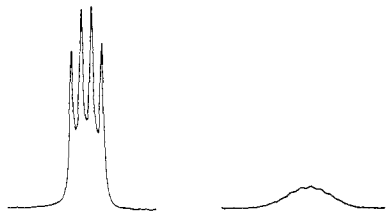

Figure 2. Experimental ^{11}B nmr spectrum (32.1 MHz) of a 40% solution of B_5H_9 in C_6F_6 showing a $J_{^{11}B^{11}B}$ of 19.4 Hz and a chemical shift between the base and the apex of 40.8 ppm. The C_6F_6 furnished the signal for the ^{19}F lock.

line width of 5–7 Hz instead of 40–60 Hz, the predicted ^1H noise-decoupled spectrum of B_5H_9 would be a quartet with a broad base for the basal borons and a poorly resolved multiplet for the apex boron. This prediction is in good agreement with the experimental spectrum of B_5H_9 given in Figure 2.

The value of $J_{^{11}B^{11}B}$ extracted from the experimental spectrum is 19.4 ± 0.2 Hz. It should be clear from the experiment presented here that with efficient proton decoupling and the inclusion of ^{10}B decoupling, one should be able to determine boron–boron coupling constants in many of the boron hydrides. These coupling constants and the improved precision should be an invaluable aid to structure identification in boron chemistry. It is also interesting to note that this increased resolution may in some cases eliminate the need for high-field nmr spectra of some of the boron hydrides.

In addition to the experimental significance mentioned above, there is a corresponding theoretical significance. Until now there has been no need to predict boron–boron coupling constants. However, these coupling constants can provide critical tests to theories of spin coupling constants, *e.g.*, the finite perturbation approach of Pople and coworkers.[7]

Work is currently in progress to assess the experimental and theoretical consequences of these interesting results.

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(7) J. A. Pople, J. W. McIver, Jr., and N. S. Ostlund, *J. Chem. Phys.*, 49, 2960, 2965 (1968).

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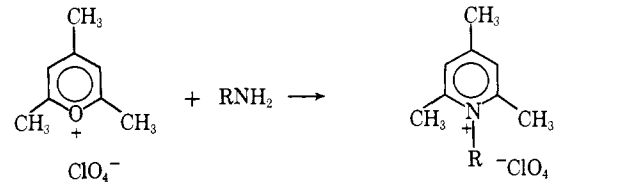
Chemical Modification of Proteins by Pyrylium Salts

Sir:

A large number of reagents are available for the modification of amino groups in proteins,[1] but interest in the development of reagents with special selectivity continues. We have developed an amino group reagent with apparent steric selectivity. The reaction of pyrylium salts with primary amines to form the corresponding N-substituted pyridinium salts is well known,[2] but has not been applied to proteins until now. Our initial studies with α-chymotrypsin, a protein of known amino acid sequence[3] and three-dimensional structure,[4] indicate that reaction with a 2,6-disubstituted pyrylium salt results in modification of only a fraction of the available amino groups, presumably those which are particularly exposed.

When 2,4,6-trimethylpyrylium perchlorate[5] (I), or the more water-soluble chloroferrate, reacts with an excess of *n*-butylamine in aqueous solution at pH 9.0 (carbonate buffer) at 21°, over a period of a few hours the uv absorbance at 240 nm due to the pyrylium salt[6] decreases, and the absorbance at 268 nm due to the product pyridinium salt IIa increases. Similarly, reaction of bovine α-chymotrypsin with an excess of I



I

IIa, R = n-C_4H_9
b, R = $(CH_2)_4CHNH_2COOH$
c, R = $CH(COOH)(CH_2)_4NH_2$

at pH 9.0 at 20° results in a gradual decrease in the pyrylium salt absorbance at 240 nm and an increase in the absorbance at 268 nm. The rate constant for the reaction of chymotrypsin with the pyrylium salt[8] is about 2×10^{-3} M^{-1} sec^{-1}, similar to that for the reaction of *n*-butylamine with the pyrylium salt, 8×10^{-4} M^{-1} sec^{-1}. The reaction of chymotrypsin with the pyrylium salt follows good first-order kinetics to at least 90% completion, and no evidence is seen for a slower reaction following this first one.

