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

By Gordon Lowe,* Barry V. L. Potter, and Brian S. Sproat

(*The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY*)
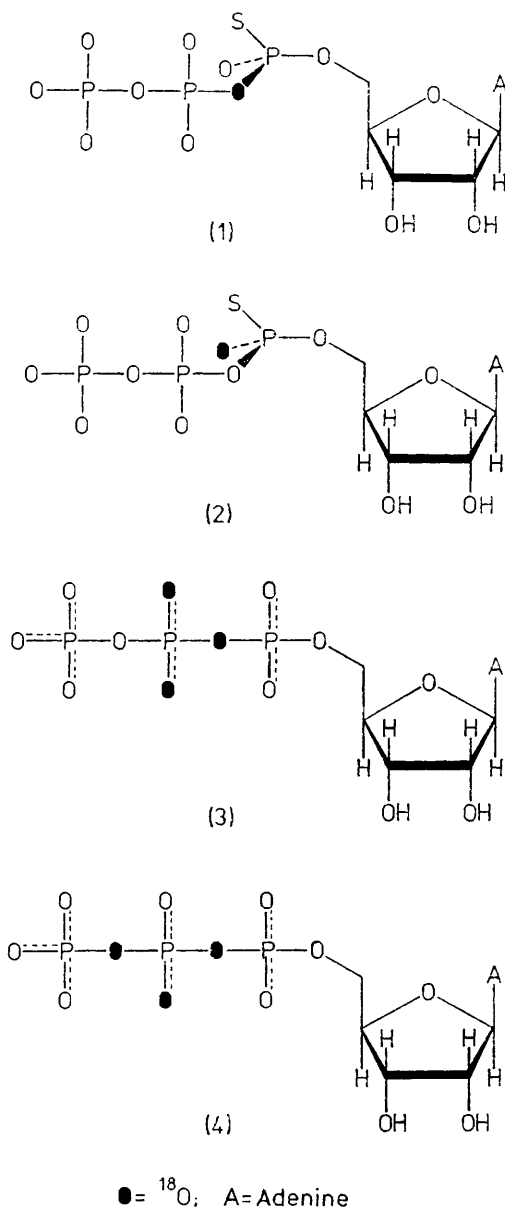
and William E. Hull

(*Bruker-Physik AG, D-7512 Rheinstetten-Fo, Am Silberstreifen, Karlsruhe, West Germany*)

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

Although the coupling constant $^{1}J(^{17}O^{31}P)$, has been measured for several phosphorus derivatives by ^{17}O n.m.r. spectroscopy,[1] there appears to be no report of it being determined by ^{31}P n.m.r. spectroscopy, presumably due to the quadrupolar line broadening caused by ^{17}O on the ^{31}P resonance. In order to substantiate this assumption, phosphorus oxychloride containing 2 atom % ^{16}O, 44 atom % ^{17}O, and 54 atom % ^{18}O,[2] was converted into isotopically labelled trimethyl phosphate with methanol. The ^{31}P n.m.r. spectrum at 36·43 MHz consisted of a sharp line ($\Delta v_{\frac{1}{2}}$ 0·4 Hz) due to trimethyl [^{18}O]phosphate (and presumably the unresolved trimethyl [^{16}O]phosphate) together with six very broad ($\Delta v_{\frac{1}{2}}$ 90 Hz) but equally spaced lines due to trimethyl [^{17}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}O has a nuclear spin quantum number of 5/2; the observed coupling constant $^{1}J(^{17}O^{31}P)$ 156 Hz is in reasonable agreement with the value of 165 Hz determined by ^{17}O n.m.r. spectroscopy.[1] Although it is possible therefore to determine $^{1}J(^{17}O^{31}P)$ by ^{31}P n.m.r. spectroscopy in favourable cases, we expect ^{17}O in ^{31}P n.m.r. spectroscopy will find its most valuable application in quadrupolar line broadening when directly bonded to phosphorus, since the residual ^{31}P(^{16}O) or ^{31}P(^{18}O) signals will be at the minimum between two lines of the ^{31}P(^{17}O) spectrum, allowing integration of the ^{31}P(^{16}O) and ^{31}P(^{18}O) signals and hence the ^{17}O content to be estimated from the loss of signal otherwise expected.†

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

(1)

(2)

(3)

(4)

● = ^{18}O;   A = Adenine

phosphate. The isotope enrichment in each ester was ca. 50 atom %, so that two peaks of approximately equal intensity were observed in the ^{31}P n.m.r. spectrum of each ester. In order to observe the isotope shift in a phosphorus–oxygen single bond, adenosine 5′[$\alpha\beta$-^{18}O, β-^{18}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.[5] The recovered ATP contained therefore a mixture of (3) and (4) enabling the isotope shift on P$_\gamma$ of ^{18}O in the P$_\beta$–O–P$_\gamma$ bridge to be measured.

