On the Bromine Oxidation of Hypophosphate: a $^{31}P(^{18}O)$ Positional Isotope Investigation

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Hypophosphate is oxidised quantitatively by bromine in ^{18}O-labelled water to [^{18}O]pyrophosphate; the ^{18}O label is located exclusively in the non-bridging position.

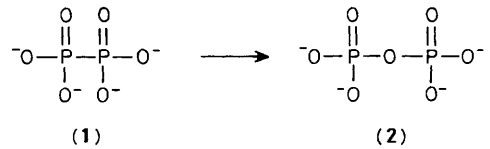
Hypophosphate (**1**) was first described by Salzer,[1] who made a qualitative observation that the tetrasodium salt is easily oxidised by aqueous bromine to give pyrophosphate (**2**), Scheme 1. Subsequent investigators, even before the structure of (**1**) had been established, found that in the pH range 7—9 the now firmly established P–P bond of (**1**) is oxidised rapidly and quantitatively by bromine to the P–O–P system of (**2**).[2] The pH dependence of this reaction has been rationalised by Palmer,[3] who showed hypobromous acid to be the active oxidant.

Several suggestions for the mechanism of this unusual insertion reaction have been put forward. Van Wazer proposed that the formation of the P–O–P system takes place *via* the d orbitals on the phosphorus atoms before the P–P bond is broken.[4] Palmer has put forward a mechanism involving the hydration of two molecules of monomeric metaphosphate,[3] and Schülke has oxidised hypophosphate in the presence of various nucleophiles to give substituted pyrophosphates, with the proposal that pyrophosphate is formed by an intramolecular reaction.[5] Until now, however, the fundamental question of whether the oxygen atom of hypobromous acid is incorporated into the bridging or non-bridging positions of pyrophosphate with appropriate mechanistic implications has not been able to be addressed owing to the lack of suitable analytical methods.

The recent discovery that ^{18}O bonded to phosphorus gives rise to an isotope shift in the ^{31}P n.m.r. spectrum[6] which is dependent on bond order[7] has led to its extensive exploitation in investigations of the enzymology of phosphoryl transfer reactions.[8] During the course of this, methods for the synthesis of oxygen-labelled pyrophosphates in the bridging and non-bridging positions have been developed.[9—11]

A $^{31}P(^{18}O)$ positional isotope investigation offers a simple method for the solution of the hypophosphate oxidation problem, since the problem is reduced to determining the location of a newly introduced ^{18}O isotope from the oxidant, *i.e.* whether it is in a bridging or non-bridging position. This can easily be accomplished by ^{31}P n.m.r. spectroscopy.

Trisodium diphosphite was synthesised according to Blaser,[12] and oxidised to hypophosphate using iodine in aqueous pyridine. The crystalline tetrasodium salt (23 μmol) and sodium hydrogen carbonate (290 μmol) were dissolved with warming in ^{18}O-labelled water (50 μl, 98 atom %) and bromine was added dropwise until a permanent faint yellow colouration was obtained. ^{31}P N.m.r. spectroscopy demonstrated quantitative conversion into [^{18}O]pyrophosphate [Figure 1(a)]. Addition of an equivalent amount of authentic tetrasodium pyrophosphate resulted in the ^{31}P n.m.r. spectrum shown in Figure 1(b). From this spectrum it can be clearly seen that only one ^{18}O isotope has been introduced, and the isotope shift of 1.73 Hz (0.011 p.p.m.) observed correlates with that measured by Kenyon *et al.* for pyrophosphate containing ^{18}O in the non-bridging position,



(**1**) (**2**)

Scheme 1. *Reagent:* Br_2–aq. $NaHCO_3$.

(5),[9] which is the exclusive product. Any ^{18}O bridge-labelled material (6) would have shown an isotope shift of 3.10 Hz (0.019 p.p.m.).

In the absence of any bridge-labelled material we may rule out the mechanism proposed by Van Wazer,[4] since it is clear that the new bridging oxygen is not derived from solvent water *via* bromate. The hydration of two reactive molecules of monomeric metaphosphate, as also proposed,[3] might be expected to produce either exclusively bridge-labelled pyrophosphate in the case of a simultaneous linking together of two molecules, or a mixture of bridging and non-bridging material as a result of rotational scrambling of the intermediate phosphate before P–O–P formation. The present data exclude these processes. A similar mechanism consistent with these data would involve the attack of a water molecule on the outside face of one of the pair of metaphosphates, followed by capture of the remaining metaphosphate by an original oxygen atom without any concomitant tumbling of the phosphate intermediate and consequent incorporation of a proportion of ^{18}O into the bridge. However, in the light of the high reactivity of monomeric metaphosphate,[13] and in view of the lack of incorporation of ^{32}P label into pyrophosphate when the oxidation is performed in the presence of [^{32}P]phosphate[5] (the tendency of monomeric metaphosphate to transfer to phosphate even in the presence of a large excess of water has been demonstrated[14]), and the small amount of inorganic phosphate (<3%) produced by the oxidation, any mechanism involving free metaphosphate does not appear to be an attractive possibility.

