reveal these to be strongly affected by its presence. Relative to the corresponding shifts in β-CAR a *shielding* of 0.547 ppm is observed for $H_{1'}$, whereas a *deshielding* of 0.154 ppm is observed for $H_{2'}$.

The relative magnitudes of the carboxylate contribution to the overall $H_{1'}$ and $H_{2'}$ magnetic shielding constants are reasonable in view of the larger distance of the latter hydrogen from the 6 position in the syn conformation. The fact that the contributions are of opposite sign may be surprising at first sight but is readily rationalized if the predominant shielding mechanism is assumed to result from the electric field associated with the 6 substituent.[11] Examination of space-filling molecular models indicates that the rotation of the carboxyl moiety is severely restricted owing to steric interactions with the $H_{1'}$ hydrogen. The most favored orientation, which minimizes these effects, appears to be that in which the plane of the carboxyl is essentially perpendicular to the uracil moiety. Assuming this relative orientation of base and carboxyl and an overall syn conformation for the molecule, simple Buckingham electric field calculations[12] predict a diamagnetic contribution for $H_{1'}$ and a somewhat attenuated paramagnetic contribution to $H_{2'}$. Moreover, the opposite result is predicted if an anti model is chosen.

The orotidine moiety is implicated in the sequence of enzymic reactions which convert orotic acid (6-carboxyuracil) to orotidylic acid[13] and finally in a decarboxylative step to uridylic acid. Little information is available concerning the active site conformation, mode of binding of substrate, and mechanism of the conversion. Binding of the negatively charged carboxyl group to the active site has been proposed.[13] The present results giving details of relevant molecular conformations may be of importance toward a better understanding of the problem. It is interesting to note that the substrate of the decarboxylative step (orotidylic acid) may safely be predicted to exist in its syn conformation, whereas the product (uridylic acid) is anti.[14] More data are now being collected.[14a]

(11) That the shielding arises largely from a "through-space" mechanism rather than from inductive effects through the σ-bond network is a very reasonable assumption in view of the considerably smaller carboxylate effect on the H_5 resonances. (Compare data for U and O.) More detailed calculations of course would include inductive and magnetic anisotropic effects but would lead to qualitatively identical predictions.

(12) A. D. Buckingham, *Can. J. Chem.*, **38**, 300 (1960).

(13) J. Imsande and P. Handler in "The Enzymes," Vol. 5, P. D. Boyer, H. Lardy, and K. Myrback, Ed., Academic Press, New York, N. Y., 1961, p 302.

(14) We do not expect 5′-phosphorylation to result in rotation of O into its anti range of sugar-base torsion angles, in view of our knowledge of other pairs of pyrimidine nucleosides and nucleotides.

(14a) NOTE ADDED IN PROOF. After submission of this manuscript, Schweizer, *et al.* (M. P. Schweizer, J. T. Witkowski, and R. K. Robins, *J. Amer. Chem. Soc.*, **93**, 277 (1971)), reported their observations of the 2-keto anisotropic effects upon the ribose chemical shifts of a number of pyrimidine nucleosides. The authors demonstrated that a qualitative determination of their syn *vs.* anti conformation could be based on these specific shielding and deshielding phenomena alone.

(15) I am grateful to Drs. H. Dugas, B. J. Blackburn, R. K. Robins, and I. C. P. Smith for communicating their views and data on β-CAR prior to publication and to a National Research Council of Canada grant which made this work possible. Kindly address correspondence to the University of Manitoba.

Frank E. Hruska[15]

Chemistry Departments, The University of Winnipeg and The University of Manitoba, Winnipeg, Manitoba, Canada

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A Novel Rearrangement in a Class of Stereochemically Nonrigid Five-Coordinate Complexes

Sir:

Stereochemical nonrigidity has been presumed for many five-coordinate transition metal complexes;[1-3] however, the phenomenon was not rigorously established for a member of this group until the studies of Udovich and Clark.[4,5] In these and all other investigations of five-coordinate complexes, only a Berry[6] type of rearrangement has been seriously considered.[1-3,7-13] We report here the first observation of limiting, slow exchange nmr spectra for a class of transition metal hydrides of the type HML_4. We propose for these nonrigid hydrides a rearrangement mechanism which comprises a hydrogen atom traverse of faces in the ML_4 tetrahedral substructure.

