Cyclic Pentaoxyphosphoranes as Models for cAMP Action. An *ab Initio* Approach[1,2]

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Received February 9, 1994®

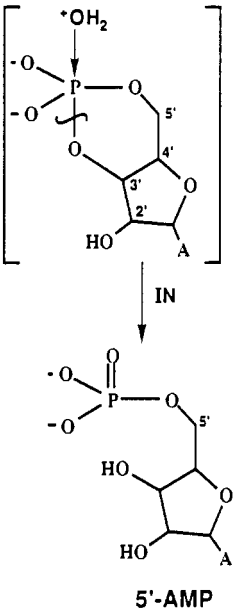
Ab initio molecular orbital calculations on monocyclic pentaoxyphosphorane molecules as model states in cyclic AMP action are carried out. Five-membered-ring systems are included for comparison. Minimum energy geometries in trigonal bipyramidal configurations that position the rings in axial–equatorial (a–e) sites are compared with those for diequatorial (e–e) ring locations. The models are made increasingly more complex approaching proposed intermediates in enzymatic hydrolysis of cAMP. These contain ribose rings *trans*-fused to the saturated six-membered ring of the pentacoordinated phosphorus state. All calculations show the e–e ring location to be higher in energy than the a–e ring arrangement. The computed energies and P–O bond lengths are compared with VT NMR activation energies for ligand exchange and with X-ray structural studies, respectively, on pentaoxyphosphoranes. The computations strongly support proposals for in-line enzymatic hydrolysis of cAMP with the ring positioned at a–e sites in a boat conformation.

Introduction

Proposals exist in the literature describing activated states in cAMP (cyclic adenosine monophosphate) action where trigonal bipyramidal (TBP) geometries are invoked. For example, in enzymatic[3–5] and nonenzymatic[6] hydrolysis of cAMP by phosphodiesterases (Schemes 1 and 2), axial–equatorial (a–e) placement of the six-membered ring of cAMP in TBP geometries is envisioned. The enzymatic hydrolysis leads to 5′-AMP,[7–10] whereas nonenzymatic hydrolysis yields 3′-AMP.[6] Both proceed by inversion of configuration. In contrast, cAMP action with protein kinases, where ring opening is not involved, is proposed to proceed via diequatorial (e–e) ring placement in a TBP activated state (Scheme 3).[11–14]

Our synthesis[15–23] of cyclic oxyphosphoranes as models for activated states in nucleophilic substitution reactions of tetracoordinate phosphorus and subsequent X-ray and NMR studies have revealed that six-membered rings of cyclic pentaoxy derivatives normally are positioned at a–e locations of a TBP

Scheme 1. Enzymatic Hydrolysis of cAMP



5′-AMP

Formation of 5′-AMP with inversion favors in-line attack opposite the 3′-oxygen atom with phosphodiesterases.[4]

* Abstract published in *Advance ACS Abstracts,* June 1, 1994.

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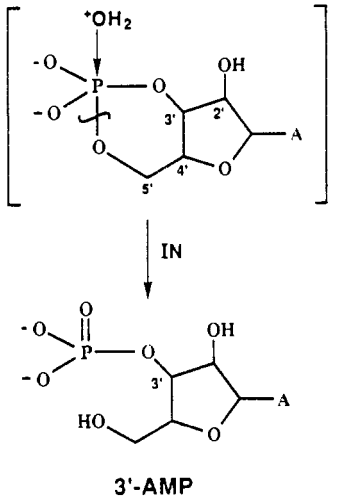
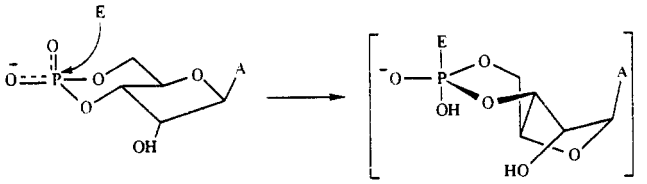
Scheme 2. Nonenzymatic Hydrolysis of cAMP



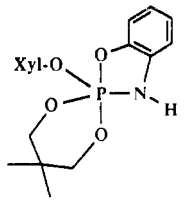
Formation of 3'-AMP with inversion of configuration favors in-line attack of H_2O opposite the 5'-oxygen atom.[6]

