Proton, Carbon, and Phosphorus Nuclear Magnetic Resonance and Conformational Studies on Phosphate Esters

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The conformational free energies of phosphate and phosphoryloxymethyl groups on a cyclohexane ring were 0.94 and 0.88 kcal mol^{-1} in deuterium oxide at pD 5 and 0.20 and 1.09 kcal mol^{-1} in [$^2H_6$]dimethyl sulphoxide, respectively. For the rotational isomerism of the C–O axis of phosphate esters, $^3J_{PH}$ coupling constants indicate that the conformers with a planar *trans* arrangement of P–O and C(1)–CH_2 bonds on primary phosphates or of P–O and C(1)–C(2 or 6) bonds on secondary phosphates predominated. The carbon magnetic shieldings in the vicinity of the phosphate ester function are interpreted in terms of an electronic exchange process.

As simple models of sugar phosphates and nucleotides, cyclohexyl, cyclopentyl, cyclohexylmethyl, and cyclopentylmethyl phosphates were prepared and conformational analysis of these compounds was carried out using proton, carbon, and phosphorus n.m.r. spectroscopy. To the best of our knowledge, there are no examples of conformational analysis of simple phosphate esters in the literature though there exist a large number of publications on that of nucleotides[1] and sugar phosphates.[2]

The proton spectra of the compounds synthesised are very complex even at 300 MHz because of small differences in chemical shift and spin–spin coupling constants. However, the proton signals (H_a, H_b, and H_c) of all compounds in Table 2 did not overlap each other. Therefore, the first-order analysis of the spectra was performed only on the protons near to a phosphate group by the homo spin–spin decoupling method.[3] The carbon spectra were obtained by the usual complete proton decoupling method and the signals were assigned by comparison with those of the parent alcohols and by the carbon–phosphorus coupling. Unfortunately both 4-*trans*- (**9**) and -*cis*-t-butylcyclohexylmethyl phosphate (**8**) were not sufficiently soluble to obtain a ^{13}C n.m.r. spectrum in acidic deuterium oxide.

The pH profiles of the proton spectra shown in the Figure indicate that the first and second pK_a values of the phosphates under examination were very close to those of inorganic phosphates (2.21 and 7.21).[4]

The relationship between dihedral angle and proton spin–spin coupling constant $^3J_{HH}$ has been used successfully in conformational analysis. As the original Karplus equation gave conflicting results in the present case, the more general equation proposed by Haasnoot and his co-workers, which includes corrections for the electronegativity of substituents,[5] was used. For the use of this equation, the difference electronegativity of phosphate ester was determined from the chemical-shift difference between methyl and methylene protons of ethyl phosphate (**1**) (Table 1).[6] Since a cyclopentyl ring is flexible, the spin–spin coupling constants $^3J_{HC-CH}$ were used to determine the conformations of the cyclopentyl phosphates as shown in Table 2. By the Haasnoot equation a ^3J value of 7.4 Hz for the cyclopentylmethyl phosphate (**3**) gave −46 (counterclockwise), 17 (clockwise), 111 (clockwise), and 162° (clockwise) dihedral angles as shown in Table 2. Since the angle H_a–C–H_b must approximate to 120°, it follows that the values for the dihedral angles must be 17 and 111°—contrary to expectation. As the Haasnoot equation does not take ring strain into account these values cannot be rationalised simply by the angle deviation caused by the strain imposed by a five-membered ring.

