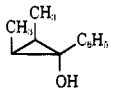
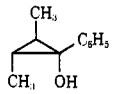
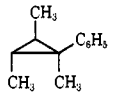
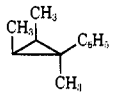
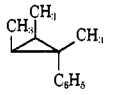
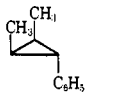
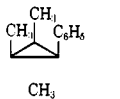
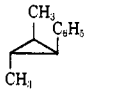
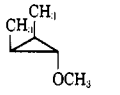
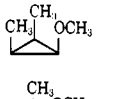
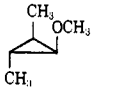
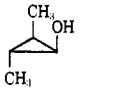
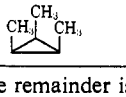
by reduction and synthesis of the resultant ether making use of the Cram–Karabatsos rules (eq 3). The stereochemical results of the ring openings are shown in Table I.

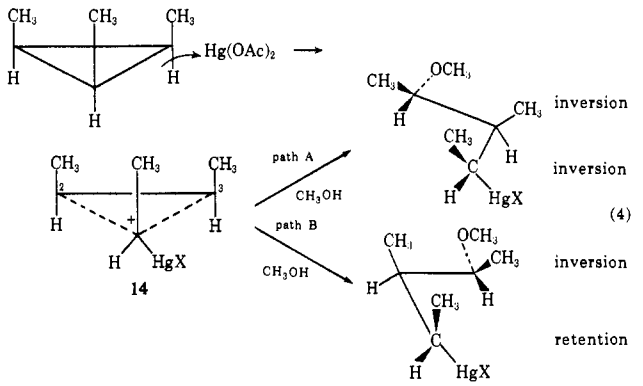
Table I. The Stereochemistry of Cyclopropane Ring Opening with Electrophilic Mercury

Compound	No.	Stereochemistry of electrophilic attack (% inversion)[a]	Stereochemistry of nucleophilic attack (% inversion)[a]
	**1**	100[b]	
	**2**	100[b]	
	**3**	100	100
	**4**	100	100
	**5**	100	100
	**6**	72	75
	**7**	82	91
	**8**	12	90
	**9**	95	
	**10**	40	
	**11**	10	
	**12**	<5	
	**13**	62	100

[a] The remainder is inversion. [b] Reference 3.

The pattern of stereochemistry observed is consistent with a mechanism in which mercuric acetate attacks the least substituted bond in the molecule, with ring opening occurring in the direction of the most stable carbonium ion. If bonds are equally substituted, then a bond with cis substituents is more easily attacked than one with trans substituents. Thus attack at the C2–C3 bond with ring opening to the benzylic cation gives exclusively inversion for electrophilic attack on **1–5**. Compounds **8**, **11**, and **12**, in which the C2–C3 bond is trans, give retention by attack on the disubstituted cis bond. In each case where the stereochemistry of the nucleophile can be determined, it occurs nearly exclusively with inversion of configuration.

In *cis,cis*-1,2,3-trimethylcyclopropane (**13**) all ring bonds are identically substituted, and so the stereochemistry of the reaction of this molecule with mercuric trifluoroacetate in methanol can give insight into the events occurring after attack on one of the ring bonds. We find that its reaction with the electrophile occurs with 62% inversion and 38% retention of configuration and with 100% inversion of configuration by the nucleophile. We believe these results are best accounted for by edge attack on the ring[3] leading to a corner-mercurated cyclopropane ring **14** (eq 4). Attack on **14** by



(4)

solvent from the rear of C2 or C3 leads naturally to nearly equal amounts of electrophilic inversion (path A) or retention (path B), respectively. Further studies with the other electrophiles are in progress.

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Biochemical Importance of the Binding of Phosphate by Arginyl Groups. Model Compounds Containing Methylguanidinium Ion

Sir:

The number of ways in which phosphate esters participate crucially in life processes is legion. We report here some observations on the way in which phosphate groups are bound by guanidinium ions, including those constituting the side chains of arginyl residues in the enzyme *Staphylococcus* nuclease, which we believe to have fundamental importance and possibly wide relevance in structural biochemistry.

