: 8.67, -$\underline{P}O_3^{2-}$; 23.19, H$\underline{P}O_2^-$ $^1J_{pp}$, 469 Hz). ^{17}O- and ^{18}O - labelled water (23.2 atom % ^{16}O, 45.6 atom% ^{17}O, 31.2 atom % ^{18}O; 98 atom % respectively) were obtained from Amersham, U.K. ^{31}P NMR spectra were obtained on 24.15 MHz Jeol FX60, 121.5 MHz Bruker AM300 or 162 MHz Bruker WH 400 spectrometers with broad band proton decoupling. ^{31}P Chemical shifts are relative to external H_3PO_4, a positive shift denotes a signal downfield from this reference.

Preparation of Hypophosphate (2) by Oxidation of Diphosphite (1)

To trisodium diphosphite dodecahydrate (250 mg, 0.58 mmol) dissolved with warming in water (5ml) was added dropwise a saturated solution of iodine in pyridine. When no more decolourisation occurred the reaction mixture was examined by low field ^{31}P NMR spectroscopy which showed hypohosphate (δ s, 13.1 ppm) to be the sole product. The solution was basified with 2M NaOH to pH 10.5 and left to crystallize. The first crop gave 170mg crystalline hypophosphate decahydrate (67.7%).

Preparation of [^{18}O]-Hypophosphate (4)

Trisodium diphosphite (20mg, 0.094 mmol) was dissolved in $H_2^{18}O$ (100 μl) with warming and was oxidised as described for the unlabelled compound. [^{31}P NMR (D_2O) δ s, 12,26, -$\underline{P}O_3^{2-}$; s, 12.25, $^{2-}O_2^{18}O\underline{PP}O_3^{2-}$ AB line]

Preparation of [^{17}O]-Hypophosphate (3)

Trisodium diphosphite (25mg, 0.118 mmol) was dissolved with warming in $H_2^{17}O$ (200μl). A saturated solution of iodine in dry pyridine was added dropwise with shaking and warming until no

more decolourisation of iodine was observed (ca. 3 drops). Low field ^{31}P NMR showed complete oxidation to hypophosphate had occurred (δ, s , 13.1 p.p.m.) ^{17}O NMR (D_2O) δ, s, 129.3 ppm.

Conversion of (3) into [^{17}O]-Pyrophosphate (5,6)

To a sample of (3) (*ca.* 25 mg) dissolved in water (200 μl) was added sodium bicarbonate (50mg, 0.6 mmol). Bromine was added dropwise with mild warming until a permanent faint yellow colouration was obtained. The reaction mixture was made pH 10.5 with sodium hydroxide and ^{31}P NMR spectroscopy demonstrated quantitative conversion into pyrophosphate [d, s , -4.900, [18]O - labelled pyrophosphate, unresolved; δ, -4.897, $^2J_{pp}$,18.94 Hz, 2- $O_2^{17}OP\underline{P}O_3$ $^{2-}$

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