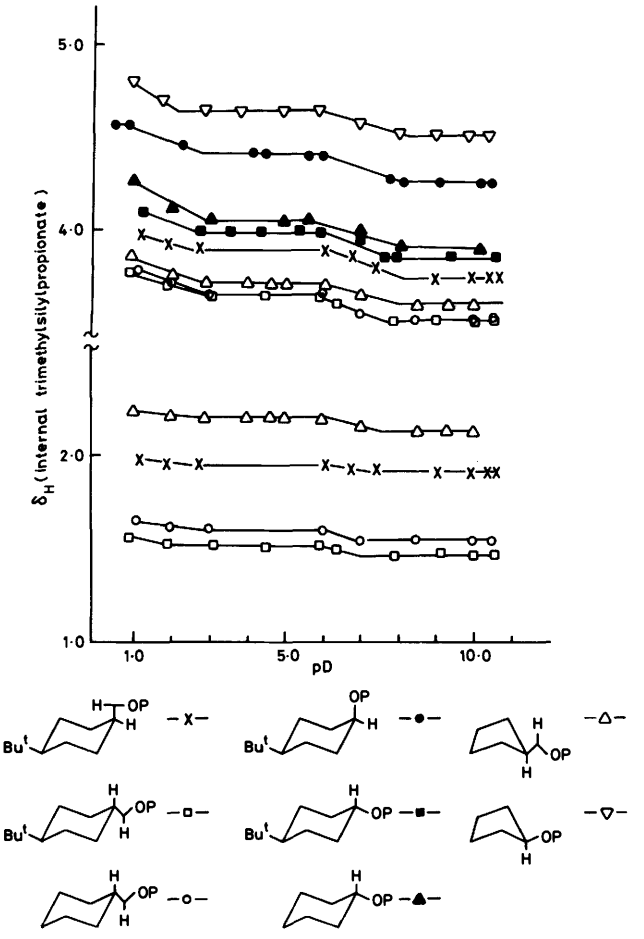


Figure. pH *vs.* Proton chemical-shift profile of phosphate esters. Signals between 1.5 and 2.5 p.p.m. are assigned to ring protons of primary phosphates

A similar procedure was used for the determination of the corresponding dihedral angles of cyclopentyl phosphate (**2**), which apparently show that the phosphate and phosphoryloxymethyl groups of cyclopentyl derivatives are mainly located at the *quasi*-equatorial position on the tip of the envelope as is usual in a mono-substituted cyclopentane.[7a]

Since the n.m.r. solvents suitable for phosphate esters were limited to deuterium oxide and [$^2H_6$]dimethyl sulphoxide and

since low-temperature measurements could not be taken from these solutions, the conformational free energies of phosphate and phosphoryloxymethyl groups on a cyclohexane ring were estimated by the comparison of the proton chemical shifts of the dynamic systems with those of the t-butyl-substituted rigid analogues in acidic (pD less than 1.5), monobasic (pD 5), and dibasic (pD 10) forms in deuterium oxide, and in the acidic form in [$^2H_6$]dimethyl sulphoxide (Table 3). In deuterium oxide, the conformation of the anionic forms is largely fixed in a chair form

Table 1. Proton chemical shifts of ethyl phosphate (**1**) in deuterium oxide and in [$^2H_6$]dimethyl sulphoxide

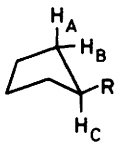
Solvent	δ_{CH_2}	J_{PH}/Hz	δ_{CH_3}	J_{HH}/Hz
pD < 1.5	4.05	7.1	1.29	7.1
pD 5	3.89	7.1	1.24	7.1
pD 10	3.80	7.1	1.20	7.1
Me_2SO	3.89	7.1	1.21	7.1

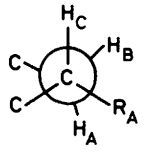
CH_3CH_2–OP
(1)

Table 2. $^3J_{HH}$ Constants and dihedral angles of cyclopentyl derivatives

	Solvent	J/Hz	Dihedral angle/°
(3)	Me_2SO	J_{AC} 7.4	162 or 111
		J_{BC} 7.4	17
	D_2O	J_{AC} 7.4	162 or 111
		J_{BC} 7.4	17
(2)[a]	Me_2SO	J_{AC} 4.2	158
		J_{BC} 4.2	28
	D_2O	J_{AC} 6.0	162
		J_{BC} 3.0	31

[a] In the steepest region (J 5—6 Hz) a 1° difference in dihedral angle makes a difference of 0.4 Hz in the ^3J constant.





