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CYCLIC PHOSPHATE AND PHOSPHITE TRIESTERS - A P^{31} N.M.R. STUDY

by

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The rapid degradation of ribonucleic acid on treatment with alkali was suggested by Fono[1] to be due to the cis hydroxyl group at C-2' of the ribose residues. The intermediate formation of nucleoside-2',3' cyclic phosphates, postulated by Brown and Todd[2a,b], was proved by the isolation of such compounds from hydrolysates of ribonucleic acid using mild alkali or pancreatic ribonuclease[3]. The extensive studies of Westheimer on cyclic esters of oxy-acids of phosphorus and sulphur led to the conjecture that variations in $d\pi$-$p\pi$ bonding might be responsible for the major part of the kinetic acceleration in the hydrolysis of five-membered cyclic sulphates and phosphates, and would explain why this acceleration is largely absent in the six-membered rings or when attack occurs at carbon[4].

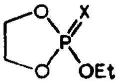
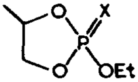
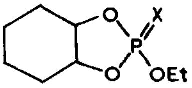
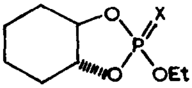
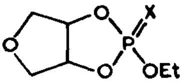
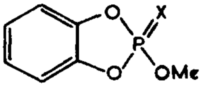
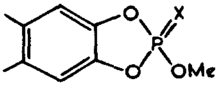
Chemical shifts in P^{31} nuclear magnetic resonance absorption are a function of the magnetic shielding of the phosphorus nucleus by electrons, and shifts between homologous compounds have been attributed to changes in the importance of ionic and double-bonded structures[5]. Therefore, a number of cyclic phosphate and phosphite tri-esters have been prepared and their P^{31} nuclear magnetic resonance spectra recorded.

Methods and Results. Cyclic phosphite esters were prepared by ester interchange between the appropriate diol and triethyl phosphite[6a, b] and purified by repeated distillation. Satisfactory analyses were obtained for new compounds. The cyclic phosphites were oxidised[7] by dinitrogen tetroxide in methylene dichloride solution at -80°, and the cyclic phosphate isolated by removal of excess reagent and solvent under reduced pressure. Spectra of five-membered esters were determined both on the freshly prepared crude oxidation products and on redistilled samples; those of six-membered esters were determined after distillation. Cytidine-2',3' methyl phosphate and methyl thymidine-3',5' phosphate were prepared from the known cyclic nucleotides in anhydrous methanolic solution by brief treatment with ethereal diazomethane. (The n.m.r. spectrum of the former indicated a 50% conversion into the acyclic ester after standing 18 hours at 0°C). N.m.r. spectra were determined at 16.2 Mc/sec. with a Varian Associates 4300B spectrometer and 12 in. electromagnet with flux stabilisation and non-spinning 10 mm. samples. Chemical shifts are given in p.p.m. relative to 85% orthophosphoric acid, with accuracy ± 1.0 p.p.m.

Discussion. The chemical shifts for the five-membered phosphate esters I - V give a mean value -15.4 (standard deviation ±2.0). This is significantly different both from the mean value +8.13 (±1.1) for the six-membered esters IX-XIV, and from the value +0.5 (± 1.5) observed[12] for acyclic phosphate triesters (cf. ref. 13). Both the nucleoside cyclic phosphates have chemical shifts 3-4 p.p.m. to lower field than the group mean value which can be attributed either to inductive effects of the furanose ring (cf. phosphate V) or to the pyrimidine ring.