The ^{19}F (84.66 MHz) and ^1H (90 MHz) nmr spectra of $HOs(PF_3)_4^-$ [14,15] and $HRh(PF_3)_4$[15] at three temperatures are shown in Figures 1 and 2. Similar spectra were obtained for $HRu(PF_3)_4^-$, $HCo(PF_3)_4$, and $HIr(PF_3)_4$[15] in the same temperature range. Chlorodifluoromethane was the solvent for the low-temperature studies.

The proton and fluorine nmr spectra establish the spectroscopic equivalence of the phosphorus and fluorine nuclei at 25°. The proton spectra consist of a group of 13 sets of quintets (of proper binomial distribution), with an additional doublet splitting due to Rh–H coupling for the rhodium complex. Values for J_{PH}, J_{FH}, and J_{RhH} can be obtained from the spectra and are given in Table I. The ^{19}F spectra are complex, and, in the absence of a complete analysis, the only additional information contained in the high-temperature limit spectrum is an approximate value for the J_{PF} coupling constant (about 1250 Hz in all cases). The proton spectrum of $HCo(PF_3)_4$ is broad at room temperature due to quadrupole relaxation of the ^{59}Co nucleus. On cooling to −60° (chlorodifluoromethane solution) the spectrum begins to sharpen and the structure can be observed down to −110°. On further

(1) E. L. Muetterties and R. A. Schunn, *Quart. Rev. Chem. Soc.*, 20, 245 (1966).

(2) E. L. Muetterties, *Accounts Chem. Res.*, **3**, 266 (1970).

(3) E. L. Muetterties, *Rec. Chem. Progr.*, **31**, 51 (1970).

(4) C. A. Udovich and R. J. Clark, *J. Amer. Chem. Soc.*, **91**, 526 (1969).

(5) C. A. Udovich, R. J. Clark, and H. Haas, *Inorg. Chem.*, **8**, 1066 (1969).

(6) R. S. Berry, *J. Chem. Phys.*, **32**, 933 (1960).

(7) F. H. Westheimer, *Accounts Chem. Res.*, **1**, 70 (1968).

(8) (a) G. Zon, K. A. DeBruin, K. Naumann, and K. Mislow, *J. Amer. Chem. Soc.*, **91**, 7023 (1969); (b) K. A. DeBruin, G. Zon, K. Naumann, and K. Mislow, *ibid.*, **91**, 7027 (1969).

(9) J. W. Faller and A. S. Anderson, *ibid.*, **92**, 5852 (1970).

(10) J. R. Shapley and J. A. Osborn, *ibid.*, **92**, 6976 (1970).

(11) Alternatives have been described; *cf.* E. L. Muetterties, *ibid.*, **91**, 4115 (1969).

(12) W. Mahler and E. L. Muetterties (*Inorg. Chem.*, **4**, 1520 (1965)) have proposed a primarily fluorine atom motion for the rearrangements in Cl_2PF_3 and Br_2PF_3. However, there is no experimental justification for this alternative proposal.

(13) The turnstile mechanism recently proposed by L. Ugi, *et al.* [*Angew. Chem., Int. Ed. Engl.*, **9**, 703 (1970)] is permutationally indistinguishable from the Berry rearrangement, and there is as yet no rationale that would justify serious consideration of this essentially equivalent mechanism.

(14) T. Kruck (*Z. Anorg. Allgem. Chem.*, **371**, 1 (1969)) reported the spectrum of $HOs(PF_3)_4^-$ to consist of two quartets. This reported spectrum is due to a complex other than $HOs(PF_3)_4^-$, produced in the neutralization reaction described by Kruck.

(15) T. Kruck (*Angew. Chem., Int. Ed. Engl.*, **6**, 53 (1967)) has described the preparation and general properties of trifluorophosphine complexes of transition metals.

Table I. Nmr Parameters for HML_4[a-c]

	$HCo(PF_3)_4$	$HRh(PF_3)_4$	$HIr(PF_3)_4$	$HRu(PF_3)_4^-$	$HOs(PF_3)_4^-$
High-temperature limit					
$\|J_{HP}\|$	5.0	57.0	31.0	9.25	3.75
$\|J_{HF}\|$	9.75	16.5	14.75	16.5	15.0
$\|J_{HM}\|$		5.5			
δ_H	12.68	9.94	12.11	9.43	11.51
δ_F	−64.7	−67.6	−57.6	−77.9	−75.5
Low-temperature limit					
$\|J_{HP}\|_{trans}$		215.0	170.0	~75.0	
δ_{axial}	−65.6	−63.7	−51.8	−72.4	−69.5
δ_{equat}	−63.7	−67.8	−57.5	−78.5	−76.1
$\Delta G^{\pm}$, kcal mol^{-1}	5.5	9.0	10.0	7.0	8.0

