Tetrahedron Letters,Vol.30,No.17,pp 2195-2198,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

PENTACOVALENT PHOSPHORUS-CONTAINING MODEL OF A P(V) CYCLIC NUCLEOTIDE INTERMEDIATE. NON-CHAIR CONFORMATION OF THE PHOSPHORUS-CONTAINING RING

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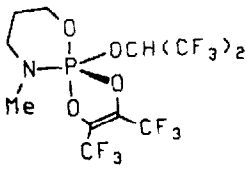
Summary: A cyclic nucleotide model system (**3**) for P(V) cAMP-substrate or cAMP-enzyme adducts is shown by ^{1}H NMR analysis to be totally in a twist conformation (**3b**).

The stereochemistry of hydrolysis of cAMP by phosphodiesterases (PDE) has been well-studied,[1] as have the structural requirements for the binding of cAMP to the active sites of both protein kinases and PDE's.[2] However, little if any attention has been given to possible changes in the conformation of the phosphate ring on binding or the potential conformations of the pentacovalent phosphorus P(V) enzyme-cAMP or substrate-cAMP adducts proposed as intermediates in the interactions of cAMP with both of these enzyme systems.[2,3]

Recently we showed that Karplus-like $^3J_{HP}$ coupling relationships are operative for P(V) coordination and that in solution the 1,3,2-oxazaphosphorinane ring of **1** populates a



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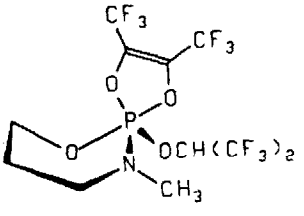

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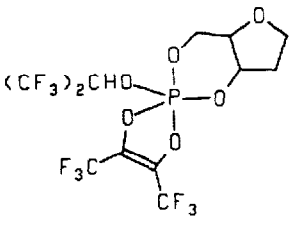

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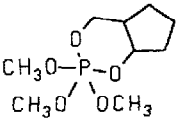

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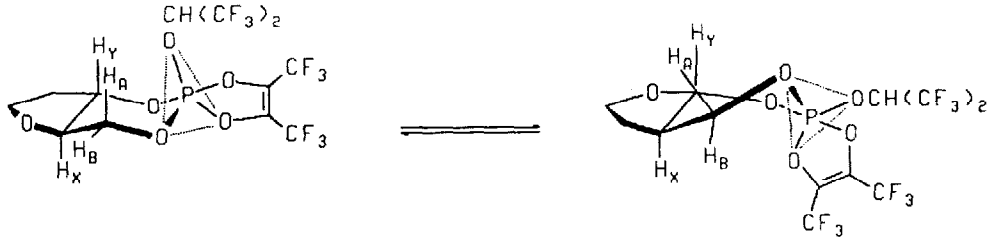
non-chair conformation approximated by structure **2**.[4] However, up to now assignments of conformation to P(V) 1,3,2-dioxaphosphorinane rings have not been made in solution. We report here that indeed the equilibrium for the system **3a** ⇌ **3b**, within experimental error, completely favors **3b**. This is of particular importance, because **3** serves as a model for cAMP itself. Thus the cyclic 3',5'-monophosphate diester corresponding to **3** has been shown[5] to possess the same 5 kcal/mole of ring strain, engendered by the trans ring fusion, attributed to cAMP which could strongly influence the conformational equilibrium.



3a **3b**

The chair-twist equilibrium for **3** is especially amenable to study as it can be readily assigned by inspection of the coupling constants J_{AP}, J_{BP}, and J_{AX}. The equatorial H_B and axial H_A in **3a** in effect exchange places and become, respectively, pseudoaxial and pseudoequatorial in **3b**. The usefulness of this approach has been amply demonstrated in studies of chair-twist equilibria for three[6a]- and four-coordinate[6b,7] analogues of **3** and with the corresponding thymidine-based cyclic nucleotide derivatives.[8]

Phosphorane **3** was readily prepared by reaction of the phosphite precursor[9] with a slight excess of hexafluorobiacetyl for 6 h at 0°. Use of starting phosphite of cis/trans ratio 11/1 (RO_{axial}/RO_{equat}) gave almost entirely a single diastereomer of highly pure **3** (^{31}P NMR), as evidenced by a major ^{31}P NMR resonance ($CDCl_3$) at δ = -49.7 and a very minor peak at δ = -49.1. Distillation gave **3** (bp, 62-63°, 0.05 mm) in >90% purity (^{31}P, ^1H, ^{13}C NMR) with the ^{31}P NMR peak ratio reduced to 2.3/1. The structures of both diastereomers of **3** were confirmed by GC/MS and ^{13}C NMR (Table I).[10] The chair-twist equilibrium shown is for the

