On the basis of chemical modification[20b,25–28] and dephosphorylation[6,29] studies, it is generally assumed that the bell-shaped k_{cat} and k_{cat}/K_M profiles obtained from pH–rate studies on pepsin substrates reflect the ionizations of two catalytically important carboxyl groups on the enzyme. However, it has recently been suggested[30,31] that there may be several closely spaced carboxyl groups, with overlapping pK values ranging from about 1 to 5, near the active site of pepsin. Since our values of pK_{E_2} and pK_{ES_2} are somewhat higher than those obtained from studies on peptide substrates, the possibility must be considered that our values reflect the ionization of a different carboxyl group than that involved in the hydrolysis of peptide substrates. Moreover, since the chemical modification studies of Lundblad and Stein[30] implicate a carboxyl group on the free enzyme with a pK near 5, it seems possible that this is the same group which is involved in BNPS hydrolysis. However, such conclusions must be regarded as tentative until more data on sulfite ester hydrolyses become available.

Acknowledgment. The support of the National Science Foundation (Grant No. GB-13208) is gratefully acknowledged.

Appendix

According to scheme 9 which postulates that both the EH_1S and EH_1H_2S forms of the Michaelis complex go to products, the expression which describes the pH dependence of k_{cat}/K_M for the pepsin-catalyzed hydrolysis of BNPS is given by eq I. This expression has the

$$\frac{k_{cat}}{K_M} = \frac{\left[\frac{k_{cat}'}{K_s'}\right]_{lim}\left(\frac{H}{K_{E_1}}\right) + \left[\frac{k_{cat}}{K_s}\right]_{lim}}{1 + \frac{H}{K_{E_1}} + \frac{K_{E_2}}{H} + \frac{K_{E_2}}{K_{E_1}}} \qquad (I)$$

same general form as eq 10 and thus predicts a leveling of k_{cat}/K_M in acid solution. However, our data seem to indicate that the pH dependence of k_{cat}/K_M is best described by a simple bell-shaped curve (see Figure 2). This result can be accommodated by eq I if one assumes that in the range of our study, $[k_{cat}/K_s]_{lim} \gg [k_{cat}'/K_s']_{lim}(H/K_{E_1})$. Thus, to a good approximation, the theoretical pH dependence of k_{cat}/K_M will be described by eq 7, *i.e.*, it will be the same as that predicted from scheme 5.

In theory, the relative magnitudes of $[k_{cat}/K_s]_{lim}$ and $[k_{cat}'/K_s']_{lim}(H/K_{E_1})$ can be estimated using the k_{cat}/K_M *vs.* pH and the k_{cat} *vs.* pH profiles obtained in our study (Figures 2 and 3, respectively). These profiles afford values for $[k_{cat}']_{lim}$, $[k_{cat}]_{lim}$, K_{E_1}, and K_{ES_1}, and since for scheme 9, $K_{ES_1}K_s = K_{E_1}K_s'$, the relative magnitudes of $[K_s]_{lim}$ and $[K_s']_{lim}$ can then be obtained. Unfortunately, the relatively large standard errors in our k_{cat} data on the acidic side of the pH profile shown in Figure 3 introduce considerable uncertainty into the values of $[k_{cat}']_{lim}$ and K_{ES_1} obtained from these data, and we therefore cannot estimate the relative magnitudes of $[k_{cat}'/K_s']_{lim}$ and $[k_{cat}/K_s]_{lim}$ to any degree of accuracy.

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Communications to the Editor

A Simple Approach to the Tetracyclo[3.3.0.0^{2,4}.0^{3,6}]oct-7-ene System. 7,8-Diazatetracyclo[3.3.0.0^{2,4}.0^{3,6}]oct-7-ene

Sir:

Studies of the $(CH)_8$ energy surface have led to great interest into the independent synthesis of the various $(CH)_8$ isomers to allow entry onto the surface at various points.[1] Recently, a rather tedious synthesis of a carboxylic acid possessing the tetracyclo[3.3.0.0^{2,4}.0^{3,6}]oct-7-ene ring system was reported.[2] We wish to report a simple approach to molecules of this structural variety. Specifically, we report the synthesis of the related nitrogen-substituted compound **1**, a molecule of considerable interest as a potential precursor of the unknown parent prismane molecule.

