zoylglycine as described above.[8] No detectable hydrolysis of the methyl ester occurred with CPA and phenylalanine. By comparison with the ^{18}O exchange rate, it could thus be determined that k_{CH_3OH}/k_{H_2O} is less than 0.0003.

Thus, the enzyme cannot incorporate methanol in the transition state of the reaction (run in either direction) for either ester or peptide substrates.[9] This suggests that removal of both protons of water is required in the transition state for hydrolysis.

From these data all together, we suggest the mechanism shown in Scheme I for the hydrolysis of peptide substrates. The glutamate carboxylate acts as a general base to deliver nucleophilic water to the carbonyl, but if this were instead methanol then the first step would simply reverse. Only a second deprotonation could drive the reaction in the forward direction, and this proton transfer might well involve the tyrosine hydroxyl[10] as a bridge between the OH and the N as we have suggested for our model system.[3]

Much controversy[11] has surrounded the question of whether Arg-145 or Zn^{2+} is the binding site for substrate carboxylate. Our mechanism indicates that *both* are true, the Zn^{2+} binding the carboxylate of one hydrolysis product which is thus the substrate for the *reverse* reaction. In general one would expect that an exopeptidase should have two alternate binding sites, separated by a distance corresponding to one residue in the substrate, as in this mechanism.

References and Notes

(1) For reviews, see (a) E. T. Kaiser and B. L. Kaiser, *Acc. Chem. Res.*, **5**, 219 (1972); (b) W. N. Lipscomb, *Tetrahedron*, **30**, 1725 (1974); (c) F. A. Quiocho and W. N. Lipscomb, *Adv. Protein Chem.*, **25**, 1 (1971).

(2) Our model studies (R. Breslow, D. E. McClure, R. S. Brown, and J. Eisenach, *J. Am. Chem. Soc.*, **97**, 194 (1975)) show that an anhydride intermediate cannot be excluded by the failure of trapping experiments.

(3) R. Breslow and D. E. McClure, *J. Am. Chem. Soc.*, preceding paper in this issue.

(4) L. M. Ginodman, N. I. Mal'tsev, and V. N. Orekhovich, *Biokhimiya*, **31**, 1073 (1966); *Biochemistry (USSR)*, **31**, 931 (1966). It is interesting that the Russian authors, despite their ^{18}O exchange results, concluded that an acyl–enzyme intermediate was unlikely on the basis of other evidence.

(5) N-Acetylphenylalanine is hydrolyzed by the enzyme rapidly enough (J. E. Snoke and H. Neurath, *J. Biol. Chem.*, **181**, 789 (1949)) to produce the phenylalanine needed to account for the reported ^{18}O exchange. Such partial hydrolysis can explain some of the data in ref 4, but not[5] that for N-acetylglycine. Of course other amino acids are likely contaminants in peptidase preparations.

(6) N-Acylglycines are not hydrolyzed at an appreciable rate by carboxypeptidase A. As expected from this fact, assuming that it is resynthesis of peptides which accounts for the catalytic effect of added amino acids on ^{18}O exchange in N-acylamino acids, glycine is not an effective catalyst.

(7) It is actually excluded, except for the possibility that acyl–enzyme hydrolysis and resynthesis are strongly *catalyzed* by enzyme-bound amino acids, but not by enzyme-bound hydroxy acids. At this point Occam's razor must be applied. Note that the effectiveness of amino acids (Table I) correlates with their expected effectiveness in peptide resynthesis, as judged by enzymatic hydrolysis rates for the corresponding N-acyl amino acid linkages. This is particularly striking in the comparison of L-leucine (acyl leucines are good substrates) with N-methyl-L-leucine (the corresponding peptides are poor substrates).

