

## The Effect of $^{17}\text{O}$ and the Magnitude of the $^{18}\text{O}$ -Isotope Shift in $^{31}\text{P}$ Nuclear Magnetic Resonance Spectroscopy

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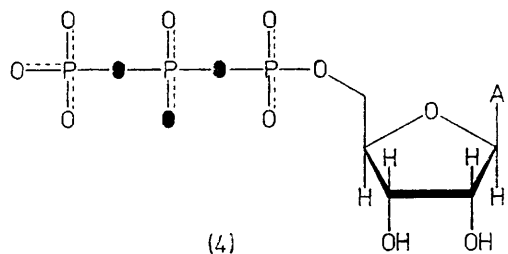
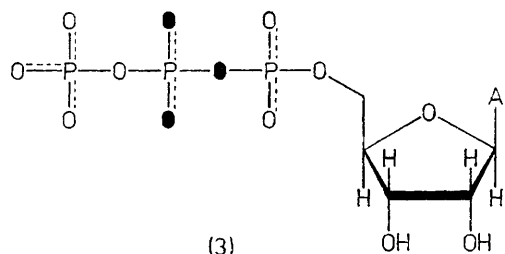
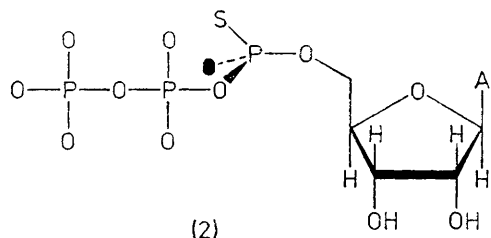
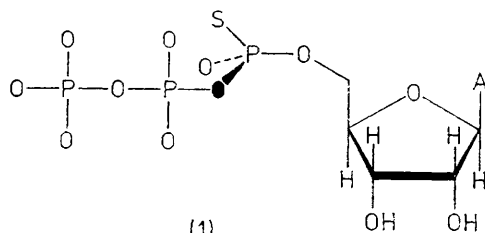
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**Summary** The effect of  $^{17}\text{O}$  directly bonded to  $^{31}\text{P}$  causes marked line broadening of the  $^{31}\text{P}$  resonance, but the coupling constant can be detected in favourable systems; the magnitude of the isotope shift caused by  $^{18}\text{O}$  directly bonded to  $^{31}\text{P}$  depends on the nature of the phosphorus to oxygen bond.

ALTHOUGH the coupling constant  $^1J(^{17}\text{O}^{31}\text{P})$ , has been measured for several phosphorus derivatives by  $^{17}\text{O}$  n.m.r. spectroscopy,<sup>1</sup> there appears to be no report of it being determined by  $^{31}\text{P}$  n.m.r. spectroscopy, presumably due to the quadrupolar line broadening caused by  $^{17}\text{O}$  on the  $^{31}\text{P}$  resonance. In order to substantiate this assumption, phosphorus oxychloride containing 2 atom %  $^{16}\text{O}$ , 44 atom %  $^{17}\text{O}$ , and 54 atom %  $^{18}\text{O}$ ,<sup>2</sup> was converted into isotopically labelled trimethyl phosphate with methanol. The  $^{31}\text{P}$  n.m.r. spectrum at 36.43 MHz consisted of a sharp line

( $\Delta\nu_{\frac{1}{2}}$  0.4 Hz) due to trimethyl [ $^{18}\text{O}$ ]phosphate (and presumably the unresolved trimethyl [ $^{16}\text{O}$ ]phosphate) together with six very broad ( $\Delta\nu_{\frac{1}{2}}$  90 Hz) but equally spaced lines due to trimethyl [ $^{17}\text{O}$ ]phosphate, three to the low field and three to the high field side of the single sharp resonance. This is in accord with expectation since  $^{17}\text{O}$  has a nuclear spin quantum number of 5/2; the observed coupling constant  $^1J(^{17}\text{O}^{31}\text{P})$  156 Hz is in reasonable agreement with the value of 165 Hz determined by  $^{17}\text{O}$  n.m.r. spectroscopy.<sup>1</sup> Although it is possible therefore to determine  $^1J(^{17}\text{O}^{31}\text{P})$  by  $^{31}\text{P}$  n.m.r. spectroscopy in favourable cases, we expect  $^{17}\text{O}$  in  $^{31}\text{P}$  n.m.r. spectroscopy will find its most valuable application in quadrupolar line broadening when directly bonded to phosphorus, since the residual  $^{31}\text{P}(^{16}\text{O})$  or  $^{31}\text{P}(^{18}\text{O})$  signals will be at the minimum between two lines of the  $^{31}\text{P}(^{17}\text{O})$  spectrum, allowing integration of the  $^{31}\text{P}(^{16}\text{O})$  and  $^{31}\text{P}(^{18}\text{O})$  signals and hence the  $^{17}\text{O}$  content to be estimated from the loss of signal otherwise expected.<sup>†</sup>

<sup>†</sup> Added in proof: Just such an application has been reported recently: M.-D. Tsai, *Biochemistry*, 1979, **18**, 1468.



