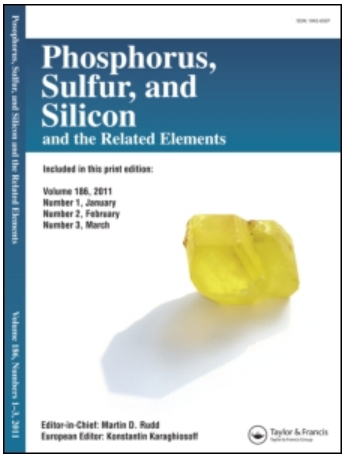

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OXYPHOSPHORANE MODELS FOR NUCLEOPHILIC DISPLACEMENTS AT Pα AND Pγ OF ADENOSINE-5′ TRIPHOSPHATE

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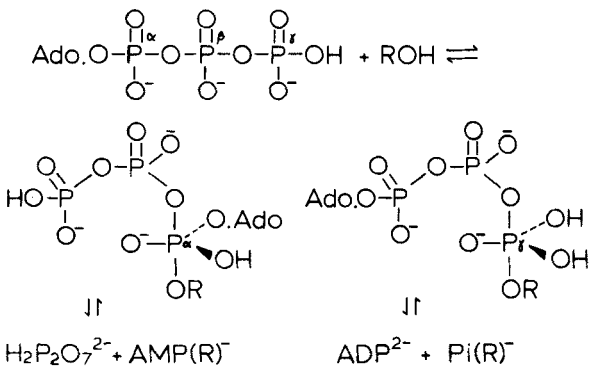
The reaction of dimethylphosphorous triethylpyrophosphoric anhydride, $(CH_3O)_2POP(O)(OC_2H_5)OP(O)(OC_2H_5)_2$, with hexafluorobiacetyl, $CF_3COCOCF_3$, yields 4,5-*bis*(trifluoromethyl)2,2-dimethoxy-2-triethylpyrophosphato-2,2-dihydro-1,3,2-dioxaphospholene. This oxyphosphorane, with a pyrophosphate group at the apical position of trigonal bipyramidal phosphorus, simulates the pentacovalent intermediate in the addition-elimination mechanism of nucleophilic displacements at the Pα and Pγ centers of adenosine-5′ tri-phosphate. The oxyphosphorane is remarkably stable in solution, although the pyrophosphate group is easily displaced by an alkoxy group in base-catalyzed reactions with alcohols.

Water, alcohols, phosphomonoesters and other nucleophiles attack mainly the Pγ and Pα electrophilic centers of nucleoside-5′ triphosphates under catalysis by enzymes of the ATPases,[2a] kinases[2b] and related types.[3,4] The basis for discussions of chemical mechanisms of these enzymic displacements[5] at P(4)[6] rests on studies of model non-enzymic reactions, although it is recognized that significant differences in mechanisms may occur under these two sets of conditions.[7] Two fundamentally different types of mechanisms are considered for displacements at P(4) in phosphate and pyrophosphate esters. (**1**) Addition-elimination via an intermediate with P(5) (oxyphosphorane).[8] (**2**) Elimination-addition via an intermediate with P(3) (monomeric metaphosphate anion.[9–14] The P(5)-mechanism applied to displacements at Pα and Pγ of ATP is illustrated in Scheme I.



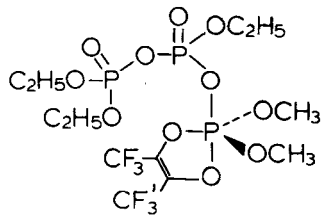
SCHEME I

The basic postulates underlying this formulation,[15,16] including problems related to ligand exchange in trigonal bipyramidal P(5) have been thorougly discussed.[17–25]

The operation of the P(5)-mechanism in displacements of phosphotriesters and phosphodiesters[26] is widely accepted in nonenzymic and enzymic[27,28] reactions. There is, however, no concensus on the operative mechanisms in displacements of phosphomonoesters, $XP(O)(OH)_2$ under different conditions; the Pγ atom of ATP is of this type.[29] Recent work from this Laboratory[30,31] has furnished evidence for the operation of both the P(5) and P(3) mechanisms in a given phosphomonoester, depending on experimental conditions, i.e. prevailing state of ionization (acid, monoanion, dianion), nature of the medium, and presence or absence of nucleophilic amines as catalysts. Moreover, the mechanism that will operate under a given set of conditions is closely related to the nature of the displaceable group, *X* in the phosphomonoester, as has been emphasized by Kirby, Jencks and co-workers.[11–13] Thus, it seems that displacements at ATPγ can occur either by P(5) or P(3) mechanisms depending on conditions, and that one of the crucial roles of the enzyme may be to insure the occurrence of that mechanistic type which is suitable for the mechanical (e.g. muscle contraction[32]), metabolic or other relevant effect.