The modified enzyme was prepared on a large scale by reaction of 10^{-3} M chymotrypsin with 5×10^{-2} M I at pH 9.0, 20° for 96 hr. The reaction mixture was concentrated and chromatographed on a column of

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(2) A. T. Balaban and C. Toma, *Tetrahedron, Suppl.*, 7, 1, 9, 27 (1967); A. N. Narkevin, G. N. Dorofeenko, and Y. A. Zhdanov, *Zh. Obshch. Khim.*, 36, 819 (1966); K. Dimroth, *Angew. Chem.*, 72, 331 (1960); K. Dimroth and K. H. Wolf, "Newer Methods in Preparative Organic Chemistry," Vol. 3, W. Foerst, Ed., Academic Press, New York, N. Y., 1964, p 357.

(3) B. S. Hartley, *Nature (London)* 201, 1284 (1964); B. S. Hartley and D. L. Kauffman, *Biochem. J.*, 101, 229 (1966); D. M. Blow, J. J. Birktoft, and B. S. Hartley, *Nature (London)*, 221, 337 (1969); B. S. Hartley, *Phil. Trans. Roy. Soc. London*, 257, 77 (1970).

(4) B. W. Matthews, P. B. Sigler, R. Henderson, and D. M. Blow, *Nature (London)*, 214, 652 (1967); P. B. Sigler, D. M. Blow, B. W. Matthews, and R. Henderson, *J. Mol. Biol.*, 35, 143 (1968); T. A. Steitz, R. Henderson, and D. M. Blow, *ibid.*, 46, 337 (1969); J. J. Birktoft, B. W. Matthews, and D. M. Blow, *Biochem. Biophys. Res. Commun.*, 36, 131 (1969); J. J. Birktoft, D. M. Blow, R. Henderson, and T. A. Steitz, *Phil. Trans. Roy. Soc. London*, 257, 67 (1970); D. M. Blow and T. A. Steitz, *Annu. Rev. Biochem.*, 39, 63 (1970).

(5) Prepared according to method II of K. Hafner and H. Kaiser, *Org. Syn.*, 44, 108 (1964).

(6) At this pH the pyrylium salt exists entirely in the open-chain "pseudobase" form.[7]

(7) J. A. Berson, *J. Amer. Chem. Soc.*, 74, 358 (1952); R. Lombard and A. Kress, *Bull. Soc. Chim. Fr.*, 1528 (1960).

(8) These rate constants were obtained from pseudo-first-order rate constants measured in the presence of a large excess of pyrylium salt. The rate constants are not very accurate due to the slow decomposition of the pyrylium salt at pH 9.

Sephadex G-50 (medium). The first two-thirds of the protein fraction was lyophilized, yielding a white solid, λ_{max} 274, 290 nm (sh).

A preliminary examination of this material was carried out by comparison of its uv spectrum with the spectra of unmodified chymotrypsin and of the compound expected from reaction of the pyrylium salt with lysine, 1-(5-carboxy-5-aminopentyl)-2,4,6-trimethylpyridinium perchlorate (IIb). Calculated spectra of the modified material were obtained by assuming that the characteristic absorption due to chymotrypsin was unchanged in the modified material and that the formation of modified lysine residues on the enzyme would result in the same absorption spectrum as that of model compound IIb. The observed spectrum of the modified material was duplicated very satisfactorily when it was assumed that 6 ± 1 amino groups were modified in each chymotrypsin molecule. Spectra of chymotrypsin, modified chymotrypsin, and a 1:6 mixture of chymotrypsin and IIb are shown in Figure 1.

Pyridinium salt IIb, the expected product of the reaction of I with the ε-amino group of a lysine residue of chymotrypsin, was synthesized by the reaction of *N*-α-carbobenzyloxylysine[9] with I in acetic acid. After removal of the protecting group by hydrogenolysis the compound was recrystallized from ethanol. The compound had the expected uv and nmr spectra and gave a positive ninhydrin test. The isomeric 1-(1-carboxy-5-aminopentyl)-2,4,6-trimethylpyridinium perchlorate (IIc) was prepared by reaction of I with lysine in ethanol and was distinct from IIb by thin-layer chromatography, ion-exchange chromatography, high-voltage electrophoresis, and nmr.