(8) $[Bz\text{-}Gly\text{-}CO^{18}OH, 76\%\ ^{18}O] = 9.8 \times 10^{-3}$ M, $[Bz\text{-}Gly\text{-}CO_2CH_3] = 9.8 \times 10^{-4}$ M, $[Phe] = 9.8 \times 10^{-4}$ M, $[CPA] = 9.8 \times 10^{-7}$ M, pH 7.5, $\mu = 0.1$, N-ethylmorpholine–acetic acid buffer, $20.0°$. Aliquots were freeze–dried and the residues chromatographed, then submitted to mass spectral and uv assay. Taking V_{hyd} as the observed initial rate of ester hydrolysis, k_0 as the first-order rate constant for ^{18}O exchange, and K_{hyd} as the equilibrium constant for hydrolysis of the ester (8×10^4 M at pH 7.5), the ratio of methanolysis and hydrolysis rate constants normalized to equal concentrations of methanol and water is

$$k_{MeOH}/k_{H_2O} = \frac{V_{hyd} \cdot 55\ M}{2k_0 K_{hyd}[ester]_{initial}}$$

(9) Other workers have apparently[1c] failed to observe methanolysis of substrates; for peptides the reaction would be endothermic. We find that *no* nucleophiles (NH_3, NH_2OH, $NaBH_4$) except H_2O are used by CPA, making this aspect of its substrate requirement completely specific.

(10) Direct evidence for the involvement of Tyr-248 is not solid, although its acetylation leads to loss[1c] of peptidase activity by CPA.

(11) Cf. D. S. Auld and B. Holmquist, *Biochemistry*, **13**, 4355 (1974), and references therein.

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Benzocyclobutadiene

Sir:

We wish to report the direct spectroscopic observation of benzocyclobutadiene. Benzocyclobutadiene has been the subject of an extensive literature.[1] A variety of methods for generating benzocyclobutadiene as a reactive intermediate is known, but direct observation of this intermediate has not been possible.[1-5]

We have developed a method for generating clean samples of benzocyclobutadiene matrix isolated in argon. The apparatus is shown in Figure 1. *cis*-1,2-Diiodobenzocyclobutene is heated to 65 °C at 10^{-6} mm by the first heater to provide a sufficient rate of sublimation. The diiodide vapor is passed over zinc powder heated to 230 °C by the second heater. The vapor is then passed through a zone cooled by acetone evaporation. Small quantities of two products, benzocyclobutadiene dimer and *trans*-diiodide, deposit in the cooled region. The matrix deposited on the cesium iodide plate is free of both dimer and *trans*-diiodide. After deposition, the vacuum shroud is rotated 90° for infrared spectroscopic observation.[6] The same procedure is used for ultraviolet spectroscopic observation except that a sapphire plate is used.[6] The infrared spectrum of benzocyclobutadiene is shown in Figure 2 and the ultraviolet spectrum in Figure 3. The species in the matrix is identified as benzocyclobutadiene by the thermal dimerization above 75 K to the known benzocyclobutadiene dimer. The disappearance of the infrared and ultraviolet absorption bands due to benzocyclobutadiene and the concurrent appearance of the absorption bands due to the dimer establish the identity of the matrix isolated species as benzocyclobutadiene. In the infrared experiment, the dimer was washed from the window and further characterized by comparison of mass spectra, ultraviolet spectra, and thin layer chromatographic behavior in five solvent systems with authentic benzocyclobutadiene dimer. Attempts to degrade benzocyclobutadiene to benzyne and acetylene by ultraviolet irradiation were unsuccessful. Benzocyclobutadiene is quite stable to irradiation in an argon matrix.
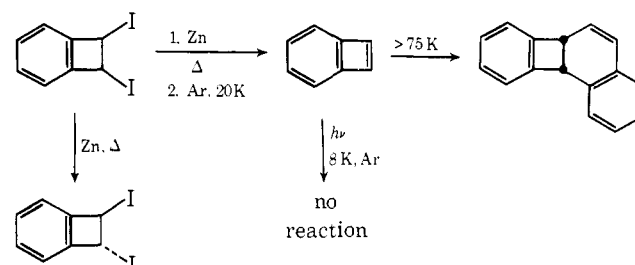
The most intense band ($737\ cm^{-1}$) in the infrared spectrum of benzocyclobutadiene is the deformation mode due to the four carbon–hydrogen bonds in a 1,2-disubstituted benzene. The band at $700\ cm^{-1}$ is probably one of the deformation modes of the olefinic carbon–hydrogen bonds (cf. 650 and $570\ cm^{-1}$ for the in-plane and out-of-plane modes for cyclobutadiene[7,8]). The ultraviolet spectrum of benzocyclobutadiene (λ_{max}^{Ar} 243, 246.5, 256, 264, 270, 281.5, and
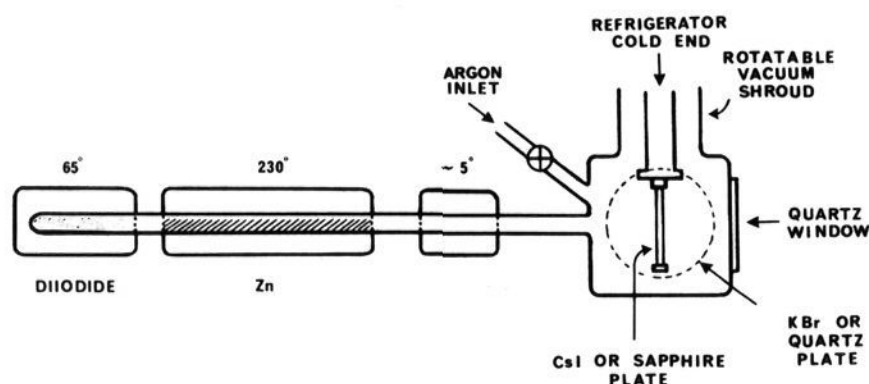
Figure 1. Apparatus for thermal generation and spectroscopic observation of benzocyclobutadiene. The system is evacuated to 10^{-6} mm during use.
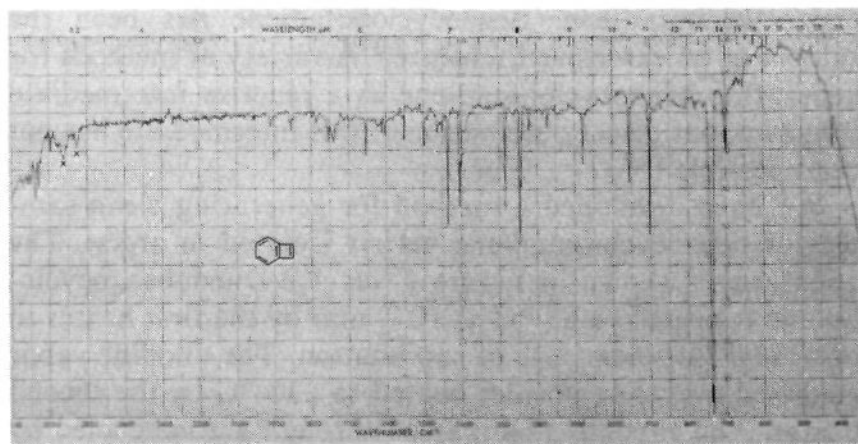

