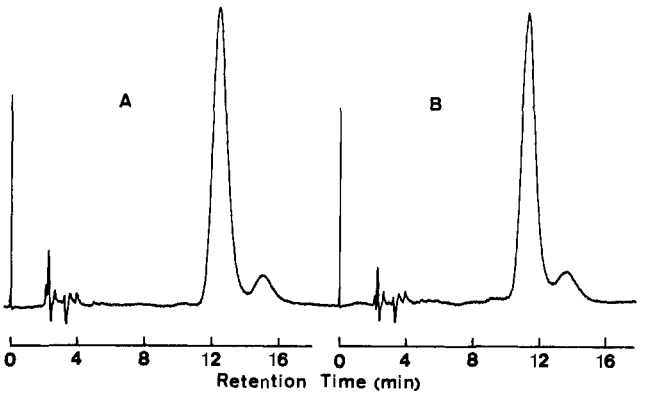

Figure 2. LC of insulins: A, porcine insulin; B, semisynthetic human insulin; column, Nucleosil $5C_{18}$, 0.4 × 20 cm; eluant, 30% CH_3CN in 5 mM tartrate buffer (pH 3.0) containing 5 mM *n*-$BuSO_3Na$ and 50 mM Na_2SO_4; detection, at 220 nm.

the desired human insulin was obtained by lyophilization (51 mg), and from another peak pure DOI was recovered in the unprotected form (37 mg). By rechromatography of the overlapping portion of the two peaks, an additional quantity of insulin was isolated, giving a total yield of 56 mg (49%). Amino acid analysis (theoretical values in parentheses):[15] Lys, 0.97 (1); His, 2.10 (2); Arg, 1.00 (1); $CySO_3H$, 5.75 (6); Asp, 3.03 (3); Thr, 2.92 (3); Ser, 2.87 (3); Glu, 7.10 (7); Pro, 1.32 (1); Gly, 4.08 (4); Ala, 1.07 (1); Val, 3.91 (4); Ile, 1.56 (2); Leu, 6.00 (6); Tyr, 3.02 (4); Phe, 2.88 (3).

Polyacrylamide gel electrophoresis and LC (Figure 2) showed that the semisynthetic insulin obtained above was as pure as crystalline porcine hormone used as starting material.[16] The sufficient purity was further confirmed by crystallization. In the assay for hypoglycemic activity in normal mice, no significant difference was found between the semisynthetic material and bovine insulin.[17] The identity of this material with natural human hormone[18] was evidenced by careful LC. Thus, a simple procedure for conversion of porcine insulin into human insulin by the two-way use of trypsin has been established. The enzymatic method developed for the coupling of DOI with octapeptide has many advantages over the existing chemical methods,[1,2] especially in (1) the reaction, which is highly specific and free of racemization; (2) the starting materials, which can be recovered for reuse; and (3) better yields and simple operation.

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(7) Aliquots from the reaction mixture were acidified, lyophilized, and treated for deprotection with TFA/anisole. After removal of the TFA the residue was analyzed on an amino acid analyzer for Lys and Lys-Val (or Arg and Arg-Val), from which the extent of peptide bond formation was calculated according to $[b/(a+b)] \times 100$ (%), where *a* stands for μmol of Lys (or Arg) and *b* for μmol of Lys-Val (or Arg-Val).
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(14) Octapeptide I: $[\alpha]^{25}_D$ −22.2 ± 0.8° (*c* 0.5, acetic acid). II: $[\alpha]^{25}_D$ −31.1 ± 0.5° (*c* 0.8, acetic acid).
(15) A sample for analysis was hydrolyzed with 6 M HCl at 110 °C for 24 h after periodate oxidation.
(16) For further purification a sample of the semisynthetic insulin was chromatographed on a QAE-Sephadex A-25 column according to Schlichtkrull et al: J. Schlichtkrull, J. Brange, A. H. Christiansen, O. Hallund, L. G. Heding, and K. H. Jørgensen, *Diabetes*, **21** (Suppl. 2), 649–656 (1972). The material isolated from a major peak was found to be homogeneous in electrophoresis and in LC.
(17) Lot 800147, Calbiochem, 27.17 units/mg.
(18) An authentic sample of human insulin (Lot 615-D63-29-C) was kindly supplied by Dr. R. E. Chance, Lilly Research Laboratories.