For more quantitative analysis, chymotrypsin and modified chymotrypsin were hydrolyzed (6 *N* HCl, 100°, 24 hr) and the products were analyzed on a Beckman Spinco amino acid analyzer. Repeated analyses showed that the two materials were identical except for the disappearance of 6 ± 1 lysine residues in the modified material. High-voltage electrophoresis of the acid hydrolysates at pH 11.03 produced only one spot in the modified material which was not present in the native enzyme. This spot was ninhydrin positive and had the same R_f as compound IIb. No ninhydrin negative spots corresponding to the product of reaction of the pyrylium salt with any of the three amino-terminal amino acids of chymotrypsin[10] (alanine, isoleucine, or cystine) were observed.

In order to establish whether the pyrylium salt might react with functional groups of the enzyme other than amino groups, possible reactions of I with imidazole, with phenol, and with ethanol in aqueous solution at pH 9.0 were investigated. In all three cases no reaction other than decomposition of I was observed in 7 days.

The modified enzyme was titrated with *trans*-cinnamoylimidazole by the procedure of Schonbaum, *et al.*[11] The active site concentration per weight of enzyme was about 90% that of the native enzyme. The activity of the modified enzyme toward *p*-nitrophenyl acetate was also about 90% that of the native enzyme.

(9) B. Bezas and L. Zervas, *J. Amer. Chem. Soc.*, 83, 719 (1961).
(10) These compounds were prepared by the reaction of I with the appropriate amino acid in acetic acid.
(11) G. R. Schonbaum, B. Zerner, and M. L. Bender, *J. Biol. Chem.*, 230, 2930 (1961).

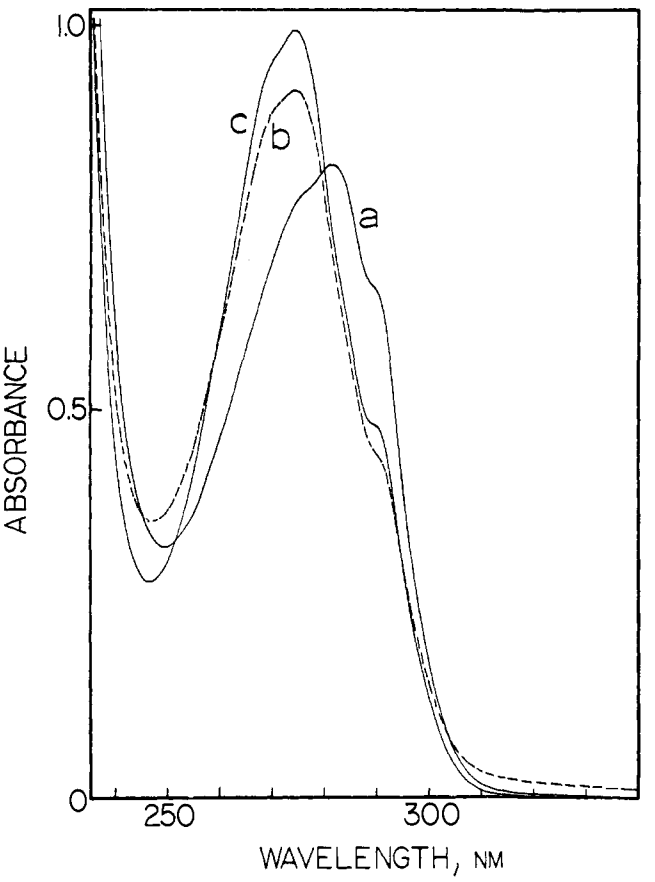

Figure 1. Uv spectra of chymotrypsin, modified chymotrypsin, and a mixture of chymotrypsin and compound IIb: a, chymotrypsin (1.67×10^{-5} *M*); b, chymotrypsin after reaction with I (1.11×10^{-5} *M*); c, 1:6 mixture of chymotrypsin (1.27×10^{-5} *M*) and IIb (7.56×10^{-5} *M*).

