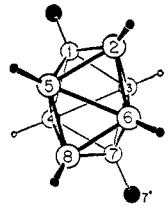

Figure 1. The structure of $B_6H_6C_2(CH_3)_2$. Methyl groups at 1′ and 7′ are attached to carbon atoms 1 and 7, respectively. Bond distances (all ±0.01 A) are C_1–B_2 = 1.50, C_1–B_3 = 1.70, C_1–B_4 = 1.60, C_1–B_5 = 1.70, B_2–B_3 = 1.77, B_2–B_5 = 1.81, B_2–B_6 = 1.70, B_3–B_4 = 1.89, B_3–B_6 = 1.84, and B_5–B_6 = 1.90 A. The B_2–B_8 distance is 2.87 A while the C_1–C_7 distance is 2.60 A. Other distances are related to these by the molecular twofold axis.

1.76 A.[9] It is even more interesting that the B_3–B_4 and B_5–B_6 distances of 1.89 and 1.90 A, respectively, are more than five standard deviations longer than the 1.84-A distances, B_3–B_6 and B_4–B_5. Thus there appears to be a tendency for both the $C_1B_3B_4C_7$ unit [$(C_1$–$C_7)/(B_3$–$B_4)$ = 1.37] and the $B_2B_5B_8B_6$ unit [$(B_2$–$B_8)/(B_5$–$B_6)$ = 1.51] to deviate slightly toward a squarelike shape, as compared with the idealized ratio of 1.62 from, for example, an icosahedron.

Molecular orbital calculations will be presented in the full paper after revision of our program to new methods[10] and after determination of other carborane structures. All previous molecular orbital studies have been carried out on idealized geometries; it will probably be of importance to reexamine these molecules theoretically when detailed geometries become available.

Note Added in Proof. The geometry of $B_8H_8^{2-}$, which is isoelectronic with $B_6H_6C_2H_2$, has been described by F. Klanberg, D. R. Eaton, L. J. Guggenberger, and E. L. Muetterties [*Inorg. Chem.*, **6**, 1271 (1967)]. The geometry and distortions from regular distances are like those found in B_8Cl_8 which has consistently been described as a molecule of D_{2d} symmetry (see ref 2).

Acknowledgment. We wish to thank M. F. Hawthorne for the sample, the Office of Naval Research for support of the research, and the National Science Foundation for a fellowship to H. V. H.

(9) T. F. Koetzle, F. E. Scarborough, and W. N. Lipscomb, submitted for publication. The framework C atoms in $B_7H_7C_2(CH_3)_2$ are at two of the three apical positions, which are each coordinated to four B atoms.

(10) M. D. Newton, F. P. Boer, and W. N. Lipscomb, *J. Am. Chem. Soc.*, **88**, 2353 (1966).

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The Reaction of Ribonuclease-A with *o*-Nitrophenyl Hydrogen Oxalate

Sir:

We report herein the initial results of our study of the reaction of *o*-nitrophenyl hydrogen oxalate anion (*o*-NPO⁻) with RNase.[1] The reaction of *p*-nitrophenyl acetate with RNase has, in our hands as well as in those of Ross and co-workers,[2] been found to be a very slow reaction comparable to the nonenzymic reaction of this ester with insulin.[3] In contrast the reaction of RNase with the negatively charged *o*-NPO⁻ proceeds in two parts (Figure 1): (1) an "initial burst" which is first order in both enzyme and substrate following Lineweaver–Burk kinetics; and (2) a slower liberation of *o*-nitrophenol in a nonenzymic bimolecular reaction of protein and substrate to completion of hydrolysis. Extrapolation of the initial-burst reaction to zero buffer provides $K_{m(app)}$ = 1.25 × 10⁻³ *M* and k_{cat} = 0.31 min⁻¹.[4] Comparison of $K_{m(app)}$ and k_{cat} for *o*-NPO⁻ to the values for cytidine 2′,3′-cyclic phosphate[5,6] indicates that the apparent binding constant for the cyclic phosphate ester is approximately four times greater and k_{cat} 100 times greater than the corresponding constants for *o*-NPO⁻ (with acetate buffer K_m = 3 × 10⁻⁴ *M* and k_{cat} = 39 min⁻¹ for the cyclic phosphate ester).

