

Table III

| Compound | | A_i^a (single temp, 26°) | A_i^a (slope of 1/T plot) |
|---|---------------------------|----------------------------|-----------------------------|
| Co(HMPA) ₂ Cl ₂ | | +1.175 × 10 ⁴ | +2.526 × 10 ⁴ |
| Ni(DAPDH) ₂ (ClO ₄) ₂ | <i>p</i> -H | +1.56 × 10 ⁵ | +1.87 × 10 ⁵ |
| | <i>m</i> -H | +8.25 × 10 ⁵ | +9.27 × 10 ⁵ |
| | CH ₃ | -1.58 × 10 ⁵ | -2.67 × 10 ⁵ |
| [Ni(4-CH ₃ pyN→O) ₆](ClO ₄) ₂ | <i>p</i> -CH ₃ | +2.205 × 10 ⁶ | +2.861 × 10 ⁶ |
| | <i>m</i> -H | +1.050 × 10 ⁶ | +1.179 × 10 ⁵ |

^a In units of hertz.

If the nonzero intercept arises in the case of nickel(II) complexes because of reaction with the solvent (as proposed for Ni(ONC₅H₄CH₃)₆²⁺) and if spectroscopic studies are available to show that the desired species is predominant (95% or better), the most accurate A_i values can be expected from a single measurement at the temperature of the spectroscopic studies. Using typical values for NiL₆²⁺, NiLX²⁺, etc., complexes, errors less than 5% in A_i should be expected from a single temperature measurement, whereas 20–30% discrepancies are noted in Table III.

In conclusion, the temperature dependence should be studied in any investigation of isotropic shifts. The potential influence of error from a nonzero intercept on the conclusions being drawn should be investigated in the manner described above. As additional systems are investigated, other factors giving rise to nonzero intercepts will undoubtedly arise.

Acknowledgment. The authors acknowledge the generous support of the National Science Foundation through Grant GP 5498.

Nuclear Magnetic Resonance Studies of Phosphate Esters. I. Conformational Study of Trisneopentyl Phosphate and Tris(β -chloroethyl) Phosphate

A. A. Bothner-By* and W.-P. Trautwein

Contribution from Mellon Institute, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213. Received August 20, 1970

Abstract: The temperature dependences of the $^3J_{\text{POCH}}$ of trisneopentyl phosphate and of the $^3J_{\text{POCH}}$ and the two $^3J_{\text{HH}}$ of tris(β -chloroethyl) phosphate have been measured. In both cases the preferred conformations for rotation about the CO bonds are those with the phosphate and alkyl groups in trans orientations. In the chloroethyl derivative the chlorine and phosphate oxygen are preferentially gauche.

The phosphate ester linkage is a common structural feature of biological molecules, including sugar phosphates, phospholipids, nucleotides, RNA's, and DNA's, etc. A study of the conformation of such molecules, centering on rotational isomerism about the P–O and O–C bonds, may yield information useful in understanding the binding of these substances to enzymes, and the mechanisms of their chemical reactions.

Studies by Sasikexharan and Lakshminarayanan¹ based on X-ray diffraction results and on empirical calculations have established that the groups on the O–C bond tend to occupy staggered positions, as in substituted ethane (Figure 1).

Rotation about the O–C bond may be studied *via* the ³¹P–H vicinal coupling constant, $^3J_{\text{POCH}}$, in suitable systems. Different values of $^3J_{\text{POCH}}$ in diverse trialkyl phosphates were observed by Axtmann, Shuler, and Eberly² in 1959. That these differences arose from conformational effects was suggested by Dudek.³

(1) V. Sasikexharan and A. V. Lakshminarayanan, *Biopolymers*, **8**, 505 (1969).

(2) R. C. Axtmann, W. E. Shuler, and J. H. Eberly, *J. Chem. Phys.*, **31**, 851 (1959).

Measurements on rigid compounds of known geometry^{4–7} have generally been concordant with the idea that the value of $^3J_{\text{POCH}}$ depends on the POCH dihedral angle, and that the form of the dependence is similar to the well-known Karplus⁸ curve for $^3J_{\text{HCC}}$.