Scheme 3. Proposed Intermediate in the Activation of Protein Kinases by cAMP[a]

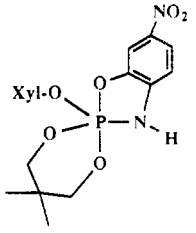


[a] Adapted from ref 3.

where, if saturated as in cAMP, the ring usually assumes a boat conformation. The prow of the boat is occupied by the apical oxygen atom, and the stern, by the opposite methylene group. If hydrogen bonding constraints are introduced, saturated six-membered rings in a–e sites of a TBP may be induced from a boat to a chair conformation, for example as found by X-ray studies in the structures of **A**[21] and **B**.[23]

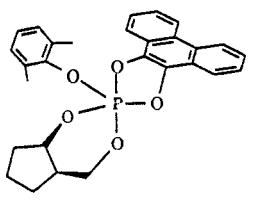


A

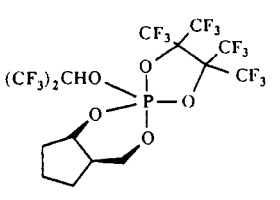


B

In further work, more closely approaching the ring system in cAMP, synthesis of pentaoxy derivatives **C**[20] and **D**[4,5] that contain

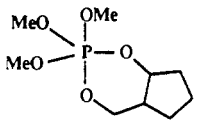


C

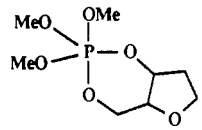


D

a five-membered ring *trans*-fused to the six-membered saturated ring shows X-ray structures having the same boat conformation located at a–e sites of a TBP. These derivatives obtained in our laboratory[20] and in Bentrude's[4,5] have the five-membered ring annelated at the position corresponding to that in the intermediate proposed in nonenzymatic hydrolysis that leads to 3'-AMP (Scheme 2). ^{13}C NMR spectra of **E**[11] and **F**[5] showed that

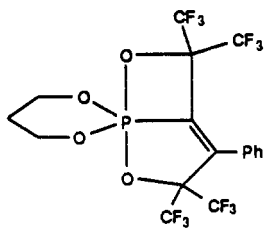


E



F

inclusion of a ribose component *trans*-fused to the six-membered ring did not alter the TBP arrangement; i.e., the ring remained positioned at a–e sites. The location of the five-membered rings was not established. They are shown here by analogy as found in the X-ray structures of **C**[20] and **D**.[4,5] The only example of a six-membered ring in an e–e location was obtained for tetraoxyphosphorane **G**[24] having a constrained bicyclic component.



G

In view of this extensive background, it became of interest to employ molecular orbital calculations to evaluate energy differences, particularly of a–e and e–e ring locations in TBP geometries. To gain confidence in the approach used, pentaoxyphosphoranes were modeled that contain five-membered saturated rings in these two locations for comparison with calculations on six-membered saturated rings. The results could then be evaluated relative to energy difference between a–e and e–e sites inferred from variable-temperature NMR studies on intramolecular ligand exchange processes (pseudorotation). The resulting activation energies correspond to barrier states having the rings in e–e sites and indicate their relative stability compared to a–e ring locations in the ground-state TBP configurations.

We report here *ab initio* calculations on the cyclic pentaoxyphosphoranes **1–8** (Chart 1) containing ring components of increasing complexity that approach proposed activated states in enzymatic interaction with cAMP.

Computational Method

The geometry of each cyclic pentacoordinated phosphorus species **1–8** (Chart 1) was fully optimized. Optimization was done at trigonal-bipyramidal minima with rings located either at axial–equatorial sites (a–e) or diequatorial locations (e–e). Calculations were carried out with the program GAUSSIAN 92[25] at the San Diego Supercomputer Center. For the phosphorus molecules, the basis set was 3-21G* (split-level valence with polarization functions on second- and third-row atoms). For the anionic phosphorus molecules, diffuse orbitals were added to the basis sets for all non-hydrogen atoms as appropriate for negatively charged species. Single-point calculations were then performed at the 6-31G* and 6-31+G* levels for the neutral and anionic molecules, respectively.