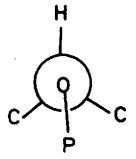
(**2**) R = OP

(**3**) R = CH_2OP

P = Phosphate

Table 4. $^3J_{PH}$ Constants and rotamer populations of secondary phosphate esters

Phosphate	Solvent	$^3J_{PH}$/Hz	I (%)	II (%)
(2)	Me_2SO	6.6	21.8	39.1
	D_2O	6.4	20.9	39.6
(4)	Me_2SO	8.4	30.5	34.8
	D_2O	8.1	29.0	35.5
(6)	Me_2SO	6.1	19.4	40.3
	D_2O	6.1	19.4	40.3
(7)	Me_2SO	10.4	40.0	30.0
	D_2O	8.1	29.0	35.5



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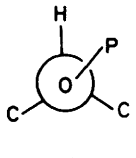

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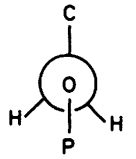
Table 5. $^3J_{PH}$ constants and rotamer populations of primary phosphate esters

Phosphate	Solvent	$^3J_{PH}$/Hz	I (%)	II (%)
(3)	Me_2SO	6.4	58.6	20.7
	pD < 1.5	6.8	54.8	22.6
	pD 5	6.6	56.8	21.6
	pD 10	5.3	69.2	15.4
(5)	Me_2SO	6.6	56.8	21.6
	pD < 1.5	6.6	56.8	21.6
	pD 5	6.4	58.6	20.7
	pD 10	5.6	66.4	16.8
(8)	Me_2SO	6.6	56.8	21.6
	pD < 1.5	6.4	58.6	20.7
	pD 5	6.4	58.6	20.7
	pD 10	6.0	62.4	18.8
(9)	Me_2SO	6.8	54.8	22.6
	pD < 1.5	6.6	56.4	21.6
	pD 5	6.4	58.6	20.7
	pD 10	5.5	67.2	16.4



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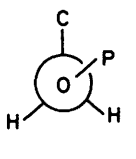

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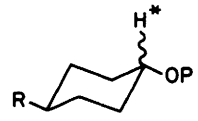
Table 3. Conformational energies of cyclohexyl and cyclohexylmethyl phosphates in deuterium oxide and [$^2H_6$]dimethyl sulphoxide

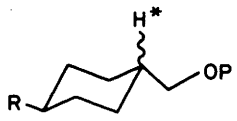
	δ_{H^*}					δ_{H^*}				
Solvent	(4)	(6)	(7)	$H^*_{eq.}:H^*_{ax.}$	$-\Delta G$/kcal mol^{-1}	(5)	(8)	(9)	$H^*_{eq.}:H^*_{ax.}$	$-\Delta G$/kcal mol^{-1}
pD < 1.5	4.25	4.57	4.09	33.3:66.7	0.41	1.63	1.97	1.54	20.9:79.1	0.79
pD 5	4.05	4.39	3.98	17.1:82.9	0.94	1.59	1.92	1.52	18.4:81.6	0.88
pD 10	3.89	4.26	3.86	7.5:92.5	1.49	1.54	1.90	1.47	16.6:83.4	0.97
Me_2SO	4.09	4.37	3.89	41.7:58.3	0.20	1.54	1.92	1.48	13.6:86.4	1.09



(**4**) R = H

(**6**) R = *cis* - Bu^t

(**7**) R = *trans* - Bu^t



(**5**) R = H

(**8**) R = *cis* - Bu^t

(**9**) R = *trans* - Bu^t

Table 6. ^{13}C Chemical shifts of phosphate esters in deuterium oxide and [$^2H_6$]dimethyl sulphoxide. J_{PC} (Hz) in parentheses

