as a series of di-*n*-butylbenzenes which are not readily accessible by conventional methods, can be obtained in one step. (2) Only C_{sp^2} halides, such as vinylic and aromatic, can be used, and especially the high reactivity of *chlorides* offers one of the most remarkable features.[18,19] (3) A bidentate diphosphine as a ligand exhibits the remarkable catalytic activity and the activity decreases in the order[21] $[NiCl_2(dpp)]$[22] > $[NiCl_2(dpe)]$ > $[NiCl_2(dmpe)]$[22] ≈ $[NiCl_2(PPh_3)_2]$ ≫ $[NiCl_2(PEt_3)_2]$ ≈ $[NiCl_2(PPh_2Me)_2]$. This order suggests that the cis configuration of two organic groups in the diorganonickel intermediate **3** is the first requisite of the catalyst. (4) Both *cis*- and *trans*-1,2-dichloroethylene react with a phenyl Grignard reagent rather nonstereospecifically to give a mixture of *cis*- and *trans*-stilbene anomalously enriched with the cis isomer.[23] (5) Diethyl ether as a solvent is definitely superior to tetrahydrofuran, in contrast with Tamura and Kochi's catalysts.[6]

Further studies are required before the stereochemical and mechanistic details can be understood, apart from the working hypothesis described above.

Investigations are continuing on the extension, refinement, and application of the promising reaction reported here.

(18) Bromides and iodides have been used so far in almost all cases.[6,8-10]

(19) It has been confirmed that the sp^2 carbon–chlorine bond undergoes a reaction of eq 1 type[11,12] and oxidative additions to low valent nickel complexes.[20]

(20) R. Ugo, *Coord. Chem. Rev.*, 3, 319 (1968); D. R. Fahey, *J. Amer. Chem. Soc.*, **92**, 402 (1970); D. H. Gerlach, A. R. Kane, G. W. Parshall, J. P. Jesson, and E. L. Muetterties, *ibid.*, **93**, 3543 (1971); M. Hidai, T. Kashiwagi, T. Ikeuchi, and Y. Uchida, *J. Organometal. Chem.*, 30, 279 (1971); see also J. H. Nelson and H. B. Jonassen, *Coord. Chem. Rev.*, **6**, 27 (1971).

(21) This order was observed in the coupling of *n*-butylmagnesium bromide with chlorobenzene.

(22) The ligands dpp and dmpe refer to $Ph_2P(CH_2)_3PPh_2$ and $Me_2PCH_2CH_2PMe_2$, respectively.

(23) This reaction may offer a facile route to *cis*-stilbene.

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Evidence for the Participation of Aspartic Acid-194 in a New Acylation–Deacylation Reaction of α-Chymotrypsin

Sir:

Circumstantial evidence based on X-ray data suggests that the unusual reactivity of the acyl group acceptor (Ser-195) of α-chymotrypsin (α-CT) would originate from its participation in a proton relay system involving an array of hydrogen bonds between Asp-102, His-57, and Ser-195.[1-4] In addition, a salt bridge between Asp-194 and Ile-16 would somehow favor a precise alignment of these residues with some key parts of substrates.[1-4] In fact, acylation of Ile-16 virtually abolishes activity.[5] However, analogous direct experimental evidence for the precise role of Asp-194 in catalysis is still lacking. We submit experimental observations which offer new perspectives on the elusive role of this acid residue in α-CT catalysis.

The potent depressant[6] and peptide bond forming reagent EEDQ[7] (1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) smoothly transforms carboxyl groups into mixed anhydrides.[7] It also inhibits, sometimes irreversibly, certain serine hydrolases including α-CT.[8] It appears that carboxyl functions act as special "recognition sites" for EEDQ.[7,8] We have confirmed this by comparing the effects of various anions on the acid-catalyzed decomposition of EEDQ to quinoline, CO_2, and ethanol. It can be seen in Figure 1 that in the pH range of 4.5–6.5, acetate is about ten times more efficient than other common anions and thus behaves as a special catalyst of these decomposition reactions. General acid catalysis of the displacement of the 2-ethoxy group by acetate would give an intermediate decomposing irreversibly to a mixed anhydride by a downhill concerted process seemingly unique to carboxyl functions[7] [and perhaps phosphate to some extent (Figure 1)]. The intermediacy of the mixed anhydride when acetate is present was readily confirmed using hydroxylamine as the trapping agent in the usual manner.