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

	At 36·43 MHz	At 162 MHz
(MeO)$_3$P^{18}O	1·27	5·83
(MeO)$_2$PO^{18}O$^-$	1·07	4·74
(MeO)PO$_2$^{18}O^{2-}	0·88	3·72
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,[7] 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.
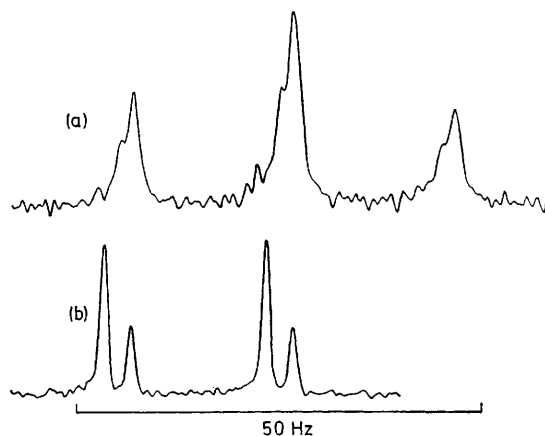

FIGURE. The ^{31}P n.m.r. spectrum (at 162 MHz) of (a) P$_\beta$ and (b) Pγ of a mixture of (3) and (4) derived by partial isotopic scrambling of (3) by pyruvate kinase.

The recent demonstration that an isotope shift in ^{31}P n.m.r. spectroscopy is observed when ^{18}O is directly bonded to phosphorus,[3] has already found extensive application.[3–5] In using this technique to determine the absolute configuration at P$_\alpha$ of the diastereoisomers of adenosine 5′-(1-thiotriphosphate), the isotope shift on both P$_\alpha$ and P$_\beta$ caused by ^{18}O in the P$_\alpha$–O–P$_\beta$ bridge in (1) was 0·6 Hz, whereas the isotope shift on P$_\alpha$ caused by the non-bridging ^{18}O in (2) was 1·1 Hz at 36·43 MHz.[6] 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}O]phosphate was made from phosphorus [^{18}O]oxychloride and methanol; partial hydrolysis of the triester gave dimethyl [^{18}O]phosphate and methyl [^{18}O]-

The ^{31}P n.m.r. spectrum of the P$_\beta$ and 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 P$_\gamma$ resonances clearly resolved at 162 MHz, but the P$_\beta$ resonances are also partially resolved, the effect being due to (3) possessing one bridging and two non-bridging ^{18}O at P$_\beta$, whereas (4) possesses two bridging and one non-bridging ^{18}O at P$_\beta$. Since the isotope shift is always to higher field, the high field triplet is assigned to P$_\beta$ of (3) and the low field triplet

is assigned to P_{β} 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.

[1] H. A. Christ, P. Diehl, H.-R. Schneider, and H. Dahn, *Helv. Chim. Acta*, 1961, **44**, 865; H. A. Christ and P. Diehl, *Helv. Phys. Acta*, 1963, **36**, 170; G. Grossmann, M. Gruner, and G. Seifert, *Z. Chem.*, 1976, **16**, 362; G. A. Gray and T. A. Albright, *J. Amer. Chem. Soc.*, 1977, **99**, 3243, H. C. E. McFarlane and W. McFarlane, *J.C.S. Chem. Comm.*, 1978, 531.

[2] P. M. Cullis and G. Lowe, *J.C.S. Chem. Comm.*, 1978, 512.

[3] 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.

[4] M. R. Webb, G. G. McDonald, and D. R. Trentham, *J. Biol. Chem.*, 1978, **253**, 2908; J. L. Bock and M. Cohn, *ibid.*, p. 4082; R. L. van Etten and J. M. Risley, *ibid.*, p. 4784; F. Jordan, J. A. Patrick, and S. Salamone, Jr., *ibid.*, 1979, **254**, 2384.

[5] G. Lowe and B. S. Sproat, *J.C.S. Chem. Comm.*, 1978, 783; *J.C.S. Perkin I*, 1978, 1622.

[6] 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.

[7] J. Kumamoto, *Spectrochim. Acta*, 1965, **21**, 345.