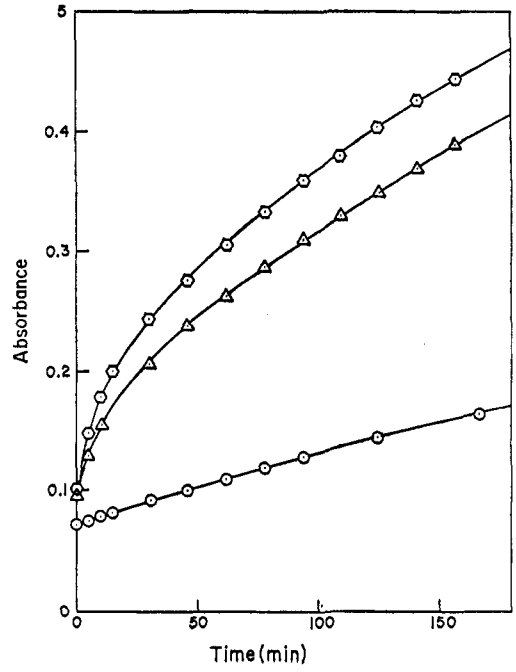

Figure 1. Plot of absorbance (at 372.5 mμ, the isosbestic point for *o*-nitrophenol and *o*-nitrophenolate) *vs.* time for the reaction of bovine ribonuclease-A with *o*-nitrophenyl hydrogen oxalate anion (4.45 × 10⁻⁴ *M*) at 30°; pH 5.9 in 0.1 *M* collidine perchlorate buffer (○, enzyme = 3.08 × 10⁻⁵ *M*; △, enzyme = 2.2 × 10⁻⁵ *M*; ○, no enzyme).

For the reaction of the RNase derivative S-protein,[7] $K_{m(app)}$ was found to be the same as for RNase while k_{cat} was found to be decreased by 40%. For Asp_{53} converted to methyl ester $K_{m(app)}$ also remains the same as for RNase, but k_{cat} is decreased by but 20%. Completely reduced RNase with all sulfhydryl groups carboxymethylated and RNase with all S–S bonds oxidized

(1) For the rational leading to this study see T. C. Bruice and B. Holmquist, *J. Am. Chem. Soc.*, **89**, 4028 (1967).

(2) C. A. Ross, A. P. Mathias, and R. B. Rabin, *Biochem. J.*, **85**, 145 (1962).

(3) E. S. Hartley and B. A. Kilby, *ibid.*, **56**, 288 (1954).

(4) $E + S \underset{}{\overset{K_{m(app)}}{\rightleftharpoons}} ES \xrightarrow{k_{cat}}$ products + E

(5) D. G. Herries, A. P. Mathias, and R. B. Rabin, *Biochem. J.*, **85**, 127 (1962).

(6) H. Witzel, *Progr. Nucleic Acid Res.*, **2**, 221 (1963).

(7) S-Protein, RNase with residues 1–20 removed by subtilisin, was found to have 6% activity toward cyclic phosphate. Considering this to be due to contamination by RNase the determined value of k_{cat} for S-protein reacting with *o*-NPO⁻ has been corrected.

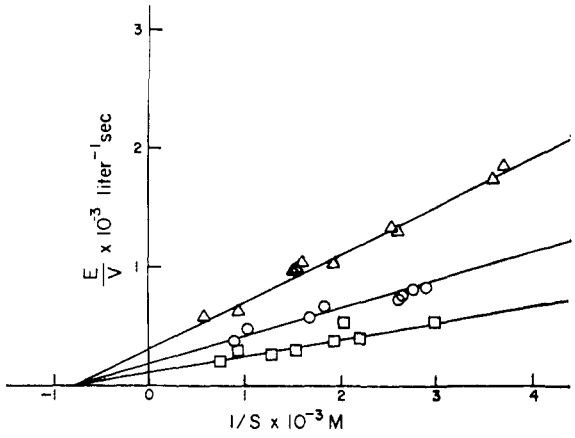

Figure 2. Plot of total enzyme divided by the initial velocity *vs.* the reciprocal of the substrate concentration for the reaction of bovine ribonuclease-A with *o*-nitrophenyl hydrogen oxalate anion at various concentrations of collidine perchlorate buffer (△, 0.2 *M*; ○, 0.1 *M*; □, 0.05 *M*) at 30° and pH 5.9.

exhibit no initial burst reaction with *o*-NPO⁻ though the reaction representing the second phase (Figure 1) is still present. His_{12}-carboxymethylated RNase (as a 2.15×10^{-5} *M* solution in 0.1 *M* phosphate buffer containing 0.05% phenol) was indistinguishable from RNase at the same concentration (for 18 kinetic runs for each).[8] Because we could not obtain His_{12}-carboxymethylated RNase as other than a dilute solution, we can say no more. His_{119}-carboxymethylated RNase exhibits an initial burst reaction smaller than that of S-protein.