When α-CT (0.2 mg/ml in 0.1 *M* NaCl) was exposed at 25° and acid pH to EEDQ at 1–5 × 10^{-5} *M*, inhibition of L-ethyl *N*-acetyltyrosinate hydrolysis (assay at pH 8) built up rapidly, the rate of inhibition appearance being strongly dependent on both EEDQ concentration and pH. Prior addition of proflavin (a known competitive inhibitor of α-CT[9]) at 4 × 10^{-4} *M* afforded effective protection against EEDQ attack. Typical reciprocal plots for EEDQ inhibition of α-CT at various pH values are shown in Figure 2. Replotting of the appropriate data as in Figure 3 produced a bell-shaped curve[10] whose characteristics allow the following conclusions; the pH_{opt} for inhibition is 5.5 ± 0.2 and two ionizing groups of respective pK_{app} 4.5 ± 0.2 and 6.3 ± 0.2 appear to control the rate. These constants are respectively characteristic of carboxyl and imidazole functions.

At alkaline pH, the EEDQ-inhibited α-CT regenerates swiftly to the extent of 85–90% within 60 min. The pH dependency of this reaction was studied in detail using inhibited α-CT rapidly freed of excess reagent by gel filtration (Sephadex-G25, 0.1 *M* NaCl, 25°, pH 5.5). The results are summarized in Figure 4 where the pH dependency of the regeneration step for the ethoxycarbonyl Ser-195 derivative[11] (prepared from *p*-nitrophenyl ethylcarbonate and α-CT according to the literature[12]) as well as that of ethoxycarbonylimidazole hydrolysis have been included. A pH dependency similar to that of ethoxycarbonylimidazole may be expected for the hydrolysis of a mixed anhydride.[13]

(1) D. M. Blow, J. J. Bivktoft, and B. S. Hartley, *Nature (London)*, **221**, 337 (1969).

(2) T. A. Steitz, R. Henderson, and D. M. Blow, *J. Mol. Biol.*, **46**, 337 (1969).

(3) D. M. Blow and T. A. Steitz, *Annu. Rev. Biochem.*, **39**, 63 (1970).

(4) D. M. Blow in "The Enzymes," Vol. III, P. D. Boyer, Ed., Academic Press, New York, N. Y., 1971, p 185.

(5) C. Ghelis, J. Labouesse, and B. Labouesse, *Biochem. Biophys. Res. Commun.*, **29**, 101 (1967).

(6) R. R. Martel, R. Berman, and B. Belleau, *Can. J. Physiol. Pharmacol.*, 47, 909 (1969).

(7) B. Belleau and G. Malek, *J. Amer. Chem. Soc.*, **90**, 1651 (1968).

(8) B. Belleau, V. DiTullio, and D. Godin, *Biochem. Pharmacol.*, **18**, 1039 (1969).

(9) R. A. Wallace, A. N. Kurtz, and C. Niemann, *Biochemistry*, **2**, 824 (1963).

(10) The curve in Figure 3 need not be corrected for reactivation because in the relevant range of pH values (4.5–6.5), the rates of inhibition are 100–200 times greater than the rates of reactivation.

(11) W. B. Melchior, Jr., and D. Fahrney, *Biochemistry*, **9**, 251 (1970).

(12) B. S. Hartley and B. A. Kilby, *Biochem. J.*, **56**, 288 (1954); A. A. Shah and K. A. Connors, *J. Pharm. Sci.*, **57**, 282 (1968).