Tsuboi, *et al.*,⁹ observed the $^3J_{\text{POCH}}$ values in a series of mono- and dialkyl phosphates in heavy water solution, and have estimated the conformational equilibria from these values, as well as the values $J_t = 28$ and $J_g = 1.5$ Hz derived from the couplings observed in methyl esters and in the rigid compound 1-phenyltrimethylene phosphate. In rigid model compounds, however, J_t has usually been smaller, of the order of 20 Hz.^{4,6}

(3) G. O. Dudek, *ibid.*, **33**, 624 (1960).

(4) L. D. Hall and R. B. Malcolm, *Chem. Ind. (London)*, 92 (1968).

(5) G. M. Blackburn, J. S. Cohen, and Lord Todd, *Tetrahedron Lett.*, 2873 (1969).

(6) J. G. Verkade and R. W. King, *Inorg. Chem.*, **1**, 948 (1962); *ibid.*, **3**, 884 (1964).

(7) R. S. Edmundson and E. W. Mitchell, *J. Chem. Soc. C*, 2091 (1968).

(8) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(9) M. Tsuboi, F. Kuriyagawa, K. Matsuo, and Y. Kyojoku, *Bull. Chem. Soc. Jap.*, **40**, 1813 (1967).

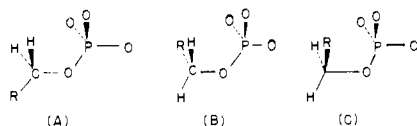


Figure 1. Stable conformations of alkyl phosphates.

In the present work, we have observed the coupling constants of trisneopentyl phosphate and tris(β -chloroethyl) phosphate as a function of temperature. It was expected that the populations of the rotamers of the compounds would change with temperature, and that the form of the resulting dependence of coupling constant on temperature would provide a test of the hypothesis that the $^3J_{\text{POCH}}$ vary with dihedral angle as previously proposed.^{3-7,9} We have also made a number of other measurements on mono- and dialkyl phosphates to investigate various points in connection with the conformational equilibria.

Experimental Section

Dimethyl and triethyl phosphate were prepared by oxidation of the corresponding phosphites with N_2O_4 in CCl_4 according to the procedure of Cox and Westheimer.¹⁰

Monomethyl and monoethyl phosphate were prepared by hydrolysis of methyl- and ethyldichloro phosphate, respectively. The chloro compounds were added to water under cooling and stirring. Then the water was evaporated under reduced pressure. Attempts to distill the phosphates were unsuccessful due to distillation and decomposition. For the nmr spectra these compounds were neutralized with the equivalent amount of NaOD in D_2O .

Neopentyl Phosphate. POCl_3 (17 g) was added to a solution of 26.4 g of neopentyl alcohol in 100 cm^3 of pyridine and 200 cm^3 of methylene chloride with stirring and cooling in an ice bath. After it had been stirred for 1 hr, the solution was washed with water and dilute HCl and dried with Na_2SO_4 . After evaporation of the CH_2Cl_2 , the residue (about 80% crude yield) was distilled at the oil pump, bp 110° (0.1 mm). The neopentyl phosphate crystallized immediately, mp $79-80^\circ$.

Trimethyl, diethyl, dibenzyl, and tris(β -chloroethyl) phosphate were of commercial origin.

Spectra. The nmr spectra were measured at 60 and 100 MHz with sweep rates of 0.1 Hz/sec. Variable-temperature studies were performed on a Varian A60 with a variable-temperature probe. Solutions (10-20%) of the compounds in the appropriate solvents were degassed and sealed under vacuum. The spectra were calibrated by the sideband technique using TMS as internal standard. Line positions were obtained by averaging the results of four spectra.

The spectra were analyzed numerically using the LAOCN3 program.¹¹ Observed and calculated line positions generally agreed within 0.05 Hz. Probable errors in coupling constants are estimated as 0.05 Hz.

Results

Temperature Dependence. The results of the analysis of the spectra of trimethyl phosphate, tris(β -chloroethyl) phosphate, and trisneopentyl phosphate taken at several temperatures are given in Table I.

The first point of importance is that no dependence of $^3J_{\text{POCH}}$ larger than experimental error is observed for trimethyl phosphate. This suggests that rotation about the P-O bond does not significantly affect the PH coupling, and that there is no intrinsic temperature dependence of the $^3J_{\text{POCH}}$.

(10) J. R. Cox and F. H. Westheimer, *J. Amer. Chem. Soc.*, **80**, 5441 (1958).

