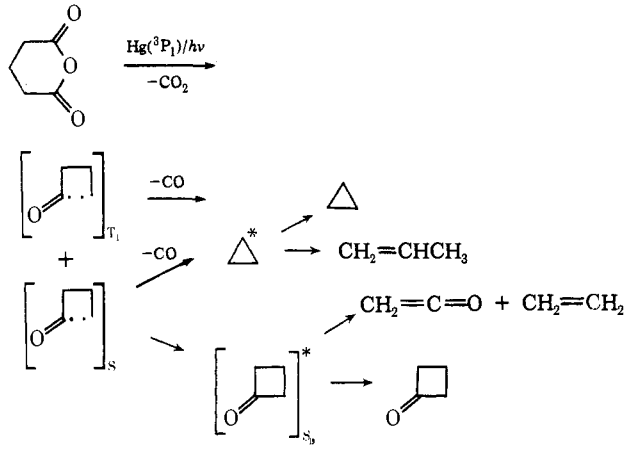
carboxylation of GA may proceed through α-cleaved cyclobutanone biradical, as shown in the mechanism of Scheme I. Because ethylene was only a minor

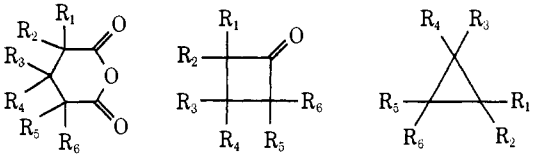
Scheme I



product in the vapor-phase photolysis, and because it was not found in the neat form photolysis, ethylene and possibly ketene were formed only through CB(S_0*). This very minor role of ethylene, a characteristic singlet state product, was first taken to indicate the triplet biradical intermediate, as in the case of benzyl esters.[7,11] However, CB(T_1) and perhaps the triplet biradical decompose with 100% efficiency and do not give rise to CB(S_0) even at 1500 Torr.[3,4] The singlet biradical undergoes the internal conversion to CB(S_0) in addition to decarbonylation as well as ethylene formation.[3,12] The biradical intermediate generated by photodecarboxylation of GA may not be so activated in comparison with the one from CB(S_1) that it undergoes decarbonylation, the energetically preferred process.[13] The absence of ethylene, thus, does not rule out the singlet biradical intermediate. The photodecarboxylation of GA may yield both singlet and triplet biradicals. The Hg($^3P_1$)-photosensitized reaction of CB yielded a significant fraction of the singlet state products.[14] In the Hg($^3P_1$)/GA system, the triplet biradical may have been formed at low pressure, but it decarbonylates. With increase of the saturation vapor pressure of GA, the singlet biradical is formed to yield CB and cyclopropane. In the condensed phases, there may be only the singlet biradical intermediate, which can partly explain the reduced cyclopropane formation relative to CB formed. The intermediacy of the singlet biradical also explains no significant effect on the yield of CB by the presence of O_2.

Photolysis of substituted GA yielded the same results as simple GA. As shown in Table II, upon photodecarboxylation and decarbonylation 2,2-dimethylglutaric anhydride yielded 2,2-dimethylcyclobutanone and 3,3-dimethylglutaric anhydride yielded 3,3-dimethylcyclobutanone in addition to 1,1-dimethylcyclopropane for both cases. In contrast with the present technique, the reported method for preparation of dimethylcyclobutanones by addition of diazomethane to dimethylketene always yields a mixture of 2,2- and 3,3-dimethylcyclobutanones.[8] The present study of photodecarboxylation was extended to a more complicated GA, *i.e.*, 3,3-tetramethyleneglutaric anhydride, which yielded 3,3-tetramethylenecyclobutanone together with spiro[2.4]heptane, CO_2, and CO. 3,3-Tetramethylenecyclobutanone was characterized by ir, mass, and nmr spectra.[15]

Table II. Photoproducts of Substituted Glutaric Anhydrides in the Vapor Phase Hg($^3P_1$) Photosensitized Reaction under the Saturation Vapor Pressure