Scheme I summarizes the synthetic sequence. Alkylation of sodium cyclopentadienide[3] with N-bromomethylbenzamide[4] at −50° followed by Diels–Alder cycloaddition with dimethyl azodicarboxylate at 0° produced dimethyl 7-*syn*-benzamidomethyl-2,3-diazabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (**2**)[5] in 78% yield. Stereochemistry at the 7 position was initially assumed to be syn based on least hindered approach

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[1] For some aspects of the $(CH)_8$ problem see H. E. Zimmerman and H. Iwamura, *J. Amer. Chem. Soc.*, **92**, 2015 (1970), and earlier references cited therein; E. Vogel, H. Kiefer, and W. R. Roth, *Angew. Chem.*, **76**, 432 (1964); R. Criegee and R. Askani, *ibid.*, **80**, 531 (1968); J. Meinwald and H. Tsuruta, *J. Amer. Chem. Soc.*, **91**, 5877 (1969); H. E. Zimmerman, J. D. Robbins, and J. Schantl, *ibid.*, **91**, 5878 (1969); L. Cassar, P. E. Eaton, and J. Halpern, *ibid.*, **92**, 6366 (1970); P. E. Eaton and T. W. Cole, Jr., *ibid.*, **86**, 962, 3157 (1964).

[2] G. W. Klumpp, W. G. J. Rietman, and J. J. Vrielink, *ibid.*, **92**, 5266 (1970).

[3] For a recent discussion of alkylations of cyclopentadienide salts see E. J. Cory, U. Koelliker, and J. Neuffer, *ibid.*, **93**, 1489 (1971).

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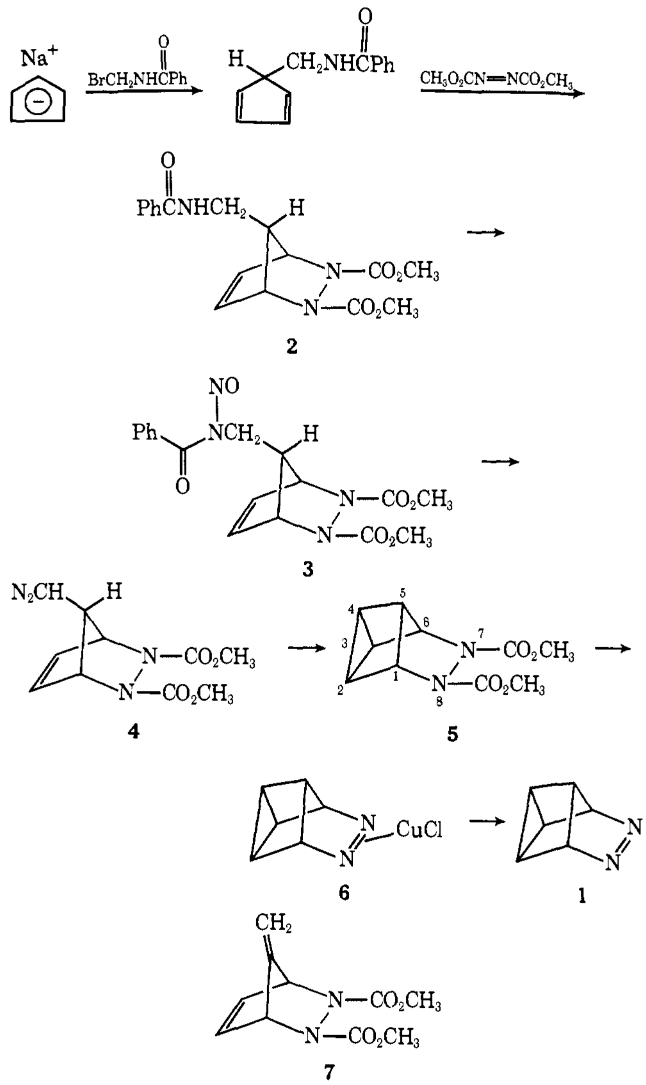
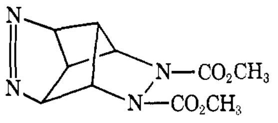
[5] All new compounds had satisfactory analytical and spectral data.