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Phosphoranes as Intermediates in the Acid Hydrolysis of Acyclic Phosphonate Esters: Evidence from Oxygen Exchange

Sir:

Convincing evidence places phosphoranes in the pathway for the hydrolysis of cyclic esters of phosphoric, phosphonic, and phosphinic acids.[1,2] However, phosphoranes with small rings form more readily than do their acyclic analogues,[3] so that some doubt had persisted as to whether the hydrolysis of acyclic esters of acids of phosphorus might not proceed by way of trigonal-bipyramidal transition states rather than by way of phosphoranes as intermediates. We now report that, when diphenyl methylphosphonate is heated with acid in 60:40 dimethoxyethane (DME)–water, exchange of oxygen with solvent takes place at ~8% of the rate of hydrolysis. This finding provides strong evidence for a phosphorane intermediate.

Diphenyl methylphosphonate enriched in ^{18}O was prepared by hydrolyzing methyltriphenoxyphosphonium triflate.[4] The labeled ester melted at 35.5–37 °C and showed an identical ^1H NMR spectrum with that of an unenriched sample.[5,6] Exchange during hydrolysis was measured mass spectrometrically; unhydrolyzed ester was recovered by ether extraction. The large increase in the peak at *m/e* 250 (the molecular ion for ester that contains ^{18}O) that occurred during hydrolysis of unlabeled ester with 71% $H_2{}^{18}O$ is shown in Figure 1. The decrease in the peak at *m/e* 250 that occurred during hydrolysis of labeled ester with ordinary water is shown in Figure 2. When the reaction is begun by hydrolyzing unlabeled ester in $H_2{}^{18}O$,

$$k_{ex}/k_h = \ln [1 - (E^*)/(E)]/\ln (E)/(E_0) \qquad (1)$$

where k_{ex} is the rate constant for exchange, k_h is that for hydrolysis, (E) is the total concentration of ester, (E_0) is its initial

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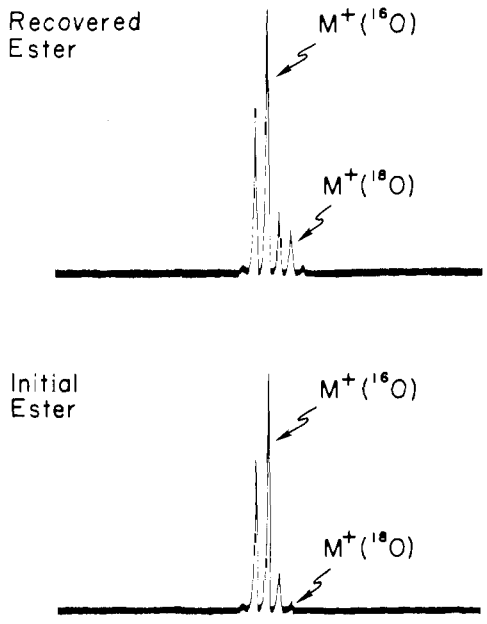


Figure 1. Mass spectra of diphenyl methylphosphonate, initially and after hydrolysis for 3 half-lives in 60:40 DME–71% $H_2{}^{18}O$ at 110 °C with 1 M triflic acid.

