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 ($\Delta G^{\ddagger}$), *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.

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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).

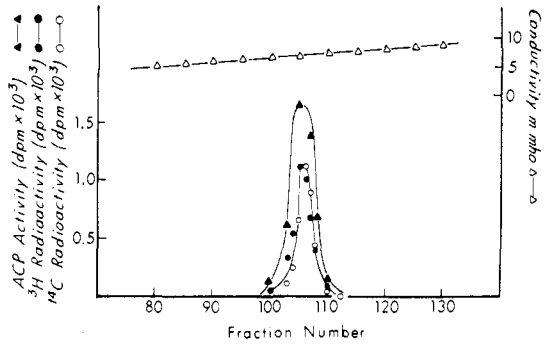

Figure 1. Identical samples of synthetic [^3H]holo-ACP were chromatographed separately on DEAE-cellulose columns (1) in the presence of authentic [^{14}C]pantetheine-ACP 1-74 and assayed for ^3H and ^{14}C radioactivity and (2) in the absence of carrier and assayed for ^3H radioactivity and ACP activity.[9] The results of these experiments have been combined.

was eluted at the position expected for ACP. The deprotection was completed by hydrogenation of the sample at atmospheric pressure with palladium oxide on barium sulfate catalyst. The prosthetic group was then introduced enzymatically[8,17] to form holo-ACP which was then purified by ion exchange chromatography on DEAE-cellulose. (Yield of protein at this stage was 0.6%, based on the initial glycine value.)

The activity of the preparation was measured by two independent assays:[18] (1) the incorporation of ^3H label into the synthetic product when the synthetic apo-ACP was treated with ACP synthetase and [^3H]pantetheine-CoA; and (2) the activity of the resulting synthetic holo-ACP in the malonyl pantetheine-CO_2 exchange reaction. The specific activities[19] in the two assays were found to be 33 and 30%, respectively, which indicated the presence of a large quantity of inactive material. It should be noted, however, that apo-ACP retained only 40% of its original activity when subjected similarly to HBr-TFA treatment and hydrogenation.

The synthetic product was found to cochromatograph with authentic 1-74 ACP[20] on ion exchange chromatography (see Figure 1) and gel filtration (Sephadex G-50).

Further work is now in progress to increase both the yield and specific activity of our preparation.

Acknowledgment. This work was supported by Grant No. AM-13025 and 5-ROI-HE10406 from U. S. Public Health Service, Grant No. GB-5142X from the National Science Foundation, and the George Murray Scholarship (University of Adelaide, Australia).

(17) The enzyme, ACP synthetase, catalyzes the incorporation of 4'-phosphopantetheine according to the following reaction

$$\text{apo-ACP} + \text{CoASH} \xrightarrow{Mg^{2+}} \text{holo-ACP} + \text{adenosine 3',5'-diphosphate}$$

(18) It has been shown that at least two of the enzymes involved, ACP synthetase and β-ketoacyl ACP synthetase, show a high degree of specificity for ACP (see ref 6 and 7).

(19) Ratio of biological activity compared with authentic holo-ACP to the total protein as measured by the Folin-Lowry assay.

(20) [^{14}C]Pantetheine-ACP 1-74 was prepared by the digestion of [^{14}C]pantetheine-ACP with carboxypeptidase A (see ref 6 and 7).

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A New Photosensitizer. Tris(2,2'-bipyridine)ruthenium(II) Chloride

Sir:

We have found that the cation $Ru(bipy)_3^{2+}$[1] photosensitizes the aquation of aqueous $PtCl_4^{2-}$. Previously, sensitized luminescence of transition metal complexes has been achieved using as donors organic molecules,[2,3] *trans*-$Cr(NH_3)_2(NCS)_4^-$[4] and $Ru(bipy)_3^{2+}$.[5] Organic donors such as various ketones and the acridinium ion have been found to sensitize the redox decomposition of Co(III) ammines[6] and the aquation of certain Cr(III) complexes[7,8] and of $Co(CN)_6^{3-}$;[9] biacetyl is reported to sensitize the aquation of $PtCl_4^{2-}$.[10]

The $Ru(bipy)_3^{2+}$-$PtCl_4^{2-}$ system is novel, however, in that it appears to represent the first case of a transition metal complex functioning as a photochemical sensitizer toward a second complex;[11] it may also represent the first example of energy transfer from a transition metal complex in room-temperature solutions. The spectroscopic properties of $Ru(bipy)_3^{2+}$ are such as to make this complex an unusually interesting and convenient sensitizer, and we wish here to propose its more general use.

The absorption and emission spectra of $Ru(bipy)_3^{2+}$ are shown in Figure 1. The prominent visible absorption feature is due to a spin-allowed charge-transfer (CT) process, and the emission arises from the lowest CT triplet state[12-14] at ~17.8 kK. The phosphorescence lifetime in room-temperature aqueous solution is ~0.7 μsec.[14] The intersystem-crossing yield is probably greater than 0.9[14-16] and the emission yield, even in fluid solutions, is relatively high. The complex is photochemically and thermally quite inert.

Preliminary experiments showed that anionic complexes such as $Cr(C_2O_4)_3^{3-}$, $Cr(CN)_6^{3-}$, $Co(C_2O_4)_3^{3-}$, and $PtCl_4^{2-}$ quench the phosphorescence in room-temperature aqueous solutions. In some cases precipitation occurs, however, and this observation plus the finding that cationic complexes do not quench well raise the possibility that the effect is a static one, quenching simply reflecting the presence of nonluminescent ion pairs. To test this question both luminescence and lifetime quenching studies were made, using $PtCl_4^{2-}$, with the results shown in Figure 2. The emission quenching was determined by means of an Aminco spectrofluorimeter and the lifetime quenching, on the same solutions, by means of a pulsed 337-nm N_2 laser (courtesy of Dr. J. H. Parks). The coincidence of the two sets of

(1) bipy = 2,2'-bipyridine.
(2) D. J. Binet, E. L. Goldberg, and L. S. Forster, *J. Phys. Chem.*, **72**, 3017 (1968).
(3) T. Ohno and S. Kato, *Bull. Chem. Soc. Jap.*, **42**, 3385 (1969).
(4) S. Chen and G. B. Porter, *J. Amer. Chem. Soc.*, **92**, 3196 (1970).
(5) I. Fujita and H. Kobayashi, *J. Chem. Phys.*, **52**, 4904 (1970).
(6) A. Vogler and A. W. Adamson, *J. Amer. Chem. Soc.*, **90**, 5943 (1968).
(7) A. W. Adamson, J. E. Martin, and F. Diomedi Camessei, *ibid.*, **91**, 7530 (1969).
(8) J. E. Martin and A. W. Adamson, *Theor. Chim. Acta*, in press.
(9) G. B. Porter, *J. Amer. Chem. Soc.*, **91**, 3981 (1969).
(10) V. S. Sastri and C. H. Langford, *ibid.*, **91**, 7533 (1969).
(11) Reference 7 notes an at best marginally successful attempt to so use a Cr(III) complex.
(12) D. M. Klassen and G. A. Crosby, *J. Chem. Phys.*, **48**, 1853 (1968).
(13) J. N. Demas and G. A. Crosby, *J. Mol. Spectrosc.*, **26**, 72 (1968).
(14) F. E. Lytle and D. M. Hercules, *J. Amer. Chem. Soc.*, **91**, 253 (1969).
(15) J. N. Demas, Ph.D. Dissertation, University of New Mexico, 1970.
(16) J. N. Demas and G. A. Crosby, *J. Amer. Chem. Soc.*, in press.