The purpose of this work is to explore the accessibility and the properties of compounds

which simulate the structure of the hypothetical P(5) intermediate in the addition-elimination mechanism of ATPα and ATPγ displacements, as illustrated by compound (**1**)[33].



1; $\delta^{31}P = -57.0, -29.0, -14.2$ ppm[33]

$\delta^{19}F = +13.1$ ppm

$\lambda CH_3 = 6.04$ ppm, $J_{HP} = 16.0$ Hz

Formula **1**

RESULTS AND DISCUSSION

Synthesis of Oxyphosphoranes

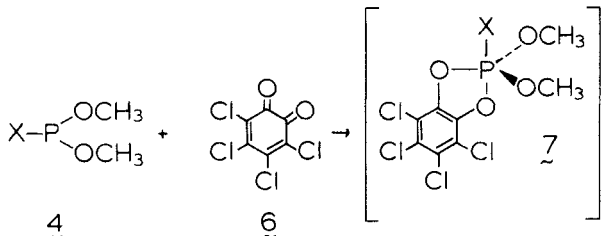
Dimethylphosphorous triethylpyrophosphoric anhydride (**4**) is prepared by the sequence of reactions outlined in Scheme II.

The P(3) atom of anhydride (**4**) adds to hexafluorobiacetyl (**5**) according to Eq. 1 forming 4,5 - *bis*(trifluoromethyl) - 2,2 - dimethoxy - 2 - triethylpyrophosphato - 2,2 - dihydro - 1,3,2 - dioxaphospholene (**1**), in about 95% yield.

The structure of the pyrophospho-oxyphosphorane (**1**) rests on its elemental analysis and on data from nmr spectroscopy to be discussed below.

$$\underset{\mathbf{4}}{XP(OCH_3)_2} + \underset{\mathbf{5}}{CF_3COCOCF_3} \rightarrow \mathbf{1} \qquad (1)$$

The success of the synthesis is due to the high reactivity of the fluorodiketone[34,35] (**5**), since the anhydride (**4**) is a relatively poor nucleophile, as would be expected from the presence of a strong electron-withdrawing pyrophosphate ligand *X* bonded to P(3). This effect is manifested in the failure of anhydride (**4**) to generate an oxyphosphorane with biacetyl, $CH_3COCOCH_3$, which is quite reactive toward more nucleophilic P(3) compounds, e.g. trialkyl phosphites.[36] The reaction of anhydride **4** with the relatively strong electrophile o-chloranil (**6**) is relatively slow at temperatures which are compatible with the stability of the corresponding oxyphosphorane, (**7**) (Scheme III). Thus, although nmr spectroscopy reveals the transient formation of (**7**) under conditions given in the Experimental Section, it has not been possible to isolate this particular oxyphosphorane.



4 **6**

X = $(C_2H_5O)_2P(O)OP(O)(OC_2H_5)O$-

SCHEME III

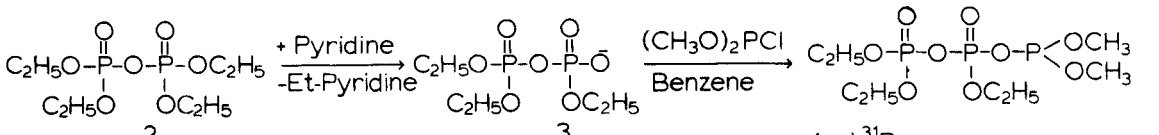
Dimethylbenzyl phosphite (**8**) is very reactive toward hexafluorobiacetyl and yields 4,5-*bis*(trifluoromethyl) - 2,2 - dimethoxy - 2 - benzoxy - 2,2 - dihydro-1,3,2-dioxaphospholene (**9**, **10**) according to Eq. 2.

$$\underset{\mathbf{8}}{C_6H_5CH_2OP(OCH_3)_2} + \underset{\mathbf{5}}{CF_3COCOCF_3} \rightarrow \mathbf{9}, \mathbf{10} \qquad (2)$$

This trialkoxy-oxyphosphorane is written as two diastereomers of not much different energy content and probably undergoing a relatively fast interconversion in solution, for reasons given in the next Section.