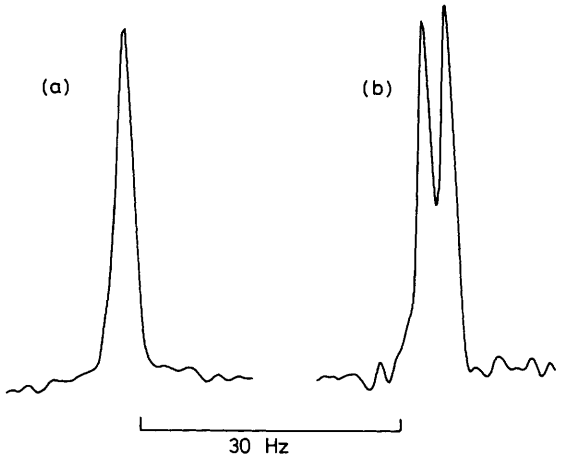
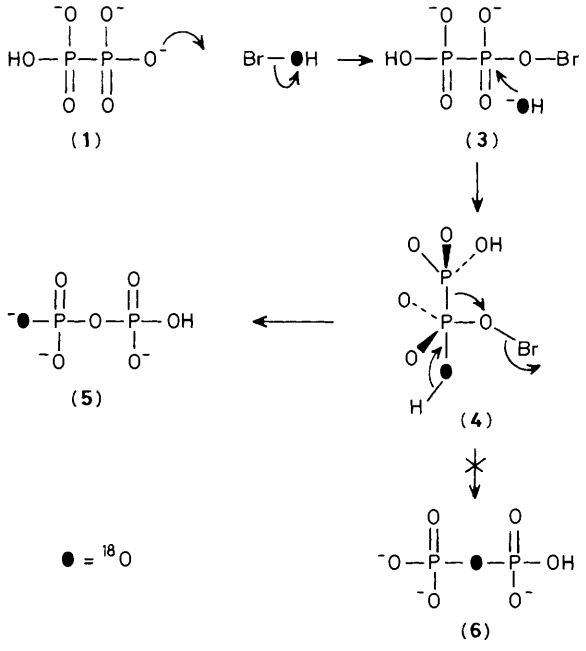


Figure 1. ^{31}P N.m.r. spectra of (a) [^{18}O]pyrophosphate (*ca.* 50 mM), δ −5.4 p.p.m. (positive shifts to high frequency of H_3PO_4) obtained *via* the oxidation of tetrasodium hypophosphate in $H_2{}^{18}O$; (b) as for (a) but after the addition of an equivalent amount of tetrasodium [^{16}O]pyrophosphate. Samples were recorded in hydrogen carbonate buffer, pH 10.5. ^{31}P N.m.r. parameters were for: (a) Bruker AM 300 operating at 121.5 MHz; sweep width, 500 Hz; pulse width, 5 μs; 4K data points; line broadening, −0.5 Hz, gaussian broadening, 0.2 Hz; no. of transients, 181; (b) Bruker WH 400 operating at 162 MHz; sweep width, 439 Hz; pulse width, 12 μs; 8K points; line broadening, −1.5 Hz; no. of transients, 344.



Scheme 2. *Reagent:* Br_2–$NaHCO_3$–$H_2{}^{18}O$.

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References

1 T. Salzer, *Liebigs Ann. Chem.*, 1877, **187**, 322.
2 B. Blaser and P. Halpern, *Z. Anorg. Allg. Chem.*, 1933, **215**, 33.
3 W. G. Palmer, *J. Chem. Soc.*, 1961, 1079.
4 J. R. Van Wazer, 'Phosphorus and its Compounds,' vol. 1, Interscience, New York, 1958, p. 410.
5 U. Schülke, *Z. Anorg. Allg. Chem.*, 1968, **361**, 225.
6 G. Lowe and B. S. Sproat, *J. Chem. Soc., Chem. Commun.*, 1978, 565; M. Cohn and A. Hu, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 200.
7 G. Lowe, B. V. L. Potter, B. S. Sproat, and W. E. Hull, *J. Chem. Soc., Chem. Commun.*, 1979, 733; M. Cohn and A. Hu, *J. Am. Chem. Soc.*, 1980, **102**, 913.
8 M. Cohn, *Annu. Rev. Biophys. Bioeng.*, 1982, **11**, 23.
9 J. A. Gerlt, M. A. Reynolds, P. C. Demou, and G. L. Kenyon, *J. Am. Chem. Soc.*, 1983, **105**, 6469; M. A. Reynolds, N. J. Oppenheimer, and G. L. Kenyon, *ibid.*, p. 6663.
10 P. M. Cullis, *J. Am. Chem. Soc.*, 1983, **105**, 7783.
11 G. Lowe and B. V. L. Potter, *J. Chem. Soc., Chem. Commun.*, 1984, 877.
12 B. Blaser, *Chem. Ber.*, 1953, **86**, 563.
13 F. H. Westheimer, *Chem. Rev.*, 1981, **81**, 313.
14 A. C. Satterthwait and F. H. Westheimer, *J. Am. Chem. Soc.*, 1978, **100**, 3197.