Table I. Selected ^{13}C NMR Parameters for **3** at 75 MHz in $CDCl_3$ at 20 °C.[10b]

	δ ^{13}C (J_{CP}, Hz)					
Diastereomer	C1	C2	C3	C4	C5	$(CF_3)_2\underline{C}H$
Major	68.3(0)	28.8 (10.5)	79.1 (6.5)	73.9 (8.1)	69.4 (9.8)	73.8 (11.0)
Minor	68.0(0)	28.8 (10.5)	79.4 (6.4)	73.0 (9.1)	68.6 (9.1)	72.4 (12.6)

diastereomer which would result from the major (cis) phosphite if reaction with $CF_3COCOCF_3$ occurs with retention of configuration at phosphorus, as it seems reasonable to assume.

The 300 MHz ^1H and 121 MHz ^{31}P NMR spectra of the distilled mixture of the diastereomers of **3** yielded the parameters of Table II. The first-order analysis used is justified by the large chemical shift differences of the coupled hydrogens. The assignments of H_A and H_B are made unequivocally on the basis of the large J_{AX} value invariably observed in cyclic 3',5'-

Table II. Selected ^1H NMR Parameters for Diasteromers of **3** at 300 MHz in $CDCl_3$ at 22 °C.

Major δH_A, 4.13	Minor δH_A, 4.09	Major δH_B, 4.63	Minor δH_B, 4.52	Major δH_X, 3.81	Minor δH_X, 3.94	Major δH_Y, 4.38	Minor δH_Y, 4.51
J_{AP}: 26.5	29.8	J_{BP}: 2.6	~0	J_{XP}: ~0	~0	J_{YP}: ~0	~0
J_{AX}: 9.3	9.2	J_{BX}: 7.0	6.8	J_{XY}: 8.7	8.8		
J_{AB}: -10.0	-9.9						

monophosphates and also for three and four-coordinated derivatives similar to **3**,[6,7] as well as those derived from thymidine,[8] regardless of the amounts of chair and twist conformations populated. The unmistakable conclusion from the data of Table I is that the twist conformation is the overwhelmingly dominant conformer populated by both diastereomers. Indeed, J_{BP} for pseudoaxial H_B is very small, (2.6, ~0 Hz). By contrast, for pseudoequatorial H_A, J_{AP} is large (26.5, 29.8 Hz), while J_{AX} (9.3, 9.2 Hz) remains large. This combination of J_{AX}, J_{BX}, J_{AP} and J_{BP} is found for similar 3- and 4-coordinate phosphorus systems with major amounts of twist forms populated.[6-8] Dreiding models indicate that the strained five/six trans ring fusion strongly biases the phosphorus-containing ring towards a twist conformation.

The data of Table II do not provide an independent basis for assigning the attachment of the ring to phosphorus, i.e. apical/equatorial or equatorial/equatorial. However, Dreiding models make it quite clear that diequatorial attachment and the accompanying large, ~120° O-P-O bond angle would result in a conformation which closely resembles a half-chair and which would have very similar H_ACOP and H_BCOP dihedral angles and consequent coupling constants. This is totally inconsistent with the J_{AP} and J_{BP} values observed. Six-membered 1,3,2-dioxa- and 1,3,2-oxazaphosphorinane rings have been generally shown to be prefentially apical/equatorial in their attachment to phosphorus,[11] and we see no evidence to suggest that the strain of the five/six trans ring fusion or any other feature of **3** has altered this principle. Inspection of Drieding models reveals that in chair conformation **3a** the RO substituent is in close proximity to the H_Y substituent. (Internuclear distance, 1.5-2.0 Å.) We suggest that the resulting destabilization is an intrinsic feature common to 1,3,2-dioxa- and oxazaphosphorinanes with apical/equatorial rings and that their normal conformation is a non-chair form such as **3b**.[12] In **3** the apical ring oxygen corresponds to the 3'-oxygen of cAMP as required for the hydrolytic cleavage of that bond by PDE to give 5'-AMP.

The findings for model system **3** do not mean that P(V) adducts of cAMP could not give the phosphorus-containing ring attached eq/eq if indeed coordination to the enzyme somehow made two of the substituents strongly apicophilic. However, until other evidence is available, it seems reasonable to think of P(V) enzyme-cAMP or substrate-cAMP adducts in terms of structure **3b**.[6]

Acknowledgement. We thank the N.C.I. of the P.H.S. for support of this work (Grant CA 11045).

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(Received in USA 11 November 1988)