[a] Parts per million upfield from TMS. [b] ^{19}F chemical shifts are measured with respect to the upfield component of chlorodifluoromethane with the exception of $HIr(PF_3)_4$ where the shifts are measured relative to 1,2-dibromotetrafluoroethane. [c] J values in hertz.

cooling, the spectrum again broadens due to the exchange process. The ^{19}F spectrum seems to be unaffected by the quadrupole relaxation. In all other cases, the spectra broaden on lowering the temperature. In the low-temperature limit, the ^{19}F spectra are consistent with a coordination sphere having three coplanar phosphorus nuclei and the hydrogen atom

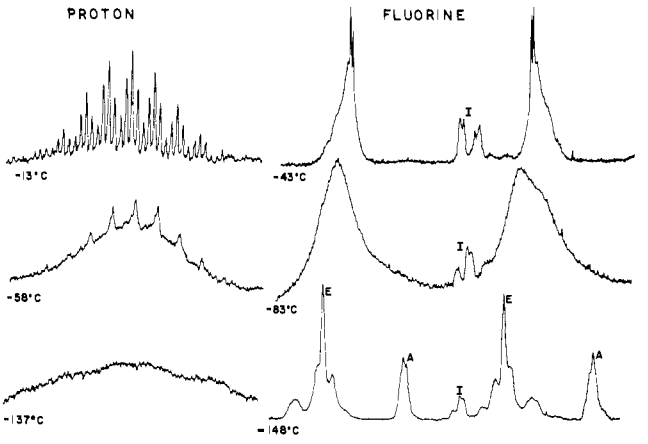

Figure 1. ^1H (90 MHz) and ^{19}F (84.66 MHz) nmr spectra for $HOs(PF_3)_4^-$ as a function of temperature: first row, acetone solution; second and third rows, $CHClF_2$ solution. I indicates impurity resonances; A and E indicate axial and equatorial fluorine resonances.

and remaining phosphorus nucleus, trans to each other, on the threefold axis of the P_3 plane. The ^{19}F resonances assigned to the axial and equatorial PF_3 groups are well separated and integration gives intensities with close to the expected 1:3 ratio. The low-temperature limit ^1H hydride spectra of $HIr(PF_3)_4$, $HRh(PF_3)_4$, and $HRu(PF_3)_4^-$ can be approximately described as complex doublets due to large H–P coupling between the axial ligands.

A preliminary analysis, with the Eyring equation,[16] of the ^{19}F nmr line shapes in the intermediate region, gives the free energies listed in Table I. If the entropies of activation are small as is usually the case for intramolecular processes, these results provide an estimate of the barrier to rearrangement. The trend shows increasing barriers with increasing atomic number and with decreasing negative formal charge. Most notable is the larger barrier change between first- and second-row transition elements. It is anticipated that $HFe(PF_3)_4^-$ will have $\Delta G^{\pm} \leqslant 5$ kcal mol^{-1}.

In the corresponding molecules of the form $M(PF_3)_5$ the equilibrium geometry is presumably trigonal bipyramidal and a Berry mechanism is operative. Measurements of the fluorine nmr of these molecules as a function of temperature for M = Fe, Ru, and Os have indicated that the complexes are fluxional on the nmr time scale at the lowest temperatures achieved (−160° in $CHClF_2$ solution). Hence it is probable that the barriers to rearrangement for these compounds are lower than for the hydrides.

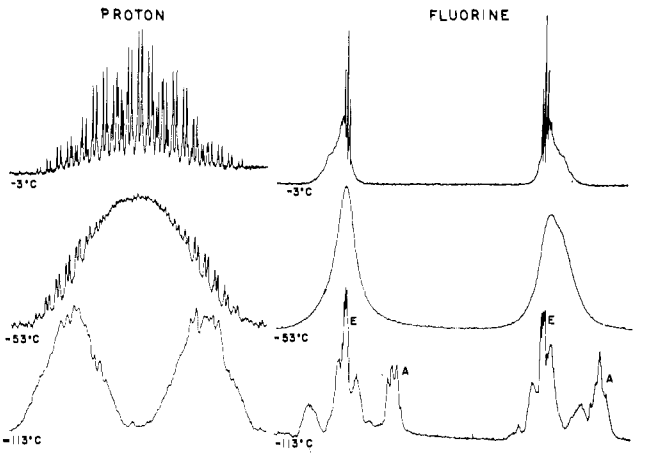

Figure 2. ^1H (90 MHz) and ^{19}F (84.66 MHz) nmr spectra for $HRh(PF_3)_4$ as a function of temperature: first row, acetone solution; second and third rows, $CHClF_2$ solution. A and E indicate axial and equatorial fluorine resonances.