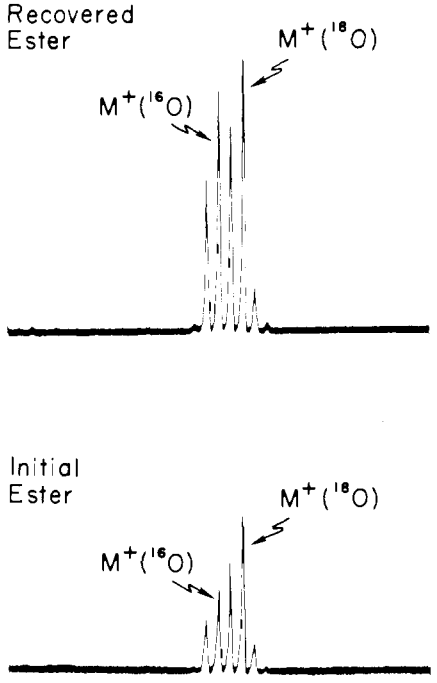


Figure 2. Mass spectra of ^{18}O-labeled diphenyl methylphosphonate, initially and after hydrolysis for 4 half-lives in 60:40 DME–H_2O at 110 °C with 1 M triflic acid.

concentration, and (E*) is the concentration of labeled ester. When the reaction is begun by hydrolyzing labeled ester in normal water, then

$$k_{ex}/k_h = \ln [(E^*)(E_0)/(E)(E^*_0)]/\ln (E)/(E_0) \quad (2)$$

The rate constants for the first step in the hydrolysis of diphenyl methylphosphonate, needed to calculate the data in Table I, are available elsewhere.[7]

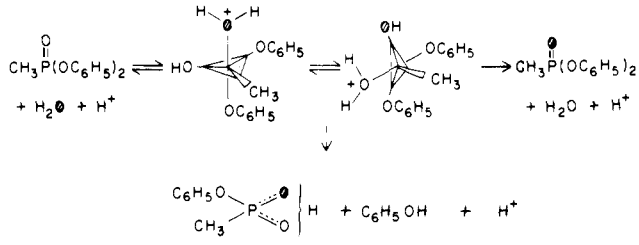
Similar experiments with labeled ester (0.001 M) were carried out in 90% water–10% dioxane at 100 °C for 1 to 2 half-lives in the presence of 1–4 M hydrochloric acid, with and without added phenol (0.008 M). The results for all of these experiments showed that the exchange takes place at ~4% of the rate of hydrolysis; the exchange was unmistakable and reproducible.

Table I. Hydrolysis of Diphenyl Methylphosphonate (0.02 M) in 60:40 DME–H_2O at 110 °C in the Presence of 1 M Triflic Acid

ester	excess % ^{18}O in water	half-lives	excess % ^{18}O initial	excess % ^{18}O final	k_{ex}/k_{hyd}
^{16}O	71	3	(0.00)	11.9 ± 0.5	0.088 ± 0.004
^{18}O	(0.00)	4	67.5	53.8 ± 0.4	0.082 ± 0.003
^{18}O	(0.00)[a]	2	67.5	59.6 ± 0.2	0.086 ± 0.003

[a] 0.09 M phenol added.

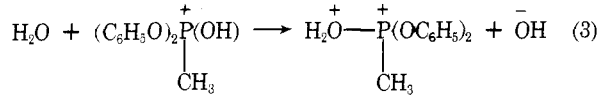
Scheme I



Exchange of oxygen atoms between the solvent and the ester can most readily be explained by the mechanism shown in Scheme I.

This scheme involves acid-catalyzed addition of water to the ester, subsequent pseudorotation, and acid-catalyzed loss of water containing a different oxygen atom, all in accordance with the rules[1] for the addition and loss of nucleophiles to and from apical positions of trigonal-bipyramidal phosphoranes. The detailed pathway for the proton transfer is at present unknown; similarly, the lifetimes of the postulated intermediates cannot yet be specified, although we believe them to be short.[7]

Although this mechanism is highly probable, others must also be considered. A direct displacement from a protonated ester would require that a water molecule eject a hydroxide ion and leave behind a dication:



This process is sufficiently unlikely to be disregarded. Alternatively, if a proton were lost from the incoming water molecule simultaneously with the attack, the data for hydrolysis and exchange would require that water displace hydroxide ion $^1/_{12}$ as rapidly as it displaced phenoxide ion; besides this process and the one above are not microscopically reversible. Furthermore, since the rates of hydrolysis and exchange are unaffected by added phenol, exchange cannot arise from hydrolysis followed by acid catalyzed reesterification.