● =  $^{18}\text{O}$ ; A = Adenine

The recent demonstration that an isotope shift in  $^{31}\text{P}$  n.m.r. spectroscopy is observed when  $^{18}\text{O}$  is directly bonded to phosphorus,<sup>3</sup> has already found extensive application.<sup>3-5</sup> In using this technique to determine the absolute configuration at  $\text{P}_\alpha$  of the diastereoisomers of adenosine 5'-(1-thiotriphosphate), the isotope shift on both  $\text{P}_\alpha$  and  $\text{P}_\beta$  caused by  $^{18}\text{O}$  in the  $\text{P}_\alpha\text{-O-P}_\beta$  bridge in (1) was 0.6 Hz, whereas the isotope shift on  $\text{P}_\alpha$  caused by the non-bridging  $^{18}\text{O}$  in (2) was 1.1 Hz at 36.43 MHz.<sup>6</sup> Since the effect of sulphur on the electronic distribution in thiophosphates is uncertain, the magnitude of the isotope shift has been investigated in simple phosphate esters.

Trimethyl [ $^{18}\text{O}$ ]phosphate was made from phosphorus [ $^{18}\text{O}$ ]oxychloride and methanol; partial hydrolysis of the triester gave dimethyl [ $^{18}\text{O}$ ]phosphate and methyl [ $^{18}\text{O}$ ]-

phosphate. The isotope enrichment in each ester was *ca.* 50 atom %, so that two peaks of approximately equal intensity were observed in the  $^{31}\text{P}$  n.m.r. spectrum of each ester. In order to observe the isotope shift in a phosphorus-oxygen single bond, adenosine 5' [ $\alpha\beta\text{-}^{18}\text{O}$ ,  $\beta\text{-}^{18}\text{O}_2$ ]triphosphate (3, fully enriched at the sites indicated) was incubated with pyruvate kinase for a period known to cause partial randomisation of label.<sup>5</sup> The recovered ATP contained therefore a mixture of (3) and (4) enabling the isotope shift on  $\text{P}_\gamma$  of  $^{18}\text{O}$  in the  $\text{P}_\beta\text{-O-P}_\gamma$  bridge to be measured.

TABLE.  $^{18}\text{O}$  Isotope shifts (Hz) in phosphate esters.

	At 36.43 MHz	At 162 MHz
$(\text{MeO})_3\text{P}^{18}\text{O}$	1.27	5.83
$(\text{MeO})_2\text{PO}^{18}\text{O}-$	1.07	4.74
$(\text{MeO})\text{PO}_2^{18}\text{O}^{2-}$	0.88	3.72
$\text{P}_\gamma$ of (4)	0.74	3.38

The isotope shifts were measured at both 36.43 and 162 MHz; the data are shown in the Table. A plot of the isotope shift against the square of the frequency of the  $A_1$  stretching mode of the phosphates,<sup>7</sup> shows a good linear relationship with a correlation coefficient of 0.985, indicating that the magnitude of the isotope shift is related to the force constant of the phosphorus-oxygen bond.

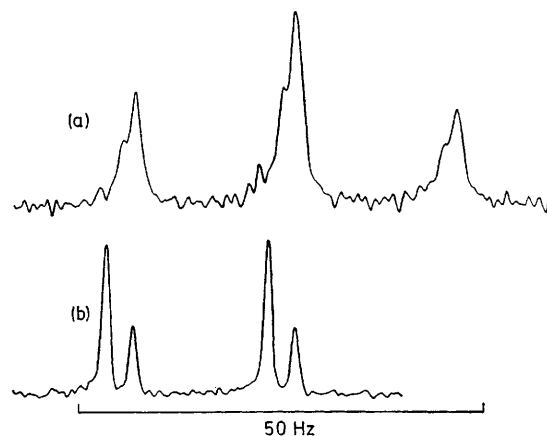


FIGURE. The  $^{31}\text{P}$  n.m.r. spectrum (at 162 MHz) of (a)  $\text{P}_\beta$  and (b)  $\text{P}_\gamma$  of a mixture of (3) and (4) derived by partial isotopic scrambling of (3) by pyruvate kinase.

The  $^{31}\text{P}$  n.m.r. spectrum of the  $\text{P}_\beta$  and  $\text{P}_\gamma$  resonances of the mixture of (3) and (4) is shown in the Figure. It is clear from this that not only are the  $\text{P}_\gamma$  resonances clearly resolved at 162 MHz, but the  $\text{P}_\beta$  resonances are also partially resolved, the effect being due to (3) possessing one bridging and two non-bridging  $^{18}\text{O}$  at  $\text{P}_\beta$ , whereas (4) possesses two bridging and one non-bridging  $^{18}\text{O}$  at  $\text{P}_\beta$ . Since the isotope shift is always to higher field, the high field triplet is assigned to  $\text{P}_\beta$  of (3) and the low field triplet

is assigned to  $P_\beta$  of (4). It seems clear that the magnitude of the isotope shift is a parameter which will enhance the range of applications of this technique.

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<sup>2</sup> P. M. Cullis and G. Lowe, *J.C.S. Chem. Comm.*, 1978, 512.

<sup>3</sup> M. Cohn and A. Hu, *Proc. Nat. Acad. Sci. U.S.A.* 1978, **75**, 200; G. Lowe and B. S. Sproat, *J.C.S. Chem. Comm.*, 1978, 565.

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<sup>5</sup> G. Lowe and B. S. Sproat, *J.C.S. Chem. Comm.*, 1978, 783; *J.C.S. Perkin I*, 1978, 1622.

<sup>6</sup> R. L. Jarvest and G. Lowe, *J.C.S. Chem. Comm.*, 1979, 364; a similar observation has been made by Dr. M. Cohn, personal communication.

<sup>7</sup> J. Kumamoto, *Spectrochim. Acta*, 1965, **21**, 345.