It was necessary to limit the calculations in this way for these cyclic derivatives as the computational time becomes prohibitive otherwise. To

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Chart 1

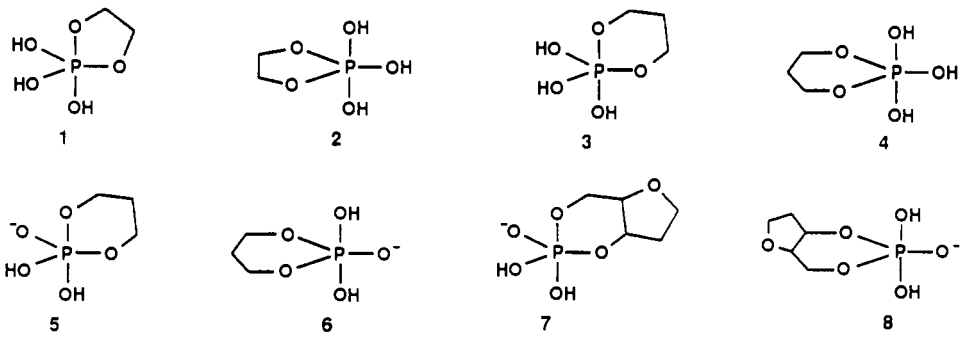

Table 1. Minimum Energies of TBP Geometries with Rings in e–e Orientations Relative to Ground-State a–e Orientations

entry	ring type	energy, kcal/mol
1	5-mem a–e	0
2	5-mem e–e	20.1,[a] 22.9[b]
3	6-mem a–e boat	0
4	6-mem e–e chair	6.6,[a] 8.7[b]
5	6-mem a–e boat	0
6	6-mem e–e chair	12.7,[a] 10.8[c]
7	6-mem a–e boat with *trans*-fused 5-mem	0
8	6-mem e–e chair with *trans*-fused 5-mem	14.4,[a] 11.9[c]

[a] Basis set 3-21G*. [b] Basis set 6-31G*//3-21G*. [c] Basis set 6-31+G*//3-21G*.

fully optimize the structures on the Cray Y-MP8/864 at the 3–21G* level, the time ranged from about 7–50 h depending on the complexity of the phosphorus species, **1–8**. The single-point calculations at the 6–31+G* level averaged about 5 h each.

Results

Table 1 lists energy differences between the higher energy e–e five- and six-membered-ring orientations relative to the ground-state a–e ring orientations for the trigonal bipyramids, **1–8**. All a–e orientations have the six-membered ring in a boat conformation. For the six-membered rings occupying e–e sites, the ring is a chair conformation. Table 2 reports calculated P–O bond distances from the minimum energy TBP structures for **1–8**. These data are compared with experimental values of P–O bond lengths obtained from molecular structures of cyclic pentaoxyphosphoranes by X-ray diffraction.[10–12] Average values are listed at the bottom of Table 2.

Discussion

Figure 1 portrays the relative energies calculated at the 3–21G* level for pentaoxyphosphoranes **1** and **2** containing a–e and e–e orientations of five-membered rings compared to those for **3** and **4** having six-membered rings. The lower energy difference for the two ring orientations in the case of the phosphoranes with the six-membered rings, **4** minus **3**, compared to that for the related five-membered rings, **2** minus **1**, partly reflects the lower strain in placing a six-membered ring diequatorial in a TBP compared to that encountered with a five-membered ring. These results agree nicely with activation energies from VT ^{19}F NMR[26] for pseudorotation of phosphoranes **H** and **I** containing these rings (Figure 2) considering the compositional differences between the theoretical and experimental species. The process occurring causes ring reorientation from a ground-state phosphorane with a ring occupying a–e sites to an activated state having the ring in e–e sites. The close energy fit provides confidence in the comparison. However, less agreement exists for the single-point calculations at the 6–31G* level which may more accurately reflect the compositional differences involved.