Several esters of acids of phosphorus had previously been hydrolyzed without observing significant oxygen exchange.[8-10] In the hydrolysis of *p*-nitrophenyl diphenylphosphinate,[9] however, both aryl groups will preferentially occupy equatorial positions,[1] so that the pseudorotation that necessarily precedes exchange can occur only by forcing one of them to become apical. The reason why a trigonal bipyramid from triphenyl phosphate[10] would not pseudorotate is less obvious. It may however depend on a difference in apicophilicities between phenoxy and hydroxy residues, which could impose a small barrier to pseudorotation; no such difficulty arises with diphenyl methylphosphonate, where the pseudorotation shown in Scheme I is a virtual process.

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A Copper(I) Derivative of a Ferraborane: Preparation, Crystal, and Molecular Structure of $Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3$

Sir:

Ferraborane analogues of hexaborane(10) represent a relatively new[1-3] and unexplored group of *nido*-metalloboranes[4] which are structural analogues[5] and isoelectronic[5,6] (in terms of electron counting rules) with B_6H_{10}.[7] We have prepared the bimetallic compound $Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3$, a derivative of $B_5H_9Fe(CO)_3$,[1] and have determined its crystal and molecular structure.

This compound is of special interest since it is unlike previously reported systems in which a metal is inserted into the basal boron–boron bond of a nido pyramid. In systems such as $Cu[P(C_6H_5)_3]_2B_5H_8$[8,9] and $Fe(CO)_4B_7H_{12}^-$ [10a] the metal replaces a bridge proton and in effect acts as a "pseudo" proton in accepting an electron pair from the boron–boron bond to form a three-center B–metal–B bond. The metal in its bridging position resides well below the basal plane of the pyramid, 1.5[9b] and 1.7 Å,[10b] respectively, for $Cu[P(C_6H_5)_3]_2B_5H_8$ and $Fe(CO)_4B_7H_{12}^-$, and there is no apparent metal–H–B bonding. In the present case, however, the inserted copper is relatively elevated (0.43 Å beneath the basal plane), not only giving a more open arrangement at the bonding site than previously observed for a metal inserted into a nido pyramid, but also bringing the copper within bonding distance of the exo hydrogen on at least one of the boron atoms adjacent to the metal. Figure 1 illustrates the coordination sphere around copper and iron in $Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3$, $Fe(CO)_3B_7H_{12}^-$,[11] and $Cu[P(C_6H_5)_3]_2B_5H_8$.[11]

The relatively "open" or "slipped" arrangement of copper at the bonding site in $Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3$ is associated with the existence of Cu–H–B bonding.[12] In terms of the formalism of electron counting rules,[7] copper can be considered to be a vertex of the cluster, with the $Cu[P(C_6H_5)_3]_2$ unit contributing two skeletal electrons. In such terms the molecule is a formal analogue of B_7H_{11}, a long sought boron hydride.[15] It is not clear, however, that this extrapolation of electron counting rules is warranted. On the other hand, in the case of $Cu[P(C_6H_5)_3]_2B_5H_8$, the molecule is clearly an analogue of B_5H_9. Since there is no Cu–H–B bridge, the $Cu[P(C_6H_5)_3]_2$ unit can be treated, formally, as a replacement for a proton, thereby contributing no electrons to the skeletal electron count.