Figure 2. Infrared spectrum of benzocyclobutadiene matrix isolated in argon at 8 K. Bands marked × were in the spectrum of the cesium iodide plate before deposition.
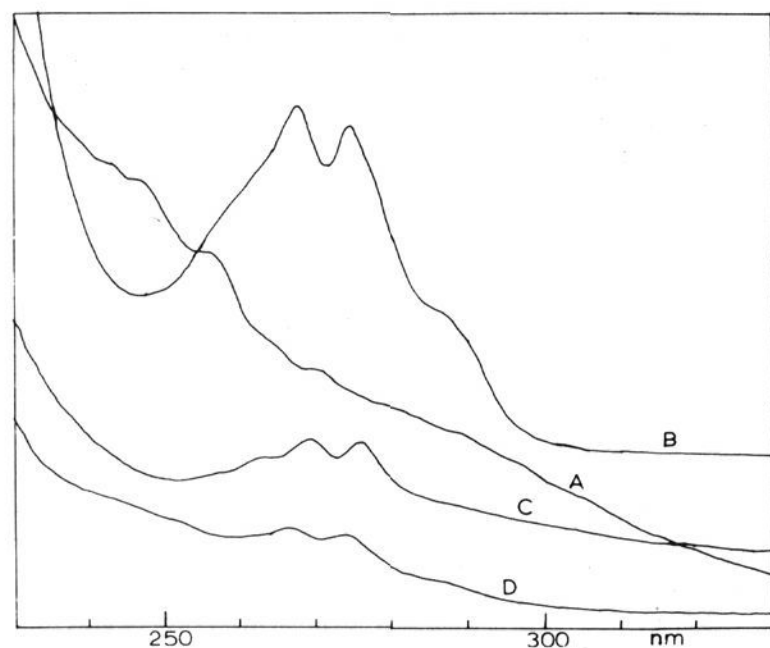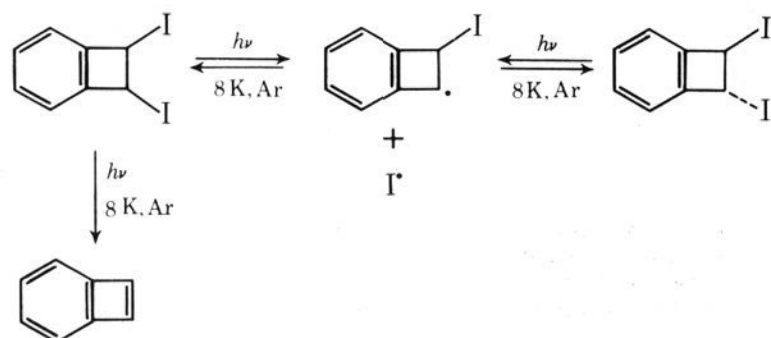