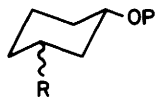
Compd.	Solvent	C-1	C-2	C-3	C-4	C-α	CMe_3	CMe_3
(1)	Me_2SO	61.2 (5.37)	16.3 (6.84)					
	pD < 1.5	64.3 (5.37)	16.3 (6.35)					
	pD 5	62.3 (5.37)	16.6 (6.83)					
	pD 10	61.0 (4.40)	17.0 (6.84)					
(2)	Me_2SO	78.2 (6.29)	33.3 (4.88)	22.6				
	pD < 1.5	81.8 (5.13)	34.3 (4.61)	23.5				
	pD 5	79.2 (5.13)	34.4 (4.40)	23.6				
	pD 10	77.5 (5.13)	34.5 (4.40)	23.7				
(3)	Me_2SO	39.6 (6.84)	24.9	28.6		69.0 (5.86)		
	pD < 1.5	40.5 (7.33)	26.0	29.5		71.5 (5.86)		
	pD 5	40.6 (6.60)	25.8	29.7		70.3 (5.13)		
	pD 10	40.8 (6.59)	25.8	29.8		69.5 (5.13)		
(4)	Me_2SO	73.8 (5.86)	33.0 (3.90)	23.1	24.8			
	pD < 1.5	77.7 (5.86)	33.8 (4.40)	24.2	25.7			
	pD 5	75.0 (5.86)	34.6 (3.66)	24.8	25.9			
	pD 10	74.3 (5.13)	34.9 (3.66)	25.1	25.9			
(5)	Me_2SO	38.0 (7.81)	28.9	25.1	26.0	70.2 (5.86)		
	pD < 1.5	39.9 (7.33)	29.7	26.3	27.1	72.6 (5.80)		
	pD 5	39.0 (6.60)	30.0	26.2	27.0	71.5 (5.13)		
	pD 10	39.2 (6.60)	30.2	26.3	27.1	70.8 (5.93)		
(6)	Me_2SO	70.4 (4.88)	31.7 (4.60)	21.1	47.3		32.5	27.6
	pD < 1.5	73.4 (5.55)	32.5 (3.78)	21.6	47.9		32.5	27.6
	pD 5	72.0 (5.13)	32.7 (3.66)	21.7	48.1		32.6	27.8
	pD 10	70.3 (4.40)	32.8 *a*	21.9	48.2		32.9	27.7
(7)	Me_2SO	74.3 (4.88)	33.7 (4.88)	25.1	46.5		31.8	27.3
	pD < 1.5	78.0 (6.59)	34.6 (3.66)	26.2	47.4		34.5	28.1
	pD 5	75.5 (5.08)	35.2 (4.39)	26.3	47.8		32.3	27.9
	pD 10	74.9 (5.13)	35.2 (3.66)	26.3	47.8		34.8	28.0
(8)	Me_2SO	32.5 (6.84)	26.8	21.5	47.6	65.6 (4.88)	32.1	27.2
	pD 5	34.1 (6.59)	28.0	22.7	49.0	66.9 (4.39)	32.8	28.0
	pD 10	34.1 (6.60)	28.2	22.6	49.0	66.3 (5.13)	32.8	28.0
(9)	Me_2SO	38.1 (6.83)	29.3	26.4	47.6	70.2 (5.86)	31.9	27.3
	pD 5	39.4 (7.33)	30.6	27.4	48.8	71.4 (5.86)	32.3	28.2
	pD 10	39.5 (6.60)	30.7	27.4	48.8	70.9 (5.13)	32.7	28.2

[a] Signals overlapped with those of dimethyl sulphoxide.

Table 7. ^{13}C Chemical shifts of phosphate esters in [$^2H_6$]dimethyl sulphoxide. J_{CP} (Hz) in parentheses

Compd.	C-1	C-2	C-3	C-4	C-5	C-6	CMe_3	Me
(10)	74.2 (5.37)	33.2 (3.91)	32.7	31.0				21.6
(11)	70.8 (4.88)	30.8 (5.86)	29.0	30.9				22.2
(12)	74.9 (5.37)	42.8 (3.42)	31.3	33.9	24.0	33.8 (5.86)		22.7
(13)	71.9 *b*	*a*	26.9	34.3	20.6	31.7 *b*		22.5
(14)	80.3 (6.35)	*a*	33.4	25.2	24.7	33.4 *b*		19.1
(15)	75.8 (5.37)	35.2 (5.03)	29.2	24.0	21.0	30.6 *b*		17.1
(16)	75.3 (5.86)	33.6 (4.88)	46.1	25.8	23.8	35.1 (4.88)	32.0	27.3
(17)	71.3 (5.37)	31.0 *b*	46.0	26.4	20.5	32.5 (5.03)	32.0	27.4

(10) R = *trans*-Me
(11) R = *cis*-Me



(12) R = *cis*-Me
(13) R = *trans*-Me
(16) R = *cis*-Bu^t
(17) R = *trans*-Bu^t

(14) R = *trans*-Me
(15) R = *cis*-Me

[a] Signals overlapped with those of dimethyl sulphoxide. [b] Coupling constants could not be obtained because of line broadening.