TABLE I

P^{31} CHEMICAL SHIFTS OF FIVE-MEMBERED ESTERS

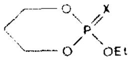
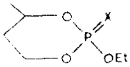
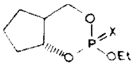
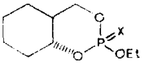
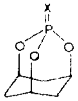
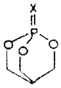
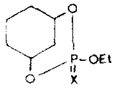
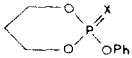
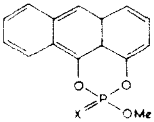
	ESTER	SOLVENT	PHOSPHITE (X=NONE)	PHOSPHATE (X=O)	$^{J}_{POCH}$ cps.
I		LIQ	−134	−17.2 (+1·6)*	10
II		"	−134	−15·8 (+2·3)	—
III		"	−136	−14.1 (+2.7)	9·5
IV		"	−139	−12·2 (+3·3)	—
V		"	−134	−17.7 (+1·5)	—
VI[a]		CCl_4	—	−12·4	12·0
VII[a]		CCl_4	—	−13·2	11·5
VIII	CYTIDINE−2′,3′ METHYL PHOSPHATE	MeOH	—	−18·5 (+1)	ca 8

* VALUES IN PARENTHESIS ARE FOR MINOR PEAKS ARISING FROM ACYCLIC PRODUCTS OF OXIDATION.

[a] PROVIDED BY T.A.KHWAJA.

TABLE 2

P^{31} CHEMICAL SHIFTS OF SIX-MEMBERED ESTERS

	ESTER	SOLVENT	PHOSPHITE (X=NONE)	PHOSPHATE (X=O)	J_{POCH} cps
IX		LIQ	−132	+7.7 (+1.5)	—
X		"	−131	+7.2	—
XI		"	−125	+7.0 (+2.3)	—
XII		"	−125	+8.5 (+1.8)	—
XIII[9]		$(CH_3)_2SO$	−137[9]	+10.4[9]	20
XIV[9]		$(CH_3)_2SO$	−91.5[9]	+7.97[9]	7[9]
XV		LIQ.	−124	POLYMERIC PRODUCT	—
XVI[10]		MeOH	—	+13	12.5
XVII[10]		$(CH_3)_2SO$	—	+13	—
XVIII[11]		$CHCl_3$	—	+15	11.5
XIX	METHYL THYMIDINE-3′,5′ PHOSPHATE	MeOH	—	+3.8	—

The five-membered cyclic esters show less electron-shielding of the phosphorus nucleus which is consistent with a diminution in the dπ-pπ double bond character of the cyclic P-O bonds in the five-membered ring. Thus, although there is at present no satisfactory mathematical correlation between chemical shift and the nature of groups bonded in phosphate esters[8], these results offer an explanation for the observed excess heat of saponification of methyl ethylene phosphate over that of dimethyl 2-hydroxyethyl phosphate (5.5 kcal/mol.)[4], and hence for the great reactivity of five-membered phosphate esters compared to six-membered and acyclic esters.

The cyclic phosphites also appear to exhibit a chemical shift characteristic of ring-size, though here the relative order is acyclic (-138$\pm$2)[12], five-membered (-135.2$\pm$2.0), and six-membered (-127.4$\pm$3.4). The groups in this case are less well defined and in particular the cage-esters XIII and XIV (X = none) have chemical shifts[9] very disparate from the mean.

The result of replacing an alkoxy- by an aryloxy-group consistently produces a change in the phosphate chemical shift to more positive values. This is in the opposite direction to that expected by consideration of inductive and diamagnetic anisotropy effects, both of which should deshield the phosphorus nucleus, but is consistent with the enhancement of dπ-pπ overlap by conjugation with the aromatic system. The fact that the increment in the five-membered phosphates VI and VII is less than half that in the six-membered phosphate XVIII agrees with the earlier deduction that π-bonding is diminished in the five-membered cyclic phosphates.

Cruickshank[14], from a study of bond lengths and bond angles, has suggested that the $d_{x^2-y^2}$ and d_{z^2} orbitals are responsible for strong π-bonding in phosphates, and has shown that the matching of available p-orbitals of O-(C) ligands with these d-orbitals can determine the π-bond orders of P-O(-C) bonds in phosphate diesters. The results presented here may provide a direct, experimental verification of this hypothesis.

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