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]

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F. A. Cotton,* E. E. Hazen, Jr.
Department of Chemistry, Texas A&M University
College Station, Texas 77843

V. W. Day, S. Larsen
J. G. Norman, Jr., S. T. K. Wong
Department of Chemistry, Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

K. H. Johnson
Department of Metallurgy and Materials Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

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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.

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

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

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

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

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

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(2) A. Allerhand, J. D. Odom, and R. E. Moll, *J. Chem. Phys.*, 50, 5037 (1969).
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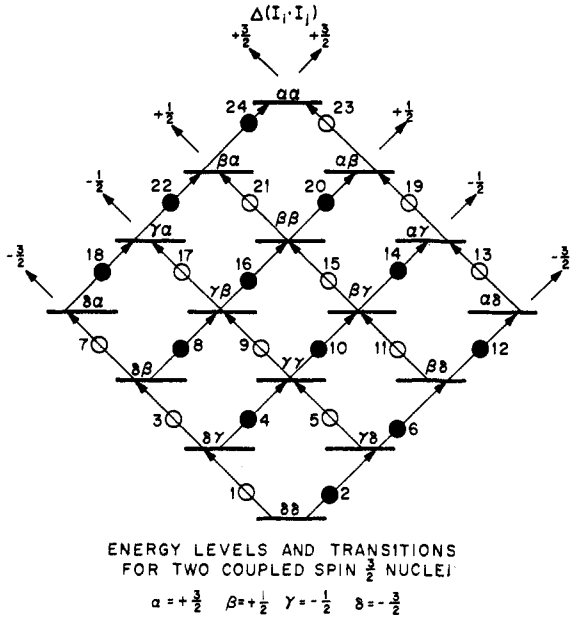

Figure 1. Energy levels and transitions for two coupled spin $^3/_2$ nuclei.

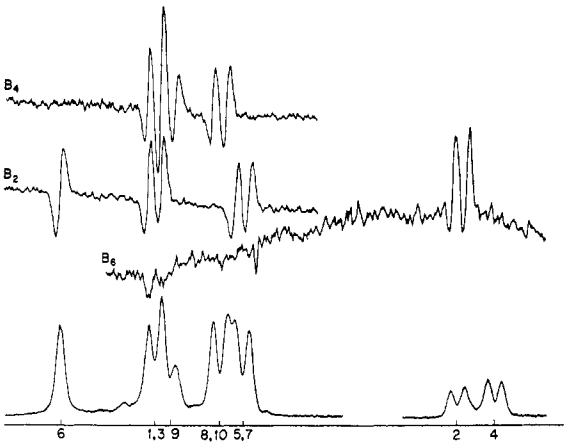

Figure 2. ^{11}B nmr and double resonance spectra of 6-methyldecaborane(14). The boron which was irradiated with H_1 while H_2 was swept through the spectrum is noted at the left side of each double resonance trace. Assignments are indicated below the single resonance spectrum. Beat signals between H_1 and H_2 are deleted.

where m_{nk} is the magnetic quantum number of the nth spin in the kth basis function in units of $\hbar$. The off-diagonal elements may be evaluated in the usual way;[3] however, all nonzero elements are of the order of $J\hbar^2$ and may be neglected in many cases of practical interest. In the approximation of $\delta \gg J$, the nmr spectrum of two inequivalent boron atoms consists of two groups of four triply degenerate lines.

If we limit ourselves to the discussion of double resonance effects due to small perturbing fields, we need consider only those effects arising from perturbation of the population of the states connected by the perturbed transition.[5] Because all transitions in the system under consideration are of equal intensity, the perturbations will be equal in magnitude. Inspection of the energy level diagram for two nuclei with spin = $^3/_2$ (Figure 1) reveals that only the outermost lines in the spectrum of spin 1 will suffer a net perturbation in intensity when the transitions due to spin 2 are perturbed by a second radiofrequency field. Furthermore, that net intensity perturbation occurs when the outermost transitions of spin 2 are perturbed. We therefore do not expect to see an effect on the central lines in a group of transitions, due to cancellation of intensity changes. In more complicated spin systems of this type we would not expect perfect cancellation but would expect to see the largest net changes in the intensities of the outermost lines in a group of transitions.

To test our predictions and at the same time demonstrate the utility of the method, we have chosen to present the double resonance spectra obtained with 6-methyldecaborane(14). The ^{11}B nmr spectrum (Figure 2) shows the singlet due to B_6 at low field, three area 1 doublets (B_2, B_4, and B_9), and three area 2 doublets ($B_{5,7}$, $B_{1,3}$, and $B_{8,10}$). Although a nearly complete assignment of chemical shifts can be proposed by analogy with decaborane(14), distinctions between $B_{5,7}$ and $B_{8,10}$ and between B_2 and B_4 cannot be made. In fact, $B_{1,3}$, $B_{5,7}$, and $B_{8,10}$ are all doublets of area 2 in a relatively narrow region and cannot be unambiguously distinguished. However, intensity considerations and three double resonance spectra (Figure 2) suffice to assign all ^{11}B chemical shifts in the observed spectrum. The area 1 doublet which shows coupling to the singlet B_6 and to two area 2 doublets is assigned to B_2. The area 1 doublet which shows coupling to two area 2 doublets and one area 1 doublet is assigned to B_4. The area 2 doublet which is coupled to both B_2 and B_4 must be assigned to $B_{1,3}$. The area 2 doublet coupled to B_2 but not to B_4 must be $B_{5,7}$ and the area 2 doublet coupled to B_4 but not to B_2 must be $B_{8,10}$. The remaining area 1 doublet must be B_9. Note that no coupling is detected between $B_{5,7}$ and B_6. We presume that the small single bond character of the three center B_5–H–B_6 bond leads to a substantial reduction of the coupling constant, making the double resonance effect undetectable under the present conditions.

Experiments of a similar nature were performed on a number of substituted decaboranes with similar results and will be discussed in a future paper. All double resonance spectra shown are the averages of 512 scans. The power in H_2 was adjusted to give optimum signal shape but was always comparable to the power required to obtain optimum signal to noise in the normal spectrum. All spectra were obtained at 80.2 MHz on saturated solutions contained in standard 5-mm sample tubes. The homogeneity was always adjusted to give better than 1 Hz resolution on an aqueous solution of $NaBH_4$ before accumulation was started.

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Richard F. Sprecher
Department of Chemistry, Carnegie-Mellon University
Pittsburgh, Pennsylvania 15213

James C. Carter*
Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15213

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