Figure 3. (A) Ultraviolet spectrum of benzocyclobutadiene matrix isolated in argon at 8 K. (B) Ultraviolet spectrum of authentic benzocyclobutadiene dimer in 95% ethanol solution. (C) Ultraviolet spectrum of sample A after warming above 75 K. (D) Ultraviolet spectrum of the product recovered from the cesium iodide window after warming the sample shown in Figure 2 to room temperature. Vertical axis is optical density. The relative positions of A, B, C, and D are arbitrary and do not show relative optical densities.

289 nm) shows considerable structure over a broad range. The rapidly rising baseline in the ultraviolet spectrum is due to light scattering by the argon. On warming, the benzocy-

clobutadiene dimer absorption appears (Figure 3). In this process, the argon is pumped off, and light scattering is less serious.

Irradiation of *cis*-diiodide matrix isolated in argon at 8 K gives two primary products, *trans*-diiodide (major) and benzocyclobutadiene (minor). Similar irradiation of the *trans*-diiodide gives only *cis*-diiodide as a primary product. When the irradiations are monitored by ESR a free radical (presumably the 2-iodobenzocyclobutyl radical) signal is observed.
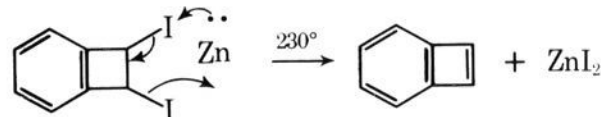
It is instructive to note that the zinc-induced elimination is much more facile with the *cis*- than with the *trans*-diiodide. This observation suggests the possibility of a cyclic elimination.

References and Notes

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Hydrozirconation. V. γ,δ-Unsaturated Aldehydes and Halides from 1,3-Dienes via Organozirconium(IV) Intermediates

Sir:

The hydride $(\eta^5\text{-}C_5H_5)_2Zr(H)Cl$ (**1**) can be used to prepare reactive precursors of a variety of alkyl or alkenyl organic compounds from olefins or acetylenes, respectively. The overall mode of addition of **1** to these unsaturated hydrocarbons in many ways parallels reactions known for several main group or transition metal hydrides. We find now, however, that the course of addition of **1** to 1,3-dienes is significantly different from that observed for these other classes of hydrides. In contrast to boron[1] or aluminum[2] hydrides which often doubly metalate 1,3-dienes or give a mixture of products, or to most transition metal hydrides which add 1,4 or 1,2 to yield allylic complexes,[3,4] **1** reacts with a variety of 1,3-dienes via 1,2-addition to the sterically less hindered olefinic unit of the substrate to give γ,δ-unsaturated (homoallylic) complexes in high yield (80–90%, reaction 1). These compounds, in turn, can be used to prepare desirable organic products, γ,δ-unsaturated aldehydes or halides, under mild conditions.

1,3-Dienes react more slowly with **1** than do terminal olefins (relative rate ca. 1:50); the γ,δ-unsaturated zirconium