Ligand Exchange in the Oxyphosphoranes

The ^{19}F nmr spectra of the two types of P(5) derivatives, (**1**) and (**9**), (**10**), in $CDCl_3$ solutions at 35°C exhibit only one signal. In principle, this signal should be a doublet due to F, P coupling;



2 **3**

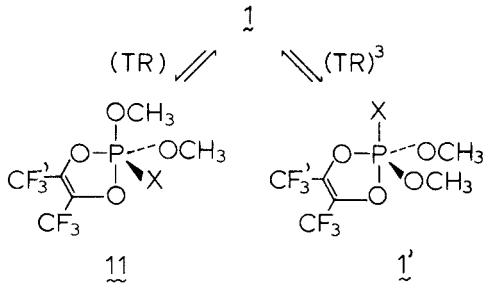
4; $\delta^{31}P = +130.3, -23.0, -14.3$ ppm

$\lambda CH_3 = 6.30$ ppm, $J_{HP} = 10.8$ Hz

SCHEME II

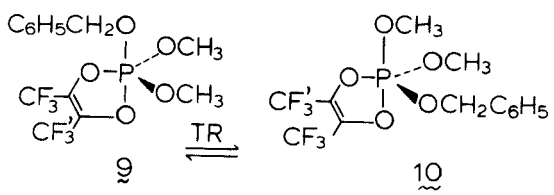
however, it is known[37] that this type of J_{FCCOP} coupling constant is relatively small and the signals appear as sharp singlet at 94.1 MHz. The two CF_3- groups in formula (**1**) are magnetically non-equivalent. For reasons already discussed[18,37] it is assumed that the appearance of one ^{19}F nmr signal in the spectra of these compounds is due to relatively fast[38] regular permutational isomerization, i.e. to a fast exchange of ligands occurring by intermolecular bond deformations. Two mechanisms have been suggested for this type of ligand exchange: *pseudorotation*[17] and *turnstile rotation*[18] (TR).

Scheme IV illustrates the exchange of position of the two CF_3- groups in (**1**) by the single TR utilizing the five-membered ring as the ligand-pair.[39] This step would lead to a new isomer (**11**) which has the strongly apicophilic[16] pyrophosphate ligand *X* in an equatorial position, and should therefore be of considerably higher energy content than isomer (**1**). The two CF_3- groups in (**1**) can also exchange positions by a triple turnstile rotation, $(TR)^3$, utilizing the same ligand-pair and leading to formula (**1′**), which is equivalent to (**1**) and retains the apicophilic pyrophosphate in the apical position. In this interpretation, the oxyphosphorane is adequately represented as a rapidly interconverting pair of equivalent structures (**1**) $\rightleftharpoons$ (**1′**), with state (**11**) not populated to any operationally significant extent.



SCHEME IV

The situation is different in the benzoxy-oxy phosphorane (**9**), (**10**). The two diastereomers generated by single TR have comparable energies with the difference stemming from modest steric and electronic effects. Hence, an adequate representation of the oxyphosphorane in solution should include both isomers. The J_{HCOP} coupling constants of oxyphosphoranes (**1**) and (**9**), (**10**) differ by a small but possibly significant amount. Methoxy groups in equatorial positions of oxyphosphoranes give rise to larger *HCOP* coupling than the same groups in the apical positions. The observed difference in J_{HP} values of the two oxyphosphoranes is, therefore, consistent with the picture presented above, where a methoxy-group in the benzoxy-oxyphosphorane has a finite apical residence time.