(13) T. C. Bruice and S. Benkovic, "Bio-organic Mechanisms," Vol. I, W. A. Benjamin, New York, N. Y., 1966, p 4. We are grateful to a

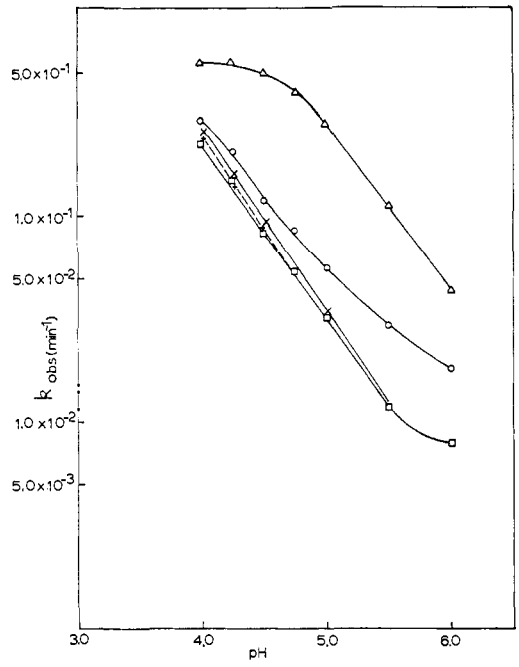

Figure 1. Effects of pH and anions at 25° on rate of quinoline formation (absorbance at 312 nm) from acid-catalyzed decomposition of EEDQ at initial concentrations of 8.5×10^{-5} *M* in 0.1 *M* NaCl: (□) control; (+) 0.02 *M* Na_2SO_4; (×) 0.02 *M* $NaClO_4$; (○) 0.02 *M* Na_2HPO_4; (△) 0.02 *M* AcONa.

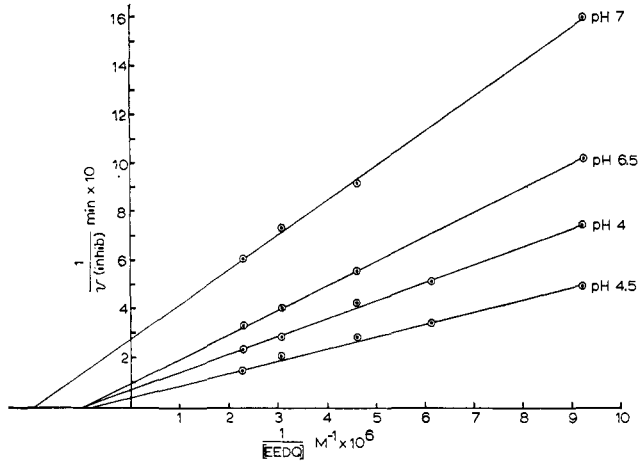

Figure 2. Reciprocal plots at typical pH values for velocity of α-CT inhibition (L-N-Ac-Tyr-OEt as substrate, assay at pH 8, 0.1 *M* NaCl, 25°) by EEDQ at various initial concentrations (v = initial velocities of inhibition build-up expressed as fractions of the total α-CT inhibited per min).

It is clear that regeneration of EEDQ-inhibited α-CT and hydrolysis of either a mixed anhydride[13] or ethoxycarbonylimidazole[11] have very similar pH profiles differing markedly from the pH dependency of the acyl-Ser-195 hydrolysis. When EEDQ labeled with ^{14}C in the ethoxycarbonyl moiety was used, the incorporation of label by α-CT was stoichiometric and linear with degree of inhibition. Subsequent regeneration at pH 8 caused loss of label to the extent of 88 ± 3%, the balance of stably bound label coinciding with the 12 ± 3% of unrecoverable enzyme activity. This minor side reaction was not investigated.

referee and Dr. R. M. Schultz for having called our attention to this analogy.

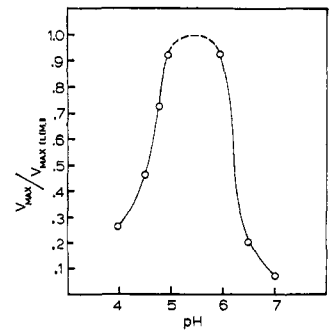

Figure 3. Plot of V_{max}/V_{max} [pH 5.5] *vs.* pH for inhibition of α-CT by EEDQ (V = maximum velocity of inhibition build-up).