(11) A. A. Bothner-By and S. M. Castellano, "Computer Program for Chemistry," D. F. DeTar, Ed., W. A. Benjamin, New York, N. Y., 1968, pp 10-39.

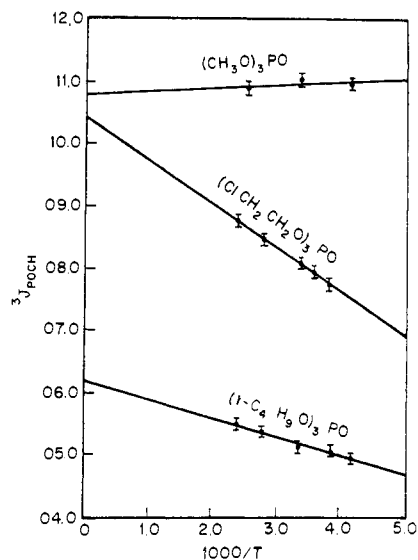


Figure 2. Plot of POCH coupling constant in three trialkyl phosphates vs. inverse absolute temperature.

In the case of tris(β -chloroethyl) phosphate, the value of $^3J_{\text{POCH}}$ shows a monotonic increase from 7.759 to 8.762 as the temperature increases from -10° to $+148^\circ$. This is consistent with the expected behavior. Since the chloromethyl is bulky, conformer A (Figure 1) is expected to predominate, with both α hydrogens in a

Table I. Chemical Shifts and Coupling Constants of Alkyl Phosphates at Different Temperatures in Chlorobenzene^a

| | Trimethyl phosphate | | | | |
|-----------------------|---|---------|---------|---------|---------|
| | $T, ^\circ\text{C}$ | | | | |
| | -30 | +26 | +128 | | |
| δ_{H} | 216.325 | 216.406 | 216.998 | | |
| J_{PH} | 10.970 | 11.047 | 10.885 | | |
| | Trineopentyl phosphate | | | | |
| | $T, ^\circ\text{C}$ | | | | |
| | -32 | -13 | +26 | +87 | +148 |
| δ_{H} | 223.940 | 223.952 | 223.858 | 224.007 | 224.398 |
| J_{PH} | 4.910 | 5.077 | 5.105 | 5.387 | 5.470 |
| | Tris(β -chloroethyl) phosphate ($\text{Cl}-\overset{1,2}{\text{CH}_2}-\overset{3,4}{\text{CH}_2}-\overset{5}{\text{O}}$) ₃ PO | | | | |
| | $T, ^\circ\text{C}$ | | | | |
| | -10 | +6 | +26 | +87 | +148 |
| $\delta_1 = \delta_2$ | 217.027 | 216.619 | 216.353 | 215.618 | 215.286 |
| $\delta_3 = \delta_4$ | 253.966 | 253.606 | 253.264 | 252.626 | 252.382 |
| J_{12} | (-12.0) | -12.942 | -12.362 | -11.744 | -12.251 |
| J_{34} | (-12.5) | -13.424 | -12.827 | -12.187 | -12.612 |
| $J_{13} = J_{24}$ | 6.563 | 6.471 | 6.458 | 6.363 | 6.297 |
| $J_{14} = J_{23}$ | 4.135 | 4.423 | 4.568 | 5.014 | 5.372 |
| $J_{15} = J_{25}$ | 0.893 | 0.957 | 0.995 | 0.885 | 0.751 |
| $J_{35} = J_{45}$ | 7.759 | 7.957 | 8.093 | 8.467 | 8.762 |

^a 60-MHz proton spectra. Chemical shifts in hertz downfield from TMS. Coupling constants in hertz.

gauche relationship to phosphorus. At higher temperature, conformers B and C will become more populated, and the α hydrogens will spend a greater proportion of their time in a trans orientation with respect to the phosphorus. A rough extrapolation to infinite temperature may be made by plotting $^3J_{\text{POCH}}$ against $1/T$ as in Figure 2. The extrapolated value, 10.4 Hz, is close to that of trimethyl phosphate, and suggests that tris(β -

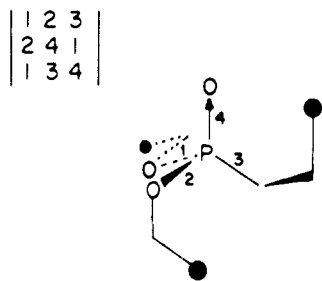


Figure 3. Conformation and notation for trisneopentyl phosphate. The black knobs represent *tert*-butyl groups.

