hydration" of 3-methyluridine is remarkable, because the comparable *photohydration* has an energy requirement of *ca.* 40 kcal/mol.[11] The rate enhancement must be due to a new kind of intramolecular 6,5′ interaction only observable after alkylation of the N-3 position. The conformational requirements for maximum effect are quite strict: while 3-methyl-2′,3′-isopropylideneuridine is very alkali labile, the analogous 2′,3′-cyclic phosphate, which differs by bond angles, ring strain, and opportunity for 3′,5′ interaction, was found to be surprisingly stable.

Incubation of 3-methylnucleotides and poly-3-methylnucleotides with alkali showed that poly-3-methyluridylic acid and 3-methyluridine dinucleotide were broken down slowly in 0.1 *N* NaOH aqueous solution as compared to 3-methyl-2′,3′-*O*-isopropylideneuridine. Apparently depolymerization precedes base degradation. Since nucleophilic reactions are greatly promoted by addition of DMSO,[12,13] considerable rate enhancement of the base-catalyzed degradation of poly-3-methyluridylic acid was observed in a mixture of water–DMSO (40:60 v/v). DMSO accelerates both depolymerization of poly-3-methyluridylic acid and nucleophilic attack by base. Alkaline hydrolysis of N-methylated polyuridylic acid to mononucleotides has met with difficulties before[14] and in the light of these findings may not be possible.

In view of the importance of methylation in the process of viral infection[15] the possible base lability of 3-methyluridine and other methylated pyrimidines and purines[1] becomes a matter of interest and concern.

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(16) Associate in the Visiting Program of the U. S. Public Health Service, 1966–1970.
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High-Resolution Boron Nuclear Magnetic Resonance. I. Pentaborane(9)

Sir:

The ^{11}B nmr spectra of boron hydrides usually show well-resolved splitting of peaks due to coupling between boron and terminal hydrogens. Except for diborane(6) and tetraborane(10) additional fine structure is usually not observed in the separate resonances.[1] This paucity of fine structure has been attributed to quadrupole relaxation effects of the ^{11}B nucleus.[2,3]

However, Williams *et al.*,[4] speculated that the broadness of the lines in that portion of the ^{11}B nmr spectrum representing the resonance of the apex boron of pentaborane(9) (I) was not due to nuclear quadrupole broadening but resulted from unresolved boron–boron coupling. More recently, the measurement of ^{10}B and ^{11}B spin–lattice relaxation times in diborane(6) and pentaborane(9) led to the conclusion that the ^{11}B nmr line widths should be only several hertz at room temperature and that the observed line widths were, in all likelihood, due to complex unresolved splittings.[5]

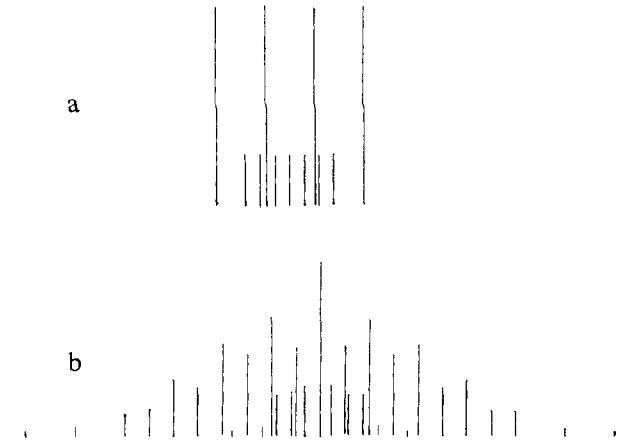
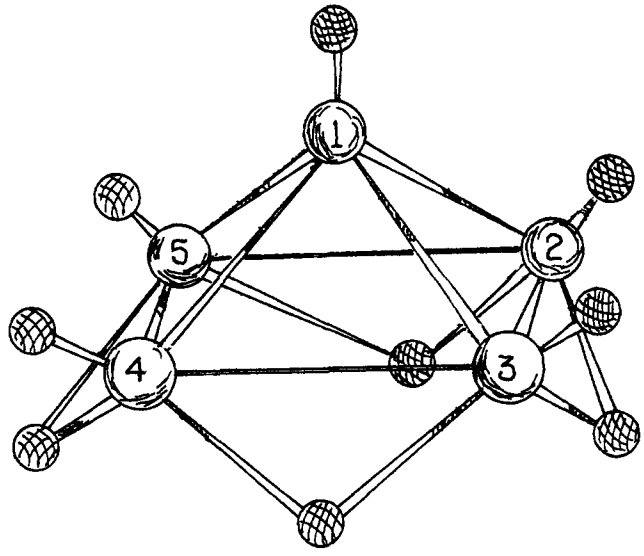


Figure 1. Predicted ^{11}B nmr spectrum of B_5H_9 assuming a value of $J_{^{11}B^{11}B}$ of 20 Hz: (a) basal boron resonance; (b) apex boron resonance.



I, B_5H_9

It is therefore of interest to reexamine the ^{11}B nmr spectrum of pentaborane(9) under high-resolution conditions in order to critically evaluate the hypothesis stated above. In order to obtain high-resolution ^{11}B spectra it is necessary to remove *all* proton coupling to boron, since the B_5H_9 spin system is very complicated. Assuming a particular value for $J_{^{11}B^{11}B}$ and that the ^1H noise-decoupled[6] spectrum of isotopically normal B_5H_9 will be first order, one can easily arrive at the predicted spectra shown in Figure 1. Figure 1a depicts the spectrum of the basal borons which are deshielded with respect to the resonance of the apex boron, Figure 1b, by 40.8 ppm It is essential to note that by assuming a

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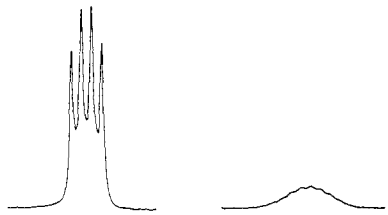

Figure 2. Experimental ^{11}B nmr spectrum (32.1 MHz) of a 40% solution of B_5H_9 in C_6F_6 showing a $J_{^{11}B^{11}B}$ of 19.4 Hz and a chemical shift between the base and the apex of 40.8 ppm. The C_6F_6 furnished the signal for the ^{19}F lock.

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.

Acknowledgment. The XL-100-15 nmr spectrometer was purchased through a National Science Foundation Department Development Grant. J. D. O. is grateful to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and P. D. E. to the University of South Carolina Committee on Research and Productive Scholarship for support of this research.

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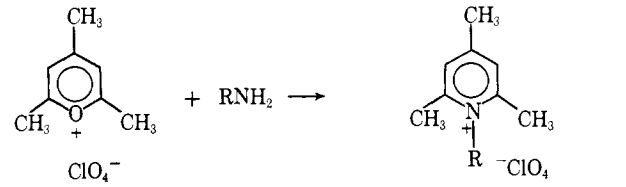
Chemical Modification of Proteins by Pyrylium Salts

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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