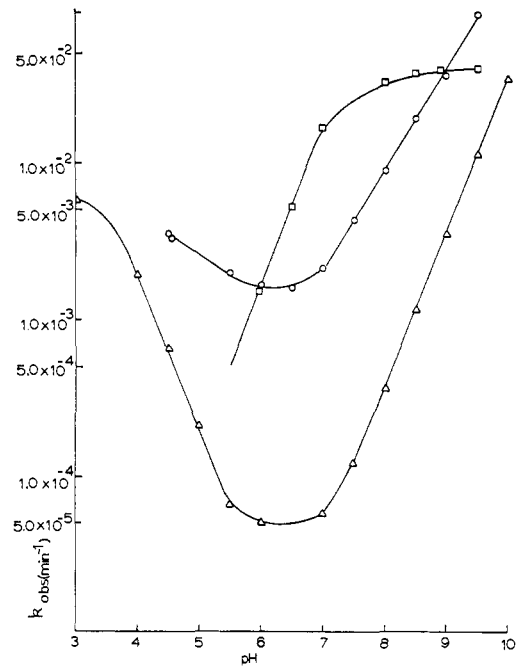

Figure 4. Plots of rate of α-CT regeneration (L-N-Ac-Tyr-OEt as substrate, assay at pH 8, 0.1 *M* NaCl, 25°) *vs.* pH (after EEDQ inhibition (○); after ethoxycarbonylation of Ser-195 (□)[12]) and rate of hydrolysis of ethoxycarbonylimidazole *vs.* pH (△).[11]

When *N*-methyl-(His-57)-α-CT[14,15] was used, *no incorporation of label was observed*, thus showing that His-57 participates in the EEDQ attack of the active center (Figure 3). Addition of excess hydroxylamine (1 *M*) to EEDQ-inhibited α-CT caused a fast and quantitative regeneration of activity both at high and low pH. Finally, the formally equivalent enzyme subtilisin[16,17] in which the Asp-194 of α-CT is substituted by a threonyl residue failed to react with EEDQ even though competitive inhibition was readily observed.[8]

Presently, one of the more attractive working hypotheses accounting for these results is as follows. (a) At acid pH, imidazolium ion (His-57) assisted solvolysis of α-CT bound EEDQ would generate the acylquinolinium cation which would pull the Asp-194 anion away from Ile-16 and rapidly transform it into the mixed anhydride (causing inactivation of the enzyme; in the presence of hydroxylamine the anhydride would

(14) Y. Nakawa and M. L. Bender, *Biochemistry*, **9**, 259 (1970).
(15) R. Henderson, *Biochem. J.*, **124**, 13 (1971).
(16) See J. Kraut in ref 4, p 5470.
(17) See F. S. Markland and E. L. Smith in ref 4, p 562.

obviously be quickly decomposed). (b) The Asp-194–Ile-16 salt bridge being destroyed, the thus relaxed active center conformation would allow the mixed anhydride to reside within the cleft where it would be shielded from the solvent. At pH >7, it would be better exposed and would suffer hydrolysis. One could also envisage more complex mechanisms where an acyl-His-57 may be formed either directly or by way of an Asp-194 mixed anhydride intermediate, but thus far all our data are consistent with the simpler (perhaps simplistic) Asp-194–mixed anhydride hypothesis. Since an acyl-Ser-195 intermediate can be clearly ruled out in the EEDQ inhibition reaction, it would seem logical to conclude that our results point to a heretofore unsuspected capacity of Asp-194 to participate directly in the active center chemistry of α-CT.

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Stereochemistry of the Photochemical Diels–Alder Reaction

Sir:

The well-known[1-3] "photochemical Diels–Alder reaction" can, in theory, be a concerted ($_{\pi}4_s + {}_{\pi}2_a$) or ($_{\pi}4_a + {}_{\pi}2_s$) cycloaddition.[4] In fact, there are many examples in which stereoselectivity is observed, but in no case has the reaction been shown to be completely under electronic symmetry control. There are three experimental reasons for this: (1) in some cases, it is not possible to determine which cis–trans isomer of the starting hexatriene actually cyclizes, since cis–trans isomerization is fast relative to cyclization (*e.g.*, eq 1); (2) in other examples, the steric restraints on the product bicyclohexene are so great that the reaction must find a route to only one bicyclohexene, whether it is the product desired by orbital symmetry control or not (*e.g.*, eq 2); or (3) the 1,3,5-hexatriene is insufficiently labeled to indicate whether the reaction is stereoselective or not (*e.g.*, eq 3).