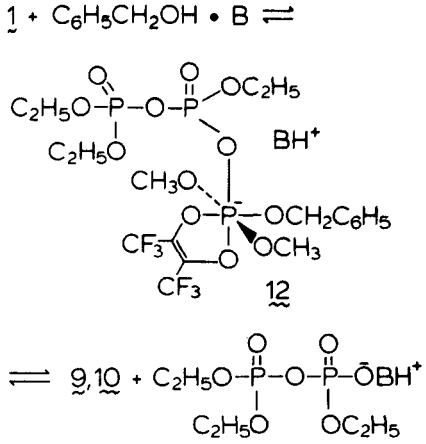
9,10; $\delta^{31}P = -48.8$ ppm ; $\delta^{19}F = +13.1$ ppm

$\nearrow CH_3 = 6.38$ ppm, $J_{HP} = 13.5$ Hz

The ^{31}P nmr spectra of both types of oxyphosphoranes exhibit three signals with the chemical shifts expected[36] from a P(5) atom and two different P(4) atoms within the pyrophospho-oxyphosphorane function.

Base-Catalyzed Nucleophilic Displacement in the Oxyphosphoranes

In the presence of the hindered base γ-collidine, benzyl alcohol displaces the pyrophosphate ligand of the oxyphosphorane (**1**) in a relatively rapid and virtually quantitative reaction carried out at 30°C in $CDCl_3$ solution. Scheme V shows the mechanism proposed for this displacement reaction at a P(5) center, where an intermediate with P(6) is postulated. This mechanism is based on our previous observations, which include the isolation of



(B = γ-Collidine)

SCHEME V

an hexaphosphoride salt analogous to (**12**).[40] The stable P(6) compound is obtained from the base-catalyzed addition of phenol to an oxyphosphorane containing the bisfluoromethyl-dioxaphospholene ring but lacking a good leaving group of the pyrophosphate-type. Elucidation of the molecular structure of the P(5) and the P(6) structures by x-ray diffraction analysis[41] supports the view that the nucleophile approaches the trigonal bipyramidal phosphorus along the equatorial plane opposite one of the endocyclic-oxygen ligands, as shown in Scheme V.

CONCLUSIONS

The remarkable stability of a pyrophospho-oxyphosphorane of type (**1**) supports the hypothesis that the Pα and the Pγ centers of ATP, in the appropriate state of ionization, can expand their covalent coordination from P(4) to P(5) during nucleophilic displacement reactions. Recent work on displacements of phosphomonoesters,[30,31] $XP(O)(OH)_2$, suggests that P(5)-intermediate formation is to be expected, on kinetic and thermodynamic grounds, only when the P(4) center has no negatively charged oxygen, or when it has one negatively charged oxygen, i.e., from the acid or the monoanion, but not from the dianion. In this respect, and with reference to Scheme I, it would seem reasonable to assume that divalent metal ions, e.g. Mg^{2+}, could contribute to the stability of P(5) by decreasing negative charge density on oxygen through electrostatic coordination with it.[42–44] However, as far as we know, there is no evidence that Mg^{2+} has a significant effect on the reactivity of ATP toward nucleophiles in the absence of enzymes. Therefore, the role of Mg^{2+} in the enzymic reactions appears to be related to the ability of the metal to engage in strong electrostatic and donor coordination with the pyrophosphate oxygens[45,46] and with the protein, in the latter case by direct ligation to amino acid residues, in particular histidine,[47,48] and by indirect hydrogen bonding via metal-ligated water. The net result of these interactions is a tight binding of the nucleoside triphosphate in the enzyme active site pocket, rather than a specific effect of Mg^{2+} on the electrophilicity of P(4). This concept does not preclude some Mg-assistance when the pyrophosphate ligand departs from the P(5) intermediate.[42–44]

The Pγ center of ATP can also, in principle, undergo displacements by elimination-addition provided that the group that is eliminated is the PO_3^- anion. This can result when the substrate is ATP^{4-} or when the proton of $ATPH^{3-}$ is on the Pβ-O-Pγ bridge-oxygen or on a non-bridging Pβ oxygen.[29] It seems plausible that differences in ATP non-enzymic vs enzymic displacements result from the ability of the enzyme to control the site of protonation. There is, in fact, increasing evidence that even under enzymic catalysis, the precise mechanism of the displacement at P(4) within a given biochemical pathway may differ significantly as a result of alterations of the native conditions, e.g. modifications of the protein, the substrate or the metal cofactor.[49–55]

EXPERIMENTAL

Analyses were performed by Galbraith Laboratories, Knoxville, Tenn. All reactions of oxyphosphoranes were carried out in anhydrous solvents.