The utility of I for modification of amino groups of enzymes is also illustrated by its reaction with acetoacetate decarboxylase. This enzyme has a lysine residue of abnormally low pK_a which forms a Schiff base with the substrate during the enzymatic decarboxylation.[12] This particular lysine reacts with acetic anhydride[13] or 2,4-dinitrophenylpropionate[14] at pH 6 to form an inactive acylated enzyme. Acetoacetate decarboxylase reacts with I (1.3×10^{-2} *M*) at 20°, pH 6 in 40 hr, to form an inactive product with a uv spectrum differing only very slightly from that of the native enzyme. The spectrum is approximately that expected for formation of 1 mol of pyridinium salt/60,000 g of enzyme. High-voltage electrophoresis of an acid hydrolysate shows the presence of pyridinium salt IIb. Although these results do not establish with certainty that we have modified only the active lysine of this enzyme, it is clear that the modification reaction is highly specific and it is likely that modification has occurred on the active lysine.

Pyrylium salts show considerable promise as selective reagents for the chemical modification of amino groups of proteins. Further aspects of this subject are being explored.

(12) S. Warren, B. Zerner, and F. H. Westheimer, *Biochemistry*, 5, 817 (1966).
(13) M. H. O'Leary and F. H. Westheimer, *ibid.*, 7, 913 (1968).
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(15) Predoctoral fellow of the National Institutes of Health (5 F01 GM 44429).

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A Novel Metal–Carbonyl–Metal Bonding System. Synthesis and Stereochemistry of $Al[W(CO)_3C_5H_5]_3(C_4H_8O)_3$

Sir:

We wish to report the synthesis, characterization, and stereochemistry of the transition metal carbonyl derivative $Al[W(CO)_3C_5H_5]_3(C_4H_8O)_3$ (**1**), the most stable member of a new series of group VIb complexes of the $Al(m)_3$ type; only one brief account of such a complex, $Al[Cr(CO)_3C_5H_5]_3$, has been reported.[1] Evidence from similar group III systems[2,3] suggests the possibility of metal–metal bonding,[4] and additional interest in these systems arises from some recently reported organoaluminum complexes of metal carbonyls in which the carbonyl ligands exhibit some unusual stereochemical[5] and spectroscopic[5-7] features.

The synthesis utilized the metal-exchange reaction previously employed for the convenient preparation of transition metal derivatives of zinc and cadmium;[8] at all stages, reactants and products were kept under an argon atmosphere. Solutions of **1** were prepared by stirring *ca.* 0.05 *M* solutions of $Hg[W(CO)_3C_5H_5]_2$ in tetrahydrofuran (THF) with excess powdered aluminum metal or powdered aluminum amalgam at room temperature for 15 min. The reaction was essentially quantitative; filtration yielded a light yellow, extremely air-sensitive solution of **1** which was used for subsequent reactions.[9]

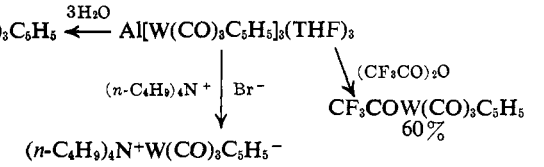
The infrared spectrum of **1** in THF and in the solid state were very similar (Table I) and exhibited some unusually low carbonyl stretching absorptions. In dimethylformamide (DMF), however, **1** appeared to be highly dissociated;[10] only the spectrum of $W(CO)_3C_5H_5^-$ was observed. Several reactions of **1** in THF (Scheme I) are characteristic of this anion; even though the ir data rule out its presence in significant concentration, it is readily available chemically, as indicated by its displacement by nucleophiles such as DMF and bromide ion,[11] and by its ready conversion to the hydride[12] or to the trifluoroacetyl derivative.[13]

Table I. Infrared Spectra of $Al[C_5H_5W(CO)_3]_3(THF)_3$ in the Carbonyl Stretching Region

Medium	Absorptions, cm^{-1} [a]
THF solution	1942 (0.9), 1854 (1.0), 1655 (0.2), 1591 (0.7), 1567 (0.6), 1548 (sh, 0.3)
DMF solution	1891 (0.9), 1773 (broad, 1.0)
Nujol mull	1934 (1.0), 1921 (0.9), 1850 (0.9), 1824 (1.0), 1670 (0.6), 1605 (0.8), 1570 (0.9)

[a] Relative intensities are given in parentheses.