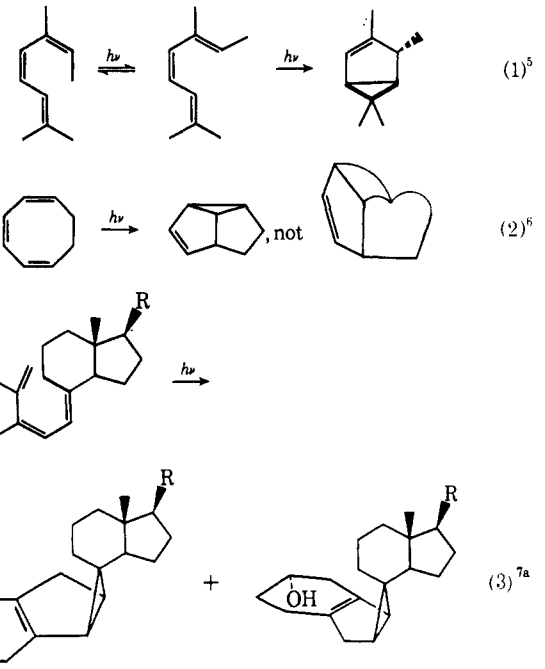
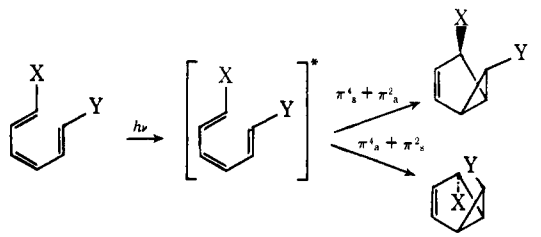


Table I

Reactant	Ref	Temp, ~°C	% products[a] 4	5	6	7	8	9	10	11	12	13	Unknown compounds
5	11b	20	16	50	3	5	0	20	1	4	0	0	0
5	11b	−70	0	50	0	0	0	50	0	0	0	0	0
5	11a	20	25	50	6	11	0	0	4	2	0	0	2
4 + 3% **5**	11b	20	50	0	11	20	0	2	3	13	0	0	1
4	11b	−70	50	0	0	0	14	6	0	0	8	20	2
4 + 5% **5**	11a	20	50	3	12	21	0	0	5	8	0	0	1

[a] All new compounds (**6**, **7**, **8**, **10**, and **11**) were identified and characterized by means of their nmr, uv, and low- and high-resolution mass spectra. The ir spectrum of **8** is characteristic of an allene (1950 cm^{-1}).



We report here the photoinduced Diels–Alder-like cyclization of 1,3,5-hexatrienes[7b] which cis–trans isomerize immeasurably slowly relative to their rate of cyclization, which have no strong steric restraints on closure to bicyclohexenes, and which are adequately labeled to indicate any stereoselectivity. The benzo-

(1) A. Padwa and S. Clough, *J. Amer. Chem. Soc.*, **92**, 5803 (1970), and references cited within.
(2) (a) L. Ulrich, H.-J. Hansen, and H. Schmid, *Helv. Chim. Acta*, **53**, 1323 (1970); (b) J. Meinwald and D. A. Seeley, *Tetrahedron Lett.*, 3739, 3743 (1970).
(3) (a) H. Kleinhuis, R. L. C. Wijting, and E. Havinga, *ibid.*, 255 (1971); (b) K. Salisbury, *ibid.*, 737 (1971).
(4) R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, **8**, 814 (1969).
(5) K. J. Crowley, *J. Org. Chem.*, 33, 3679 (1968).
(6) W. R. Roth and B. Peltzer, *Angew. Chem.*, **76**, 378 (1964); *Justus Liebigs Ann. Chem.*, **685**, 56 (1965); J. Zirner and S. Winstein, *Proc. Chem. Soc.*, 235 (1964); O. L. Chapman, G. W. Borden, R. W. King, and B. Winkler, *J. Amer. Chem. Soc.*, **86**, 2660 (1964).
(7) (a) Reference 4 and references cited therein; (b) actually pentaenes which have orbital symmetries exactly analogous to 1,3,5-hexatrienes; see A. Streitwieser, Jr., and J. I. Brauman, "Supplemental Tables of Molecular Orbital Calculations," Vol. 1, Pergamon Press, New York, N. Y., 1965, p 71.