Reaction of tetraethyl pyrophosphate with pyridine. Tetraethyl pyrophosphate (**2**) (6.74 g, 23 mmol) dissolved in pyridine (3.6 g, 46 mmol) was stirred at 85°C for 6 hr. Unreacted pyrophosphate and pyridine were removed by extraction with 3 × 15 mL of ether. The residue was kept 30 min at 1 mm to give 8.0 g (90% yield) of *N*-ethylpyridinium triethyl pyrophosphate (**3**; $\delta^{31}P = -10.2, -11.4$ ppm; multiples at $\tau = 8.66$ (CH_3, 9H) and 5.84 (CH_2, 6H); multiplet at $\tau = 8.29$ (CH_3, 3H), multiplet at 5.0 (CH_2, 2H).

Reaction of dimethyl phosphorochloridite with N-ethylpyridinium triethyl pyrophosphate (**3**) Dimethyl phosphorochloridite[56] (3.0 g, 23 mmol) dissolved in 10 mL of benzene was added dropwise over 25 min to *N*-ethylpyridinium triethyl pyrophosphate (**3**), (8.0 g, 23 mmol) suspended in 40 mL of benzene at 5°C. The mixture was stirred an additional 20 min at 5°C and then filtered. The filtrate was evaporated at 30°C (30 mm), to give dimethylphosphorous triethylpyrophosphoric anhydride (**4**) in 75% yield.

Anal. Calcd. for $C_8H_{21}O_6P_3$: C, 27.1; H, 5.9; P, 26.3. Found: C, 26.9; H, 5.9; P, 26.0. Attempts to distill the anhydride (**4**) at 100°C (0.1 mm) resulted in decomposition.

Reaction of hexafluorobiacetyl (**5**) *with dimethylphosphorous triethylpyrophosphoric anhydride* (**4**) The α-diketone (**5**) (0.23 g, 1.2 mmol) dissolved in 10 mL of cold CH_2Cl_2 was added dropwise with stirring over a 50 min period to the anhydride (**4**) (0.49 g, 1 mmol) dissolved in 20 mL of CH_2Cl_2 and cooled at −78°C. The mixture was kept 30 min at −78°C and allowed to reach 20°C; the solvent was evaporated at 30°C (30 mm). The residue was kept 30 min at 20°C (0.1 mm) to yield 4,5-*bis* (trifluoromethyl)-2,2-dimethoxy-2-triethylpyrophosphato-2,2-dihydro-1,3,2-dioxaphospholene (**1**), in 95% of the theoretical yield. The analytical data were obtained on this preparation without further purification.

Anal. Calcd. for $C_{12}H_{21}O_{11}F_6P_3$: C, 26.3; H, 3.8. Found: C, 25.6; H, 4.7.

Preparation of dimethylbenzyl phosphite Benzyl alcohol (6.7 g, 62 mmol) and pyridine (5 mL, 62 mmol) dissolved in 20 mL of benzene were added dropwise to a stirred solution of dimethyl phosphorochloridite (7.7 g, 62 mmol) in 100 mL benzene over 30 min at 5°C. The filtrate was evaporated at 30°C (35 mm). The residue was submitted to short path distillation. Dimethylbenzyl phosphite (**8**) was isolated in 85% yield, b.p. 73–75°C (0.1 mm); $\delta^{31}P = +140.0$ ppm; $\tau = 6.50$ ($J_{HP} = 10.8$ Hz) and 5.10 ($J_{HP} = 8.0$ Hz) ppm.

Reaction of hexafluorobiacetyl (**5**) *with dimethylbenzyl phosphite* (**8**) A cold solution of hexafluorobiacetyl (**5**) (0.2 g, 1 mmol) dissolved in 10 mL of CH_2Cl_2 was added to a solution of dimethylbenzyl phosphite (**8**) (0.2 g, 1 mmol), in 35 mL of CH_2Cl_2 at −78°C over 40 min. The solution was stirred another 10 min at −78°C and allowed to warm to 20°C. The solvent was evaporated at 20°C (30 mm). The residue was kept 30 min at 20°C (0.1 mm) to give 4,5-*bis*(trifluoromethyl)-2,2-dimethoxy-2-benzoxy-2,2-dihydro-1,3,2-dioxaphospholene (**9**, **10**) in 95% yield.