In the course of refinement and interpretation of the structure of the ternary *Staph.* nuclease–thymidine di-

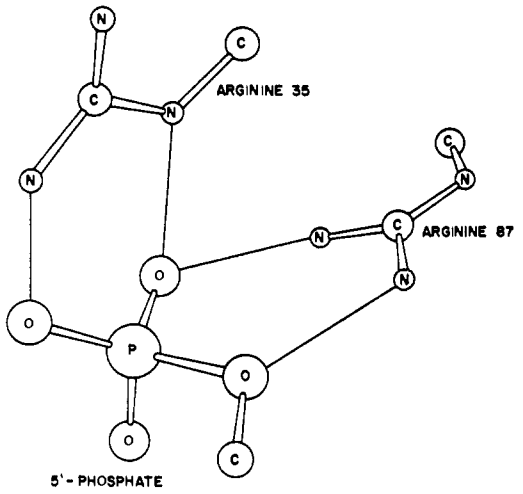

Figure 1. The interaction of the guanidino groups of Arg-35 and Arg-87 with the 5′-phosphate group in the complex of *Staph.* nuclease with thymidine 3′,5′-diphosphate and calcium ion. See ref 1 for additional details.

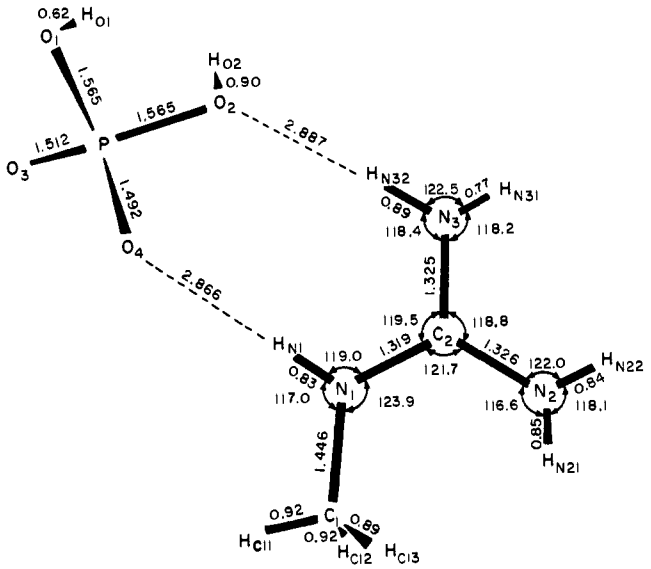

Figure 2. A diagram showing the hydrogen bond interactions between methylguanidinium (MGD) ions and $H_2PO_4^-$ ions in $(MGD)H_2PO_4$.

phosphate–calcium complex[1] we have observed that the guanidino groups of Arg-35 and Arg-87 play a vital and specific role in binding the inhibitor by their H-bonding interactions with the 5′-phosphate group. Figure 1 shows these interactions.

The resolution and accuracy attainable in the structure of a medium size protein are limited. Thus, in order to get a more accurate and detailed view of this type of interaction, a model system was sought. Arginine phosphate was passed over since it was considered likely to have a very complex and perhaps entirely uninformative structure; this in fact is the case.[2,3] Instead, it was decided to examine one or more methylguanidinium (MGD) phosphates.

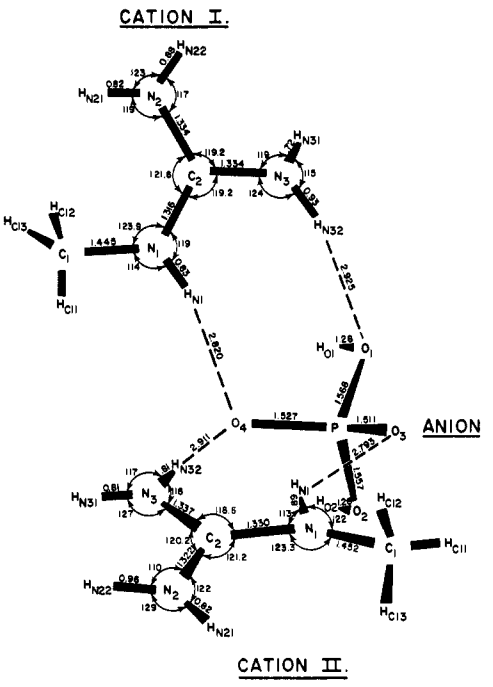

Figure 3. The interaction of the two methylguanidinium (MGD) ions with the HPO_4^{2-} ion in $(MGD)_2HPO_4$. The atoms denoted H_{O1} and H_{O2} are half-hydrogen atoms owing to a disorder required by the twofold axis.