by the phosphate group assuming an equatorial position as can be seen in cyclohexanecarboxylic acid.[7b] The energy differences observed in the ionic states of cyclohexylmethyl phosphate (**5**) were less than those of cyclohexyl phosphate (**4**) because of the indirect influence of the phosphate group through the methylene group. In dimethyl sulphoxide, the behaviour of these groups was different from that in water. The conformational energy of phosphoryloxymethyl was large whereas that of the phosphate was small.

The rotational isomerism about the C–O axis of the phosphate esters was also examined. When the $^3J_{CP}$ constant was used to estimate the rotamer distribution, the population of conformer (I) (P_I) (see Table 4) of cyclopentyl phosphate (**2**), for instance, was found to be in the range 4—58% because of wide variations of the Karplus relationship between $^3J_{CP}$ and the dihedral angle previously published.[8] In contrast to the above estimation, the values obtained for P_I for the same ester using the $^3J_{PH}$ values previously published now fall in the range 14—24%. The Karplus constants estimated by Lee and co-workers were used in this paper.[9] In the case of (**2**) and cyclohexyl phosphate (**4**), the $^3J_{PH}$ constants obtained for both were found to be approximately equal and independent of change in pH to the solution as shown in Tables 4 and 5. On the other hand, the $^3J_{PH}$ constants for the phosphoryloxymethyl series did vary with changing pH. Since the relationship used here is not affected to any great extent by the solution conditions (see Table 1),[10] the observed $^3J_{PH}$ values primarily show that the effect exerted by the bulkiness of the phosphate ester part of the cyclohexylmethyl derivatives was decreased with increased acidity and was smallest in dimethyl sulphoxide; this parallels the change in conformational energy of (**4**) as obtained from the proton chemical shifts. Therefore, the structure of solvation around a phosphate ester in dimethyl sulphoxide was independent of whether the ester group is attached to a primary or secondary carbon atom.

The ^{13}C chemical shifts of the phosphates under various conditions are listed in Tables 6 and 7. The typical chemical-shift differences between the esters and the corresponding parent alcohols[11] are as follows. In the case of primary esters, the α-carbon of cyclohexylmethyl phosphate (**5**) showed downfield shifts of 1.9, 4.3, 3.1, and 2.5 p.p.m. in dimethyl sulphoxide, and at pD < 1.5, 5, and 10, respectively, and the β-carbon of (**5**) showed an upfield shift of 2.6, 1.6, 1.5, and 1.3 p.p.m. in dimethyl sulphoxide, and at pD < 1.5, 5, and 10, respectively. The other carbons showed shifts of < 1 p.p.m. In the case of secondary esters, the α-carbon of cyclohexyl phosphate (**4**) showed downfield shifts of 3.8, 7.7, 5.0, and 4.3 p.p.m. in dimethyl sulphoxide, and at pD < 1.5, 5.0, and 10, respectively, whereas the β-carbon of (**4**) showed an upfield shift of 2.5, 1.7, 0.9, and 0.6 p.p.m. in dimethyl sulphoxide, and pD < 1.5, 5, and 10, respectively. These differences in chemical shift can be rationalised as follows. In aqueous solution, the acidic form of the ester can exchange its acidic phosphate hydrogens too rapidly for the n.m.r. time-scale to resolve the process and the three oxygen atoms of the phosphate group become equivalent. In this instance the dibasic phosphate ester can be considered to be symmetrical. As a result, it is only the electric charges on the phosphate group which determine the δ_C values in aqueous solution. However dimethyl sulphoxide behaves as a hydrogen-bond acceptor, so the acidic hydrogens on a phosphate group in dimethyl sulphoxide cannot exchange rapidly with respect to the n.m.r. time-scale. Therefore the phosphate group becomes less symmetrical, and the hydrogen bonding fixes the direction of P–O–H bond close to a solvent

Table 8. ^{31}P Chemical shifts of phosphate esters in deuterium oxide (p.p.m.)