Anal. Calcd. for $C_{13}H_{13}O_5F_6P$: C, 39.6; H, 3.3; P, 7.9. Found: C, 39.3; H, 3.4; P, 7.6.

Reaction of 4,5-Bis(trifluoromethyl)-2,2-dimethoxy-2-triethylpyrophosphato-2,2-dihydro-1,3,2-dioxaphospholene (**1**) *with Benzyl Alcohol in the Presence of γ-Collidine* Benzyl alcohol (0.03 mL, 0.3 mmol) and γ-collidine (0.05 mL, 0.4 mmol) were added to the phospholene (**1**) (0.16 g, 0.3 mmol) dissolved in 0.2 mL $CDCl_3$ at 30°C. The reaction was monitored by ^1H nmr. The displacement of the pyrophosphate group by benzyl alcohol was complete within 20 min. The product was shown to be 4,5-*bis*(trifluoromethyl)-2,2-dimethoxy-2-benzoxy-2,2-dihydro-1,3,2-dioxaphospholene (**9**, **10**), by comparison with an authentic sample.

Relative Reactivities of the Phosphite-Pyrophosphate Anhydride **4** *Toward Hexafluorobiacetyl* (**5**), *o-Chloranil* (**6**) *and Biacetyl* A 0.1 M dichloromethane solution of the carbonyl compound was added over a 15 min period to a stirred 0.05 M dichloromethane solution of anhydride **4** at −78°C (equimolar amounts).

The fluorodiketone reaction was complete in minutes and the oxyphosphorane **1** was stable for at least 10 days at −25°C in solution.

The o-quinone reaction proceeded at impractically slow rates at temperatures below 0°C. At 25°C, the red color of the quinone solution persisted for about 10 min. Nmr spectroscopy showed the transient formation of the oxyphosphorane **7**, which underwent decomposition. The structure of the decomposition products was not established; one of them had a ^{31}P nmr signal at −1.0 ppm suggestive of methyl tetrachloro-o-phenylene phosphate.

No reaction was noted between the anhydride **4** and biacetyl, $CH_3COCOCH_3$ within 30 min at 25°C. Spectroscopy disclosed the formation of a complex mixture of products after 16 hr at 25°C.

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21. A P(5) intermediate may or may not have a sufficiently long life-time to allow apical-equatorial ligand exchange by regular permutational isomerization (RPI), i.e. by intramolecular bond deformations. In either case the P(5) intermediate can be formed by attack of the nucleophile *trans* (in-line) or *cis* (adjacent) to the leaving group. Evidently, if apical entrance-departure is a prerequisite for displacement (Ref. 15) *cis*-attack requires an exchange of the leaving group from equatorial to apical position. This exchange can be achieved by RPI and also by a much slower irregular permutational isomerization (IPI), i.e. by the rupture and reformation of bonds at the P(5) stage. Displacements by *trans*-attack do not require an exchange of ligands, although they may also involve ligand exchange by multiple RPI or by IPI (Ref. 19, 20). Some authors have equated the "in-line mechanism" with a S_N2 displacement at P(4) via a five-coordinate *transition state* analogous to the Walden-inversion at carbon, but it is not always clear when this is intended (e.g., five solid lines indicative of full covalent bonds sometimes illustrate the S_N2 "transition state"). Although such a transition state is operationally

indistinguishable from a P(5) intermediate that gives no direct or indirect indication of its existence, there is at present no evidence for Walden inversion-type of mechanism in P(4), while there is much experimental support for oxyphosphorane intermediates, including their direct observation (Ref. 22–25). It should be emphasized that a P(5) intermediate generated by in-line or adjacent attack *can* have the same consequences depending on the type of operative RPI; e.g., both can lead to inversion of configuration of P(4) and to the single-labeling of P(4) from $^{18}OH_2$ in hydrolysis.

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