The first compound investigated crystallographically was $(MGD)H_2PO_4$. It did prove to be a model (Figure 2), albeit a somewhat inexact one, for the arginine–phosphate binding in the enzyme. The second compound, $(MGD)_2HPO_4$, is a remarkably close facsimile of the enzymic system; the relevant part of its structure is shown in Figure 3. In all three structures it is clear that the guanidino group is remarkably well suited by its own structure to interact strongly through two hydrogen bonds with the phosphate group.

In *Staph.* nuclease the guanidino groups appear to have a function beyond that of merely binding inhibitor or substrate, important though that undoubtedly is. The 5′-phosphate group of the inhibitor evidently occupies the same position as the phosphate group which is hydrolytically severed from the sugar in the actual substrate.[4] The important additional function of Arg-35 and Arg-87 is to accelerate the hydrolysis reaction. This activity may be described in either of two complementary ways. The two guanidino groups neutralize negative charge on the phosphate and partially polarize it by drawing negative charge from the phosphorus atom, thus facilitating nucleophilic attack at that atom. Alternatively, the multiple H-bond interactions can be thought of as lowering the energy of a 5-coordinate transition state formed by attack of OH^- on P (the pH for maximum rate, 8.4, suggests OH^- rather than H_2O attack).[1,4]

The effect of guanidino groups on phosphate has been studied theoretically by calculations using the SCF $X\alpha$ scattered wave method.[5] Calculations were made for

(1) A. Arnone, C. J. Bier, F. A. Cotton, V. W. Day, E. E. Hazen, Jr., D. C. Richardson, J. S. Richardson, and A. Yonath, *J. Biol. Chem.*, **246**, 2302 (1971); F. A. Cotton, C. J. Bier, V. W. Day, E. E. Hazen, Jr., and S. Larsen, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 243 (1971).

(2) K. Aoki, K. Nagano, and Y. Iitaka, *Acta Crystallogr., Sect. B*, **27**, 11 (1971).

(3) W. Saenger and K. G. Wagner, *ibid.*, **28**, 2237 (1972).

(4) C. B. Anfinsen, P. Cuatrecasas, and H. Taniuchi, *Enzymes, 3rd Ed.*, **4**, 177 (1971).

(5) For accounts of this method see (a) J. C. Slater and K. H. Johnson, *Phys. Rev. B*, **5**, 844 (1972); (b) K. H. Johnson, J. G. Norman, Jr., and J. W. D. Connolly in "Computational Methods for Large Molecules and Localized States in Solids," F. Herman, A. D. McLean, and R. K. Nesbet, Ed., Plenum Press, New York, N. Y., 1972.

PO_4^{3-}, HPO_4^{2-}, $C(NH_2)_3^+$, and an aggregate $[HPO_4 \cdot 2C(NH_2)_3]$ having a structure similar to those in Figures 1–3. The results buttress the notion that the guanidino–phosphate interactions decrease negative charge on phosphate (all orbitals on HPO_4^{2-} are significantly down-shifted) and redistribute it so as to render phosphorus more positive (by *ca.* 0.6 e$^-$).

In addition to the particular example of the structural and kinetic importance of phosphate binding by arginyl residues afforded by *Staph.* nuclease, we suggest that similar interactions may have importance elsewhere. Whenever phosphate-containing molecules are bound to proteins the possibility of arginyl involvement may exist though it does not always occur.[6,7] For the nucleoproteins,[8] especially the protamines with their sequences of consecutive arginyl residues and the arginine-rich histones, binding to DNA may well involve Arg–phosphate interactions of the type we have observed. Other instances may occur in the binding of ATP and various enzyme cofactors to proteins and enzymes as well as in the enzyme activity of other nucleases, phosphatases, or in phosphate synthetases.

The ability of guanidino groups to bind phosphates may be important for other enzymes. For example, chemical modification of alkaline phosphatase of *E. coli* with α-dicarbonyl compounds (*e.g.*, 2,3-butanedione, phenylglyoxal) has demonstrated the presence of a functional arginyl residue.[9] Such modifications inactivate the enzyme and a competitive inhibitor, phosphate, prevents the inactivation. The data are consistent with the essential role of an arginyl residue in the enzymatic mechanism of alkaline phosphatase, possibly as a binding site for the negatively charged phosphate group of the substrate.[10]

(6) F. M. Richards and H. W. Wyckoff, *Enzymes, 3rd Ed.*, **4**, 647 (1971).
(7) A. Arnone, *Nature (London)*, **237**, 146 (1972).
(8) D. M. P. Phillips, Ed., "Histones and Nucleohistones," Plenum Press, New York, N. Y., 1971; S. C. R. Elgin, S. C. Froehner, J. E. Smart, and J. Bonner, *Advan. Cell Mol. Biol.*, **1**, 1 (1971).
(9) Private communication from F. Daemen, F. Riordan, and B. L. Vallee.
(10) This research was supported by the National Institutes of Health under Grant No. GM-13300.