chloroethyl) phosphate tends toward equal population of rotamers A, B, and C at high temperature. Designating the population of rotamer A by p_A , and taking $2J_g + J_t = 31.2$ (the extrapolated value), one can derive the relationship

$$p_A = (31.2 - J_g - 2J_{\text{obsd}})/(31.2 - 3J_g) \quad (1)$$

The value of J_g is not known precisely, but very probably lies in the range 1.0–3.0 Hz. The equilibrium constant for rotamer interconversion is defined as

$$K = 2p_A/(1 - p_A) \quad (2)$$

and may be evaluated by substitution of J_{obsd} in 1. The K 's which are found give a satisfactory Arrhenius plot with $\Delta H = -400$ to -500 cal (depending on the particular value of J_g chosen) and $\Delta S = 0$.

The three-bond proton-proton coupling constants in the chloroethyl group are also temperature sensitive. Following treatments previously given,¹² we identify J_{13} , which decreases with temperature, as the coupling constant between protons which are gauche oriented when the substituents Cl and $-\text{OPO}(\text{OR}_2)$ are trans oriented. The direction of the change with temperature indicates that the chloroethyl groups are most stable in a gauche configuration about the C–C bond, in accord with earlier observations.¹³ The magnitude of ΔH is sensitive to the value of J_g taken; it is estimated as 800 ± 300 cal, with $J_g \sim 2.5$ Hz and $J_t \sim 13$ Hz.

Trisneopentyl phosphate presents a more difficult case. Again there is a monotonic increase of coupling constant with temperature, indicating a preponderance of conformer with H and P in gauche relationship at lower temperature and increasing population of the less-hindered conformers at higher temperature. However, an extrapolation of ${}^3J_{\text{POCH}}$ vs. $1/T$ now yields an infinite temperature intercept at 6.2 Hz. This indicates that some conformers are excluded at the temperature reached.

With the assumption of staggered conformations, there are three energy minima for rotation about each P–O ester linkage, and three about each O–C bond, making nine possibilities per branch and $9^3 = 729$ total possibilities. A number of these are related by rotation about the P→O bond axis or reflection in one of the OPO planes. We have designated and catalogued these rotamers as follows.

The three PO ester linkages are numbered 1, 2, and 3, while the P→O linkage is numbered 4. In staggered conformations each bond fits approximately on a dia-

(12) D. Jung and A. A. Bothner-By, *J. Amer. Chem. Soc.*, **86**, 4025 (1964).

(13) R. J. Abraham and K. G. R. Pachler, *Mol. Phys.*, **9**, 165 (1963).

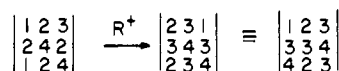


Figure 4. Rotation-degenerate conformations.

mond lattice specified by vectors 1, 2, 3, and 4 and is parallel with one of these vectors. A conformation may then be specified by a 3×3 matrix, as shown in Figure 3. Each column of the matrix represents one branch of the ester; the rows represent respectively the PO, OC, and C–CMe₃ bonds, and the digits 1, 2, 3, and 4 represent the direction of the bond in space.

Rules for deriving degenerate representations (related by rotation or reflection) are easily derived. Figure 4 represents a clockwise rotation, obtained using the cyclic permutation $1 \rightarrow 2 \rightarrow 3 \rightarrow 1$ ($4 \rightarrow 4$). After permuting the indices, the columns are permuted to restore the original order.

Consideration of the arrangement of the side chains convinces one that certain conformers are so hindered as to be unattainable. Thus all conformers represented by matrices in which two columns are of the form

$$\begin{vmatrix} l & m & \dots \\ n & n & \dots \\ m & l & \dots \end{vmatrix}$$

have the central carbons of the *tert*-butyl groups superposed, and are clearly unattainable. In the same way, rules may be derived for other interferences, for example superposition of two methyl groups, methyl group and methylene group, hydrogen and hydrogen, hydrogen and oxygen, etc. A computer program was written to aid in generating representations of all conformers, and classifying them by degree of hindrance. After elimination of all H,H superpositions, exactly nine distinguishable conformers remained. They are represented in Figure 5. If it is assumed that these conformers are equally populated in the high temperature limit, then one may deduce

$$(216J_g + 42J_t)/258 = J_{\infty} \quad (3)$$

accounting, at least in part, for the low observed value of J_{∞} , the extrapolated coupling constant. The values of J_g deduced by previous workers^{4,7,9} are consistent with eq 3; values of 25.0–30.0 and 1.5–2.5 Hz for J_t and J_g , respectively, appear to be consistent with most observations.