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Table 2. Calculated and Experimental P–O Bond Distances (Å) for Cyclic Pentaoxyphosphoranes[a]

Calculated P–O Bond Distances

	ring		P–OH		P–O⁻
entry[b]	ax	eq	ax	eq	eq
1	1.643	1.625	1.673	1.593	
				1.593	
2		1.614	1.646	1.614	
		1.614	1.646		
3	1.631	1.612	1.681	1.601	
				1.592	
4		1.603	1.657	1.615	
		1.602	1.644		
5	1.721	1.657	1.723	1.637	1.483
6		1.658	1.738		1.492
		1.658	1.686		
7	1.814	1.656	1.665	1.617	1.492
8		1.657	1.738		1.501
		1.657	1.685		

Experimental Average P–O Bond Distances[c]

five-mem rings[d]		six-mem rings[e]		P–OXyl[f]	
ax	eq	ax	eq	ax	eq
1.716(4)	1.636(4)	1.636(4)	1.589(4)	1.665(6)	1.612(6)

[a] Calculated distances from minimized structures at the 3-21G* level. [b] Entries **1** and **2** have five-membered rings while **3–8** have six-membered rings attached to phosphorus. Of the six-membered rings, **3**, **5**, and **7** have boat conformations in a–e orientations, and **4**, **6**, and **8** have chair forms in e–e sites. [c] Numbers in parentheses are standard deviations. [d] From refs 18 and 20. Four X-ray structures of cyclic pentaoxyphosphoranes with rings in a–e orientations. [e] From refs 18–20. Six X-ray structures of cyclic pentaoxyphosphoranes with rings in a–e orientations with boat forms. [f] From refs 18–20. Ten X-ray structures of cyclic pentaoxyphosphoranes with acyclic xylyloxy groups.

In modeling cAMP–enzyme interactions, it is more realistic to consider a phosphorane anion for an activated intermediate. This was done for computations at the 6–31+G* level involving **5–8** by replacing an equatorial OH group with the O^- ion. This alteration resulted in an increase in the energy difference between the higher energy e–e chair form for the ring in **6** relative to the a–e boat form found in the ground-state TBP **5** from 8.7 kcal/mol to 10.8 kcal/mol. Hence, mechanistically it would be more difficult for an enzyme to accommodate an e–e ring orientation for hydrolytic cleavage in phosphodiesterase action of cAMP relative to an a–e orientation.

Calculations show that introduction of a *trans*-fused ribose ring in the position found from the X-ray analysis of **C** and **D** lead to a further increase of the energy of the structure with the ring in an e–e orientation (**8**) relative to the one with an a–e orientation (**7**). The energy difference here is 11.9 kcal/mol, about 1 kcal/mol greater than the difference for **6** and **5** lacking ribose attachments. It seems reasonable that *trans*-annelation acts as a constraint on the six-membered ring with a consequent increase of ring strain for **8**.

P–O Bond Length Comparisons. Table 2 shows average P–O bond distances from X-ray structures[18–20] on numerous cyclic

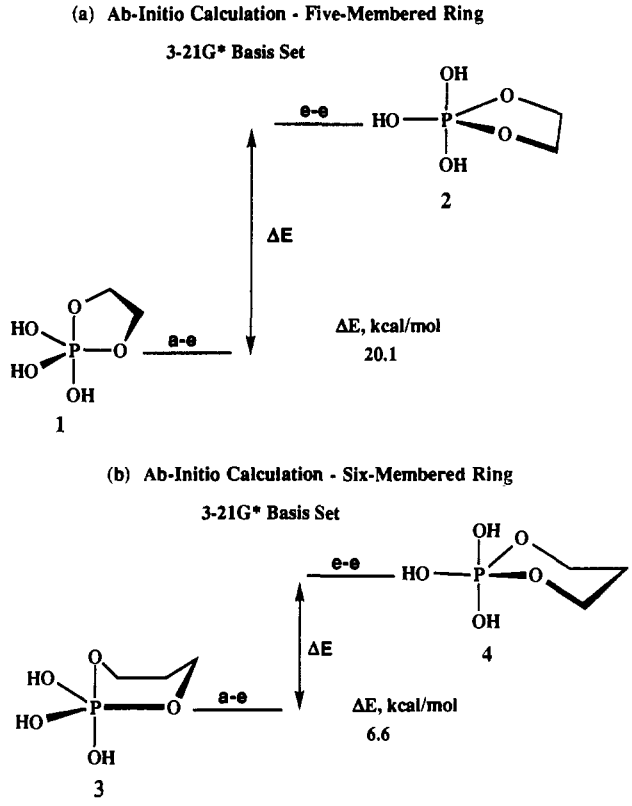

Figure 1. Computed relative energies, ΔE (kcal/mol), of the trigonal bipyramids (a) **2** minus **1** and (b) **4** minus **3**.