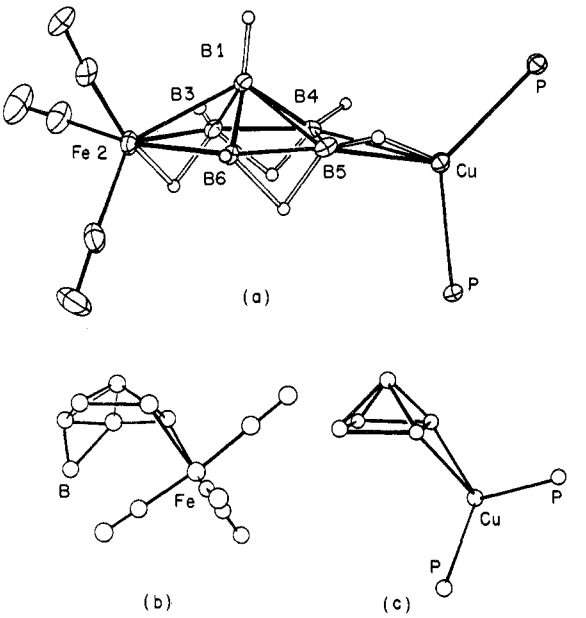

Figure 1. Comparison of the structure of $Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3$ (a) with the skeletal structure of $Fe(CO)_4B_7H_{12}^-$ (b) and $Cu[P(C_6H_5)_3]_2B_5H_8$ (c).

From the sequence of reactions

$$B_5H_9Fe(CO)_3 + KH \xrightarrow[-78\ °C]{(CH_3)_2O} K^+B_5H_8Fe(CO)_3^- + H_2 \quad (1)$$

$$K^+B_5H_8Fe(CO)_3^- + Cu[P(C_6H_5)_3]_3Cl \xrightarrow[-45\ °C]{(CH_3)_2O} Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3 + KCl + P(C_6H_5)_3 \quad (2)$$

the compound $Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3$ was prepared and isolated. It is a yellow solid which is apparently stable at room temperature in the absence of air. It does not appear to be as photosensitive as its precursors $K^+B_5H_8Fe(CO)_3^-$ and $B_5H_9Fe(CO)_3$.[1]

Crystals of $Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3$ were grown by vapor diffusion of *n*-pentane into a toluene solution at 0 °C. They are triclinic (space group $P\bar{1}$); a = 11.113 (5), b = 14.670 (8), c = 15.034 (7) Å; α = 95.60 (4), β = 121.01 (3), δ = 106.29 (4)°; Z = 2; d_{calcd} = 1.36, d_{exptl} = 1.34 g cm^3. All X-ray data was collected at −90 °C.[16]

Bond distances in the borane unit of $Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3$ are normal:[19] B–B = 1.662 (11) to 1.783 (9), B–$H_{terminal}$ = 1.02 (7) to 1.13 (7), B–H_{bridge} = 1.13 (7) to 1.30 (8) Å. Structural parameters for the $B_5H_8Fe(CO)_3$ unit are in excellent agreement with those observed for $B_5H_8Fe(CO)_3^-$.[1a] Iron–boron distances are Fe(2)–B(1) = 2.154 (7), Fe(2)–B(3) = 2.115 (10); Fe(2)–B(6) = 2.075 (7) Å. The Fe(2)–H(23) distance = 1.56 (6) Å. The site of copper insertion, the B(4)–B(5) bond is 1.662 (11) Å. Interestingly, this site is not the site of the boron–boron bond (B(3)–B(4)) in the parent anion $B_5H_8Fe(CO)_3^-$.[1] The significant difference in copper–boron distances is indicative of unsymmetrical binding of copper to the B(4)–B(5) system: Cu–B(4) = 2.274 (7), Cu–B(5) = 2.164 (8) Å.

As noted above, the remarkable structural feature of $Cu[P(C_6H_5)_3]_2B_5H_8Fe(CO)_3$ is the relatively open bonding site of the copper atom. There are no unusually short nonbonded contact distances or distorted angles to suggest a configuration forced by steric considerations. The angle P(1)–Cu–P(2) is 127.57 (8)°. The plane which is defined by B(4)–Cu–B(5) is

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