It should be pointed out that the average coupling expected for free rotation on the basis of these values is slightly less than that observed in trimethyl phosphate. Substitution of alkyl groups and other electron-withdrawing substituents reduces HCCH^{13,14} and HCCF¹⁵ vicinal couplings and may be expected to have a similar effect here. Because of the complex equilibria in-

(14) A. A. Bothner-By, *Advan. Mag. Resonance*, **1**, 195 (1965).

(15) R. J. Abraham and L. Cavalli, *Mol. Phys.*, **9**, 67 (1965).

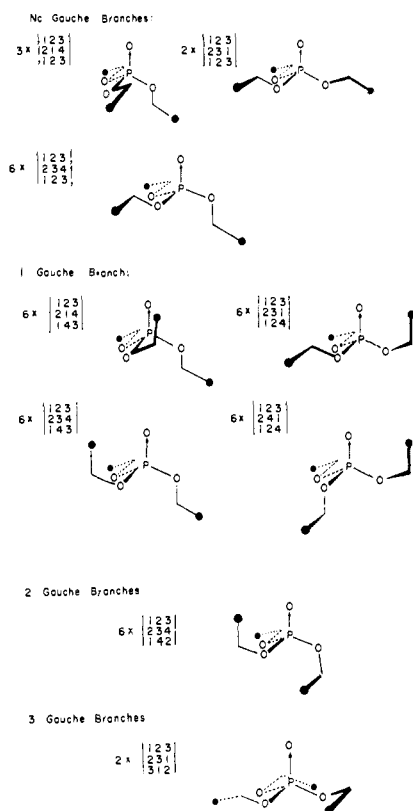


Figure 5. The nine distinguishable conformers of trisneopentyl phosphate lacking H,H or more severe superpositions.

involved, analysis in terms of a single ΔH appears unwarranted.

Effect of Ionization on Mono- and Dialkyl Phosphates. The energies of the conformers ought to depend on the state of ionization and hydration of the phosphate group. We have accordingly made some measurements of mono-, di-, and trialkyl phosphates in nonpolar solvents and in water under strongly acidic and under near-neutral conditions. The results are given in Table II.

Table II. J_{PH} of Mono-, Di-, and Trialkyl Phosphates in Different Solvents

| Phosphate | CCl_4 | $CDCl_3$ | 20% DCl | D_2O |
|-------------------------|---------|----------|---------|-------------------|
| Monomethyl ^a | | | 11.48 | 10.75 |
| Dimethyl ^a | | 11.32 | | 10.65 |
| Trimethyl ^a | | 10.96 | | 11.05 |
| Monoethyl ^a | | | 8.13 | 7.04 |
| Diethyl ^a | 8.05 | 8.00 | 8.10 | 7.15 |
| Triethyl ^a | 8.41 | | | 8.24 |
| Dibenzyl ^a | | 7.49 | | 7.20 ^b |

^a As sodium salt. ^b In pyridine.

Inspection reveals that the $^3J_{POCH}$ for the trialkyl esters are not much affected by the medium change. The mono- and diesters, on the other hand, exhibit values of $^3J_{POCH}$ which are smaller by about 1 Hz, when the phosphate groups are ionized. Under these conditions the valence shell of electrons is expected to expand somewhat, and there is probably considerable electrostatic binding of water, increasing the steric requirement of the phosphate and favoring conformers in which H is gauche to phosphate.

Conclusions

Our results are consistent with the dependence of $^3J_{POCH}$ on dihedral angle and on substitution in the same way as that well established for $^3J_{HCC}$. Alkyl phosphates show temperature-dependent coupling constants indicating a preference for conformers with phosphate and bulky alkyl groups trans oriented. Ionization and hydration of the phosphate group increase its steric requirement in this context.

Acknowledgment. This work was supported in part under Grant No. RR-00292 from the National Institutes of Health and used the NMR Facility for Biomedical Studies supported under the same grant. We are also indebted to NATO for a fellowship and the "Stiftung Volkswagenwerk" for a fellowship to one of us (W.-P. T.) which enabled him to participate in the study.