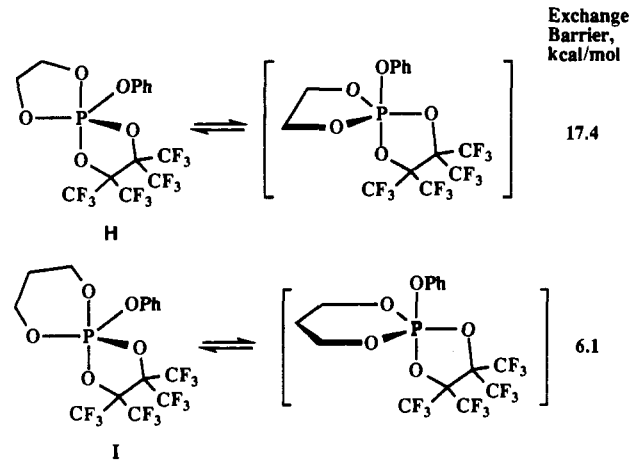

Figure 2. Intramolecular ligand exchange activation energies from VT ^{19}F NMR spectra.[26]

pentaoxyphosphoranes, footnotes *d–f*. These compare very well with computed values for minimum energy structures obtained at the 3-21G* level. Direct comparisons can be made with rings occupying a–e sites for **1** and **3**. The average values from four X-ray structures[18,20] containing five-membered rings are 1.716(4) Å for the axial P–O ring bond and 1.636(4) Å for the equatorial one. The calculated P–O ring values for **1** are 1.643 and 1.625 Å, respectively. For **3** with a six-membered ring, the experimental bond lengths average 1.636(4) Å for the axial P–O ring bond and 1.589(4) Å for the equatorial one obtained from six X-ray structures.[18–20] The respective P–O ring bond distances for the computed structure **3** are 1.631 and 1.612 Å. The agreement between experimental and calculated values is surprisingly close considering the X-ray structures are monocyclic[19] in some cases and bicyclic[18,20] in others and all contain xylyloxy groups compared to OH ligands used in the calculations.

In all cases, **1–8**, where comparisons can be made, the axial P–O bonds, as expected, are longer relative to like equatorial bonds whether the comparison involves ring P–O bonds or acyclic P–O bonds. The average experimental axial P–OXyl bond length is 1.665(6) Å, and the average equatorial one is 1.612(6) Å obtained from 10 X-ray structures.[18–20] This compares with those average values from the computed structures for **1–8** of 1.682 Å for the axial P–OH bond length and 1.608 Å for the equatorial P–OH bond.

If we leave out **6** and **8** containing e–e ring forms, the average axial P–OH bond distance drops to 1.667 Å. It is noted that introduction of the O^- ion in **5–8** results in significant lengthening of the P–O bonds, particularly for the axial P–OH bonds in **6** and **8**, relative to that computed for **4** and for the axial P–O ring bonds computed for **5** and **7** relative to that for **3**. It is reasoned that removal of a proton from an equatorial OH ligand should result in enhanced P–O bond lengthening in agreement with the *ab initio* calculations. The O^- ion acts as a group of low electronegativity and increases electron density at phosphorus. From VSEPR theory,[27] increased bond pair repulsions arise, especially between the P–O^- equatorial bond and the P–O axial bonds situated close to 90°. One might anticipate that stabilization of forms containing the P–O^- anion at active sites with accompanying lengthening of the axial bonds would activate P–O axial bond cleavage in the hydrolysis of cAMP at enzyme active sites in an in-line attack of a nucleophile such as that depicted in Scheme 1.

Conclusions. Considering the dissimilarity between the composition of the experimental and computed structures, agreement between relative energies from the two approaches for a–e and e–e ring orientations in TBP molecules and accompanying P–O bond distance comparisons is very good. The higher energies found for e–e ring orientations, relative to a–e ring occupancy, which difference increased on going successively to structures more closely related to actual enzyme site models for cAMP action, suggests the likelihood that a–e ring orientations in TBP intermediates represent preferred structures over e–e ring orientations. Also schemes of enzyme action invoking pseudorotation of cAMP intermediates where the ring rearranges to an e–e form seem unlikely.

Acknowledgment. The support of this research by the National Science Foundation (Grant CHE 91-22352) and the donors of the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged. We also thank the San Diego Supercomputer Center for generous allocation of computer time.

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