Scheme I. Synthetic Sequence



of dienophile to diene.[6] Independent generation of related compounds in the anti series[7] as well as ultimate success of the synthetic plan confirmed the assignment. Conversion of **2** to nitrosamide **3** using dinitrogen tetroxide in methylene chloride at 0° proceeded in 95% yield. Treatment of **3** with sodium methoxide in tetrahydrofuran at 0° gave a 60% yield of diazo compound **4**: nmr (CDCl$_3$) δ 2.89 (d of m, 1, J = 7 Hz, 7-CH), 3.34 (d, 1, J = 7 Hz, CHN$_2$), 3.79 (s, 6, OCH$_3$), 5.02 (m, 2, bridgehead CH), 6.48 (t, 2, J = 2 Hz, vinyl CH); ir (CH$_2$Cl$_2$) 2070 cm^{-1}. Irradiation of a crude solution of **4** with a 450-W mercury vapor lamp fitted with a Pyrex filter at −78° generated the desired tetracyclic carbamate ester **5** (28% overall isolated yield based on **3**) in addition to the product of hydrogen migration, **7** (15% overall isolated yield based on **3**). Compound **5** showed the following characteristics:[5] nmr (CDCl$_3$) δ 2.23 (m, 2, J = 5.5, 4.7, 3.3, 2.0, and 1.0 Hz, 2- and 3-CH), 2.53 (t of d, 1, J = 3.3 and 0.8 Hz, 4-CH), 2.96 (t of t of d, 1, J = 4.7, 4.7, and 0.8 Hz, 5-CH), 3.78 (s, 6, OCH$_3$), 5.00 (d of m, 2, J = 4.7, 2.0, 1.0, and 0.2 Hz, 1- and 6-CH); ir (CCl$_4$) 1748, 1709 cm^{-1}. Compound **7**[5] was compared to an authentic sample generated independently by the addition of fulvene to dimethyl azodicarboxylate.[8] Saponification of the cyclic carbamate **5** with methanolic potassium hydroxide at 25° followed directly by oxidation of the hydrazine with cupric chloride produced the brick red cuprous chloride complex **6**. Azo compound **1** was released from the complex by treatment with aqueous sodium hydroxide. Recrystallization from pentane at −78° gave a 75% yield (overall yield from **5**) of **1**[5] as a colorless hygroscopic solid: nmr (CFCl$_3$) δ 2.19 (m, 3, cyclopropyl H), 2.79 (m, 1, 5-CH), 5.74 (m, 2, 1- and 6-CH); ir (CCl$_4$) 1493 cm^{-1} (-N=N-); uv (cyclohexane) λ_{max} (log ϵ) 357 (sh, 2.37), 363 (2.46), 368 (2.46), 372 (2.49), and 381 nm (2.40).[9]

The intramolecular carbene addition to the double bond (conversion of **4** to **5**) is quite interesting in light of Hoffmann's calculations regarding the geometry for singlet carbene additions.[10] These calculations indicate that for a concerted cycloaddition the p orbital of the carbene overlaps in a σ manner *with one end* of the ethylene π bond. This lopsided configuration is unattainable in the carbene derived from **4** without severely distorting the rigid bicyclic framework. In agreement with this hypothesis, attempted thermal decomposition of **4** in the presence of copper salts failed to generate any insertion product **5**. Success of the photolysis may be attributable to the intermediacy of either a triplet carbene or to nitrogen loss from an intermediate 1,3-dipolar adduct **8**.[11]



8

Our approach to 7,8-diazatetracyclo[3.3.0.0^{2,4}.0^{3,6}]oct-7-ene is quite flexible and clearly should be applicable to the all-carbon system as well.

Acknowledgment. We wish to express our appreciation to the National Science Foundation for their generous support of our programs.

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[6] In some of the cycloadditions to 5-substituted cyclopentadienes, additions to the more hindered side have been observed. See, for example, (a) S. Winstein, M. Shatavsky, C. Norton, and R. B. Woodward, *J. Amer. Chem. Soc.*, 77, 4183 (1955); (b) S. McLean and P. Haynes, *Tetrahedron Lett.*, 2313 (1965); (c) K. L. Williamson and Y.-F. L. Hsu, *J. Amer. Chem. Soc.*, **92**, 7385 (1970).

[7] Generated by catalytic hydrogenation of dimethyl 7-alkylidene-2,3-diazabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate.[8]

[8] R. M. Cory, unpublished results in these laboratories.

[9] Compare the uv spectrum of 8,9-diazatetracyclo[4.3.0.0^{2,4}.0^{3,7}]non-8-ene; R. M. Moriarty, *J. Org. Chem.*, **28**, 2385 (1963).

[10] R. Hoffmann, *J. Amer. Chem. Soc.*, **90**, 1475 (1968).

[11] For an example of this process, see M. Schwarz, A. Besold, and E. R. Nelson, *J. Org. Chem.*, 30, 2425 (1965).

[12] Henry and Camille Dreyfus Teacher-Scholar Grant Recipient.

[13] National Science Foundation and National Institutes of Health Predoctoral Fellow.

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The Thermal and Photochemical Decomposition of 7,8-Diazatetracyclo[3.3.0.0^{2,4}.0^{3,6}]oct-7-ene. 1,2-Diazacycloocta-2,4,6,8-tetraene

Sir:

In the preceding communication,[1] we reported the synthesis of 7,8-diazatetracyclo[3.3.0.0^{2,4}.0^{3,6}]oct-7-ene

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[1] B. M. Trost and R. M. Cory, *J. Amer. Chem. Soc.*, **93**, 5572 (1971).