Stereochemical nonrigidity has also been demonstrated for the molecule $HRh[P(OC_2H_5)_3]_4$; the slow exchange limit spectra are consistent with a C_{3v} structure with $J_{RhH} = 9$ Hz, $J_{HP\,axial} = 152$ Hz, and $J_{HP\,equat}$ small. The activation parameter $\Delta G^{\pm}$ for this compound is 7.3 kcal mol^{-1}.

The solid state structures of several molecules from this HML_4 class have been established.[17-19] In all these, ignoring the position of the metal atom, the coordination sphere may be described as trigonal bi-

(16) W. F. K. Wynne-Jones and H. Eyring, *J. Chem. Phys.*, 3, 492 (1935).

(17) B. A. Frenz and J. A. Ibers, *Inorg. Chem.*, 9, 2403 (1970); $HCo(PF_3)_4$.

(18) R. W. Baker and P. Pauling, *Chem. Commun.*, 1495 (1969); $HRh[P(C_6H_5)_3]_4$.

(19) R. W. Baker, B. Ilmaier, P. J. Pauling, and R. S. Nyholm, *ibid.*, 1077 (1970); $HRh[P(C_6H_5)_3]_3[As(C_6H_5)_3]$.

pyramidal with the hydrogen nucleus, as expected, at an axial position; but, due to nonbonding repulsions, the metal nucleus is displaced from the P_3 plane.[17-19] In fact, the MP_4 substructure in many of these molecules is a regular or near-regular tetrahedron.[17-19] In light of these structures, it would seem unrealistic to consider a Berry[6] mechanism as being the dominant pathway for these rearrangements. Since the MP_4 substructure so closely approximates a regular tetrahedron in this class of compounds, the angular departure from the idealized values in a trigonal bipyramid, on which the Berry mechanism is based, is rather significant. Furthermore, a Berry rearrangement would necessarily pass a relatively high-energy state with an equatorial hydrogen ligand if spectroscopic equivalence of the phosphorus nuclei is to be achieved. This does not seem consonant with the low-activation parameters ($\Delta G^{\pm}$), *ca.* 7 kcal/mol, found for $HM(PX_3)_4$ molecules. We propose that the rearrangement mechanism consists of a hydrogen atom traverse of MP_4 "tetrahedral" faces and that the barriers largely reflect the force constants for the MP bending modes involved in the charges in the phosphorus disposition during the rearrangement. This is formally analogous to the mechanism for rearrangement in H_2ML_4 complexes.[20-22] The substantially larger free energies of activation, ~12 kcal/mol and greater, in the latter family probably reflect the added activation required to distort the ML_4 substructure toward a regular tetrahedral array. In fact, the ML_4 angles found in H_2ML_4 complexes do depart significantly from 109° 28′.[21]

Ligand character, metal size, and electronic state and the formal charge on the aggregate affect the relative nonrigidity of these HML_4 complexes, and we are systematically exploring these facets.

(20) F. N. Tebbe, P. Meakin, J. P. Jesson, and E. L. Muetterties, *J. Amer. Chem. Soc.*, **92**, 1068 (1970).
(21) P. Meakin, L. J. Guggenberger, J. P. Jesson, D. H. Gerlach, F. N. Tebbe, W. G. Peet, and E. L. Muetterties, *ibid.*, **92**, 3482 (1970).
(22) The full mechanistic analysis will be published shortly.

P. Meakin, J. P. Jesson
F. N. Tebbe, E. L. Muetterties*
Contribution No. 1778, Central Research Department
E. I. du Pont de Nemours and Company, Experimental Station
Wilmington, Delaware 19898
Received January 4, 1971

The Synthesis of a Protein with Acyl Carrier Protein Activity

Sir:

We wish to report the synthesis of *E. coli* acyl carrier protein[1-3] by the solid-phase method.[4] ACP has been implicated in all biological systems synthesizing fatty acids *de novo*;[5] the substrates involved in fatty acid biosynthesis are bound as thio esters to ACP through its prosthetic group, which is 4′-phosphopantetheine. Although ACP contains 77 residues, we chose to synthesize only the 1–74 peptide because it has been shown that the three residues at the C terminus are not required for biological activity.[6,7] The apo form of the protein was prepared, and after deprotection of the peptide, the prosthetic group was added enzymatically to form holo-ACP.[8] This product was found to be active in the malonyl pentetheine–CO_2 exchange reaction.[9]