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Double Resonance Experiments Involving Coupled Quadrupolar Nuclei. I. Boron–Boron Coupling in 6-Methyldecaborane(14)

Sir:

We are interested in making ^{11}B chemical shift assignments in the nmr spectra of substituted boron cage compounds with the ultimate goal being direct structure assignment from nmr parameters. Although specific deuteration and selective ^1H–^{11}B double resonance studies have led to some assignments, in many cases ambiguities do remain. It seemed to us that selective ^{11}B–^{11}B double resonance experiments should give the bonding information we seek and perhaps obviate the need for selective deuteration experiments.

A requirement for the success of the proposed experiment is a finite spin–spin coupling between bonded boron atoms which is not relaxed by the nuclear quadrupole moment of the boron atoms. Odom[1] has recently shown that such couplings do exist, and Allerhand[2] has found that ^{11}B relaxation times in a few higher cage compounds are long enough to allow the observation of spin–spin coupling between boron atoms. That such couplings are not evident in the spectra of many compounds is attributable to the extreme complexity of the nmr spectrum of more than a few spins when the spin quantum numbers are greater than unity. In order to illustrate the case in point and in order to build a foundation for the following discussion, we sketch the derivation of the matrix elements of the Hamiltonian for a system of two nuclei, each with spin = $^3/_2$.

If we assume the usual high-resolution nmr Hamiltonian for two spins[3]

$$\mathcal{H} = (\gamma/2\pi)[H_1I_z(1) + H_2I_z(2)] + J_{12}I(1)\cdot I(2)$$

where H is the local field, J is the coupling constant, and I is the spin operator, and choose the products of the spin functions as our basis, we can write down the matrix of the Hamiltonian in the manner given by Pople, Schneider, and Bernstein.[3] In order to do so we need the matrix elements for I for spin = $^3/_2$. From the expressions given by Davydov[4] for an angular momentum operator, we find those elements to be as shown in eq 1.

$$I_x = h/4\pi \begin{array}{c|cccc} & \alpha & \beta & \gamma & \delta \\ \hline \alpha & 0 & \sqrt{3} & 0 & 0 \\ \beta & \sqrt{3} & 0 & 2 & 0 \\ \gamma & 0 & 2 & 0 & \sqrt{3} \\ \delta & 0 & 0 & \sqrt{3} & 0 \end{array}$$

$$I_y = ih/4\pi \begin{array}{c|cccc} & \alpha & \beta & \gamma & \delta \\ \hline \alpha & 0 & -\sqrt{3} & 0 & 0 \\ \beta & \sqrt{3} & 0 & -2 & 0 \\ \gamma & 0 & 2 & 0 & -\sqrt{3} \\ \delta & 0 & 0 & \sqrt{3} & 0 \end{array} \quad (1)$$

$$I_z = h/4\pi \begin{array}{c|cccc} & \alpha & \beta & \gamma & \delta \\ \hline \alpha & 3 & 0 & 0 & 0 \\ \beta & 0 & 1 & 0 & 0 \\ \gamma & 0 & 0 & -1 & 0 \\ \delta & 0 & 0 & 0 & -3 \end{array}$$

With the aid of the usual selection rules we find the diagonal elements of the Hamiltonian matrix to be given by the following equation

$$H_{kk} = (\gamma/2\pi)(H_1m_{1k} + H_2m_{2k}) + J_{12}m_{1k}m_{2k}$$

(1) J. D. Odom, P. D. Ellis, and H. C. Walsh, *J. Amer. Chem. Soc.*, **93**, 3529 (1971).
(2) A. Allerhand, J. D. Odom, and R. E. Moll, *J. Chem. Phys.*, **50**, 5037 (1969).
(3) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959, pp 103–115.
(4) A. S. Davydov, "Quantum Mechanics," NEO Press, Ann Arbor, Mich., 1966, p 144.