The catalysis of the hydrolysis of cytidine 2′,3′-cyclic phosphate by RNase is known to be greatly dependent upon the integrity of His_{12} and His_{119}, as well as upon the tertiary structure of the enzyme which is assured by the intactness of all four disulfide bonds. Of these features the intactness of the four disulfide bonds of the S-protein is essential for *o*-NPO⁻ activity. The lack of activity of the cyanoethylated RNase suggests the involvement of an amino group in binding of *o*-NPO⁻ or in the catalytic process. The inability of oxidized RNase and reduced and carboxymethylated RNase to exhibit the initial burst and the almost complete lack of reactivity of bovine serum albumin, lysozyme, pepsin, and trypsinogen with *o*-NPO⁻ provide evidence that we are not dealing with a completely nonspecific protein reagent.

The initial burst is not subject to product inhibition at substrate levels nor inhibition by phenol (to 0.17 *M*), cytidine (at 3×10^{-4} *M*), and phosphate or chloride ion (at 1.22×10^{-3} *M*). RNase activity is known to be only weakly inhibited by cytidine,[9] and binding of phosphate and chloride ions[10,11] is also weak. Cytidylic acid, ATP ($K_i = 3.8 \times 10^{-3}$ *M*), and CTP are strong competitive inhibitors of the cyclic phosphate,[9] whereas toward *o*-NPO⁻ they are strong noncompetitive inhibitors ($K_i \cong 1.5 \times 10^{-4}$ *M*, 1×10^{-4} *M*, and 8×10^{-4} *M* for cytidylic acid,[12] ATP, and CTP, respectively). The cytidylic inhibition may be employed to determine what derivatives of RNase not possessing RNase activity still bind inhibitor. Thus, the reaction of S-protein with *o*-NPO⁻ is inhibited by cytidylic acid ($K_i = 6.6 \times 10^{-3}$ *M*). Cytidylic acid also inhibited the reaction of His_{119}-carboxymethylated RNase. From this result binding of cytidylic acid is not dependent on the intact nature of His_{12} or His_{119}. It has been reported that neither of the carboxymethylhistidine derivatives of RNase complexes with 2′-cytidylate.[13] Our data are not in accord with these results and tend to support the data of Ross, Mathias, and Rabin that cytidylic acid is bound to carboxymethylribonuclease.[14] Iodoacetate (at 1.8×10^{-3} *M*) and collidine or lithium perchlorate (for both, $K_i = 0.03$ *M*; Figure 2) are noncompetitive inhibitors of *o*-NPO⁻, whereas anions are known to be competitive inhibitors of the cyclic phosphate.[9,15,16] Thus, anionic species which are known to be competitive inhibitors of cytidine 2′,3′-cyclic phosphate are noncompetitive inhibitors of *o*-NPO⁻. It is known that anions bind to more than one site on RNase,[17-19] and the reaction of *o*-NPO⁻ likely occurs near one of these sites. The noncompetitive inhibition is probably due to the binding of cytidylic acid or other inhibitor at an anion binding site other than the active site toward *o*-NPO⁻. One might conclude that the positive binding site afforded by Arg_{10} and the group His_{12} is not essential to *o*-NPO⁻ activity.

All data reported herein have been collected at the single pH of 5.9 and at a temperature of 30° and, unless otherwise indicated, in 0.1 *M* collidine perchlorate buffer.[20,21]

(8) His_{119}-carboxymethylated RNase was found to have approximately 1% activity toward cyclic phosphate compared to the native enzyme while the His_{12} derivative had less than 1% activity.

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(13) S.-T. Yang and J. P. Hummel, *J. Biol. Chem.*, **239**, 3775 (1964).

(14) C. A. Ross, A. P. Mathias, and R. B. Rabin, *Biochem. J.*, **85**, 145 (1962).

(15) M. Irie, *J. Biochem.* (Tokyo), **37**, 355 (1965).

(16) J. P. Hummel, M. Flores, and G. Nelson, *J. Biol. Chem.*, **233**, 717 (1958).

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(18) G. I. Loeb and H. A. Saroff, *Biochemistry*, **3**, 1819 (1964).

(19) Y. P. Myer and J. A. Schellmen, *Biochim. Biophys. Acta*, **55**, 361 (1962).

(20) F. A. Long and J. G. Pritchard, *J. Am. Chem. Soc.*, **79**, 2365 (1957).

(21) We gratefully acknowledge the kindness of F. M. Richards, C. H. W. Hirs, A. R. Crestfield, and J. P. Riehm in providing us with ribonucleases, and the National Institutes of Health for financial support. In addition we wish to thank Drs. A. R. Crestfield, G. G. Hammes, S. Moore, F. M. Richards, and W. H. Stein for helpful suggestions.

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The Direct Observation of a Cationic Intermediate in a Carbonyl-Assisted Solvolysis

Sir:

The participation of the carbonyl oxygen in the solvolysis of γ-halobutyrophenones (Ia,b) has been suggested by Oae[1] and by Pasto and Serve.[2] To explain the observed rate enhancement relative to the corre-

(1) S. Oae, *J. Am. Chem. Soc.*, **78**, 4030 (1956).

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