Scheme I



Of particular interest are the carbonyl stretching absorptions of **1** in the 1540–1670-cm^{-1} region; at the time these observations were made, no precedent existed for such metal carbonyl absorptions other than the acyl–metal type.[14] The absorption band of $[C_5H_5Fe(CO)_2]_2 \cdot 2AlEt_3$ at 1682 cm^{-1} was attributed[5] to a bridging carbonyl also coordinated through oxygen to an aluminum atom. Infrared spectral data which further suggested the possibility of carbonyl oxygen coordination were reported for π-$Ph_3PC_5H_4Mo(CO)_3 \cdot AlMe_3$ (1665 cm^{-1})[6] and for n-$Bu_4N^+[W(CO)_3C_5H_5 \cdot AlPh_3]^-$ (1600 cm^{-1}).[7]

An X-ray diffraction analysis of **1** was undertaken on a nearly rectangular parallelepiped crystal (0.15 × 0.20 × 0.30 mm) sealed under argon in a thin-walled capillary tube. The diffraction symmetry was triclinic with $a = 11.419 \pm 0.003$, $b = 11.263 \pm 0.004$, $c = 16.156 \pm 0.006$ Å and $\alpha = 98.13 \pm 0.01$, $\beta = 100.55 \pm 0.01$, $\gamma = 100.23 \pm 0.01°$. Intensities were measured in a θ–2θ scan mode with Zr-filtered Mo Kα radiation on a Picker FACS-I diffractometer; of the 5589 independent reflections investigated ($\sin\theta/\lambda \leq 0.58$ Å^{-1}), a total of 4860 was statistically acceptable as observed data. The intensities were corrected for absorption and for Lorentz and polarization effects.

The coordinates of the three tungsten atoms were determined from a Patterson analysis based upon the space group $P\bar{1}$; all other atoms (excepting hydrogens) were located in two sequential Fourier syntheses. Block-diagonal least-squares refinement with anisotropic thermal parameters for all atoms (469 independent parameters) yielded a standard residual. $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2]^{1/2}$ of 0.077 was obtained for $w = 1/\sigma_{F^2}$. No attempt was made to locate hydrogen atoms.

(1) T. P. Whaley and V. Norman, U. S. Patent 3,071,493 (1963).
(2) D. J. Patmore and W. A. G. Graham, *Inorg. Chem.*, **5**, 1586 (1966).
(3) G. Schmid and H. Nöth, *J. Organometal. Chem.*, **7**, 129 (1967).
(4) For a review, see M. C. Baird, *Progr. Inorg. Chem.*, **9**, 1 (1968).
(5) N. J. Nelson, N. E. Kime, and D. F. Shriver, *J. Amer. Chem. Soc.*, **91**, 5173 (1969).
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(7) J. M. Burlitch and R. B. Petersen, *J. Organometal. Chem.*, **24**, C65 (1970).
(8) J. M. Burlitch and A. Ferrari, *Inorg. Chem.*, **9**, 563 (1970).
(9) Addition of hexane to the THF solution precipitated **1** as a microcrystalline yellow powder which was sparingly soluble in toluene. All attempts to crystallize **1** from this solvent were unsuccessful. Crystals were obtained by slow diffusion of hexane into a saturated solution of **1** in 20% THF–toluene. This material did not melt below 280° under argon but decomposed slowly starting at 170°. Elemental analyses were satisfactory for metals but consistently low for C and H.
(10) J. M. Burlitch, *J. Amer. Chem. Soc.*, **91**, 4562 (1969).
(11) J. M. Burlitch, *ibid.*, **91**, 4563 (1969).
(12) Addition of the stoichiometric quantity of water to **1** in THF constitutes a convenient, high-yield synthesis of $HW(CO)_3C_5H_5$. Isolation is simply effected by evaporation of the solvent and sublimation of the residue.
(13) R. B. King and M. B. Bisnette, *J. Organometal. Chem.*, **2**, 15 (1964).
(14) For example, the acyl carbonyl band in $CF_3COW(CO)_3C_5H_5$ was observed at 1616 cm^{-1}; see ref 13.