The synthesis was based on the stepwise addition of suitably protected amino acids to 0.5 mmol of *tert*-butyloxycarbonyl glycine esterified to 1.5 g of a 1% cross-linked polystyrene resin support. The *tert*-butyloxycarbonyl (Boc)[10] group was used for α-amino protection and the side chains were protected as follows: Asp (β-OBzl), Glu (γ-OBzl), Ser (Bzl), Thr (Bzl), Tyr (Bzl), Lys (Z), Arg (NO_2). The coupling steps were carried out with a fourfold excess of the appropriate amino acid and dicyclohexylcarbodiimide (DCC), as the coupling reagent, except for glutamine and asparagine which were added as the *p*-nitrophenyl ester. All coupling reactions were carried out twice with a reaction time of 2 hr, except for active esters which were left for 12 hr. The second coupling was conducted in a solvent system of dichloromethane and dimethylformamide (1:1, v/v) with the addition of urea (1.5 *M*).[11] Boc groups were removed by two treatments of the resin with 50% (v/v) trifluoroacetic acid (TFA) in methylene chloride, each for 20 min. Another modification of the solid-phase method[12] was that *tert*-butyl alcohol, containing 5% (v/v) dichloromethane, was used instead of ethanol in the washes to reduce possible loss at peptide chains by transesterification. Acetylation was used to terminate partially complete sequences after the coupling of residues 2, 10, 20, 47, 62, and 70.[13]

The overall yield[14] of the peptide was low (15%) but this could be attributed to the modifications that were introduced into the synthetic procedure. It was hoped that the losses, however, would be compensated for by a more homogeneous product. The peptide was cleaved from the resin with HBr and TFA[15] in the presence of anisole and methionine at 2° for 2 hr. The crude product was purified by gel filtration, and 5% of the material, as determined by the Folin-Lowry assay,[16]

(1) The abbreviations used are: ACP, acyl carrier protein; holo-ACP, acyl carrier protein holoprotein; apo-ACP, acyl carrier protein lacking the 4′-phosphopantetheine prosthetic group.
(2) P. R. Vagelos, P. W. Majerus, A. W. Alberts, A. R. Larrabee, and G. P. Ailhaud, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **25**, 1485 (1966).
(3) The complete amino acid sequence of ACP has been recently determined by T. C. Vanaman, S. J. Wakil, and R. L. Hill, *J. Biol. Chem.*, **243**, 6420 (1968).
(4) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963).
(5) P. W. Majerus and P. R. Vagelos, *Advan. Lipid Res.*, **5**, 1 (1967).
(6) P. W. Majerus, *J. Biol. Chem.*, **242**, 2325 (1967).
(7) D. J. Prescott, J. Elovson, and P. R. Vagelos, *ibid.*, **244**, 4517 (1969).
(8) J. Elovson and P. R. Vagelos, *ibid.*, **243**, 3603 (1968).
(9) P. W. Majerus, A. W. Alberts, and P. R. Vagelos, *Methods Enzymol.*, **14**, 43 (1969).
(10) Nomenclature and abbreviations follow the tentative rules of the IUPAC–IUM Commission on Biological Nomenclature, *J. Biol. Chem.*, **241**, 2491 (1966); **242**, 555 (1967).
(11) It has been suggested that these modifications give a better yield for difficult coupling reactions: F. C. Westall and A. B. Robinson, *J. Org. Chem.*, **35**, 2842 (1970).
(12) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).
(13) Investigation of the peptide chains, released by transesterification of the benzyl ester with triethylamine and methanol, showed that 15% of the peptide chains on the polymer, but none of the synthetic ACP, had been terminated with [^{14}C]acetic anhydride.
(14) The yield was based on the ratio of the single arginine (residue 5) to the amount of glycine originally esterified to the resin. The overall composition of the crude peptide was in close agreement with the reported values for ACP.[3] The values were obtained by amino acid analysis of the 1–74 peptide resin, which was hydrolyzed with HCl and propionic acid (see J. Scotchler, R. Lozier, and A. B. Robinson, *J. Org. Chem.*, **35**, 3151 (1970)).
(15) HBr–TFA was used instead of HF because apo-ACP was completely inactivated under conditions suitable for deprotection by HF. Also it was observed that HF or HF–TFA mixtures gave incomplete removal of protecting groups.
(16) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).