Phosphate	pD 5.0	pD 9.8
(1)	1.14	4.42
(3)	1.15	4.50
(5)	1.29	4.63
(9)	1.29	4.61
(8)	1.37	4.74
(2)	0.54	4.01
(4)	0.43	3.95
(7)	0.42	3.95
(6)	0.74	4.38

Positive values are downfield relative to external 85% H_3PO_4.

open space and the direction of P=O bond close to its alkyl group. Therefore, the diamagnetic screening effect by phosphate group appears on the carbon atoms in the phosphate ester.[12] 4-*cis*- and 4-*trans*-t-butylcyclohexyl phosphates (**6**) and (**7**) show the β-shift differences of carbon 1.6 and 2.3 p.p.m. upfield from the parent alcohols, respectively. The rotamer population P_t of the axial phosphate compound (**6**) was less than that of the equatorial one (**7**). This fact reflects the smaller β-difference of (**6**). A similar kind of unexpectedly slow proton exchange occurs at the *N*-terminus of amino acids in trifluoroacetic acid solutions.[13]

Comparison of the δ_C values of methyl carbons of methylcyclohexanols with those of their phosphates showed that larger shift differences were observed in the isomers whose hydroxy and phosphate groups preferentially assume an axial orientation. Taking account of the conformational energy of phosphate to be 0.20 kcal mol^{-1} in dimethyl sulphoxide, the methyl groups (1.70 kcal mol^{-1})[7b] of these phosphates may exist exclusively in the equatorial orientation in the equilibrium of two conformers. Since it is known that the axial methyl carbon appears at δ_C 17.1—17.7 and the equatorial one at δ_C 22.3—23.5, the methyl shifts of phosphates are in a good agreement with those of the equatorial one.[14] On the other hand, the larger conformational free energy of the hydroxy group (0.75 kcal mol^{-1})[7b] results in the shift to higher field of the methyl signals of cyclohexanols compared with the corresponding phosphates.

The ^{31}P chemical shifts of the present phosphates in deuterium oxide are listed in Table 8. The signals at higher pH generally appeared at lower field than those of lower pH, *i.e.* nearer that of inorganic phosphate.[15] The shifts of the primary esters were about 0.6 p.p.m. lower than those of the secondary esters. The shifts of the axially oriented esters were lower than those of the equatorially oriented ones. These tendencies are the reverse of those observed for the carbon and proton chemical shifts.[16]

Experimental

N.m.r. Measurements.—^1H and ^{31}P spectra were measured by a Bruker CXP-300 spectrometer at 300 MHz and 121 MHz, respectively, and ^{13}C spectra were measured by a JEOL PFT-100 spectrometer at 25 MHz with the complete proton decoupling method. N.m.r. standards used were sodium 2,2,3,3-[$^2H_4$]-3-(trimethylsilyl)propionate (internal) for ^1H, the strongest solvent signal in [$^2H_6$]dimethyl sulphoxide (internal, 39.5 p.p.m.) and dioxane in deuterium oxide (internal, 67.4 p.p.m.) for ^{13}C, and 85% phosphoric acid (external) in a coaxial capillary for ^{31}P.

The adjustment of pD was carried out by use of the appropriate concentration of DCl or NaOD and was checked before and after n.m.r. measurement. Obtained pD values were the direct readings of a pH meter (Hitachi-Horiba M-4) with a pH electrode for a 5 mm diameter n.m.r. tube (Fuji Chemical Measurement) without the correction for D_2O.

Synthetic Procedures.—Cyclohexanol, cyclopentanol, and cyclohexylmethanol were commercially available compounds. Cyclopentylmethanol was obtained by the $LiAlH_4$ reduction of commercially available cyclopentanecarboxylic acid. Methylcyclohexanols, t-butylcyclohexanols,[17-19] and t-butylcyclohexylmethanols[20] were synthesised according to literature methods.

Obtained alcohols were esterified by the method of Atherton.[21] Synthesised phosphate esters were recrystallised as cyclohexylamine salts in ethanol–water or acetone-water. After acidification of amine salts of phosphates by column chromatography with Dowex 50WX8, lyophilised phosphate esters showed satisfied i.r. and n.m.r. spectra.

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