Substituents	Temp, °C	Products, 10^{-4} mol/9.6 × 10^{-4} einstein: Cyclobutanones	Cyclopropanes	$R_3R_4C{=}CR_5R_6$
$R_1 = R_2 = CH_3$; $R_3 = R_4 = R_5 = R_6 = H$	165	0.53	2.86	0.098
$R_1 = R_2 = R_5 = R_6 = H$; $R_3 = R_4 = CH_3$	165	0.51	3.10	0.12
$R_1 = R_2 = R_5 = R_6 = H$; $R_3, R_4 = CH_2CH_2CH_2CH_2$	200	0.19	2.57	

Acknowledgments. The author wishes to thank Dr. H. E. Hunziker for his constant encouragement and helpful discussions and Dr. R. D. Miller for his most useful comments. The referee's comments are specially useful in understanding the mechanism.

(11) The intermediacy of the singlet biradical was pointed out by a referee.

(12) H. A. J. Carless and E. K. C. Lee, *J. Amer. Chem. Soc.*, **94**, 1 (1972).

(13) The decarbonylation process is preferred by 9.73 kcal mol^{-1} at 25° to the ethylene formation process; *cf.* D. R. Stull, E. F. Westrum, Jr., and G. C. Sinke, "The Chemical Thermodynamics of Organic Compounds," Wiley, New York, N. Y., 1969. A. T. Blades (*Can. J. Chem.*, **47**, 615 (1969)) suggested that α-cleaved cyclobutanone biradical was responsible for decarbonylation, while β-cleaved cyclobutanone biradical led to ethylene + ketene.

(14) D. C. Montague and F. S. Rowland, *J. Amer. Chem. Soc.*, **91**, 7230 (1969).

(15) Ir (CCl_4) carbonyl absorption 1780 cm^{-1}; mol wt by mass 124; nmr (CCl_4) τ 7.20 (s, 4), 8.28 (singlet with a shoulder, 8).

H. Hiraoka
IBM Research Laboratory
San Jose, California 95193
Received October 3, 1972

Subtilisin Catalysis of Nonspecific Anilide Hydrolyses

Sir:

The hydrolysis mechanism of serine proteases has often been studied using specific and nonspecific ester substrates. The same cannot be said for amide substrates since nonspecific amide substrates have not been observed to be hydrolyzed at all. We wish to report that the bacterial proteinase subtilisin[1] rapidly catalyzes the hydrolysis of two substituted acetanilides: 2,2,2-trifluoro-*N*-methyl-*p*-nitroacetanilide (I) and 2,2,2-trifluoro-*p*-nitroacetanilide (II). However, we were unable to detect subtilisin-catalyzed hydrolysis of *p*-nitroacetanilide.[2]

(1) The enzyme was purified on carboxymethylcellulose according to the method of L. Polgar and M. L. Bender, *Biochemistry*, **8**, 136 (1969).

(2) Nonproductive complexes cannot be the cause of the inactivity of subtilisin since the k_2/K_s constants quoted in Tables I and II can have no nonproductive component; see M. L. Bender and F. J. Kézdy, *Annu. Rev. Biochem.*, **34**, 49 (1965).

Table I. Subtilisin-Catalyzed Hydrolysis of 2,2,2-Trifluoro-*N*-methyl-*p*-nitroacetanilide at pH 8.42[a]

10^4[S], *M*	10^4[Inh],[b] *M*	10^5[E], *M*	Rate constant, $\times 10^3$, sec^{-1}	k_2/K_s, M^{-1} sec^{-1}
1.33	0	4.82	8.48	153
1.42	0	0	1.10	
1.31	7.74	4.72	1.10	

[a] I = 0.1 *M* Tris buffer, 0.62% acetonitrile, 25°. Kinetics were observed at 390 nm. [b] The inhibitor is phenylmethanesulfonyl fluoride.

ical explanation for the absence of subtilisin activity toward *p*-nitroacetanilide while showing large activity (comparable to that of highly specific amide substrates[14,15]) toward the 2,2,2-trifluoro analog.

Finally, it should be mentioned that the difference between rate constants shown by the *N*-methylanilide (I) and its unmethylated analog (II) is not necessarily due to the presence of the methyl group alone but may be related to ionization of the unmethylated analog[16] with a resulting loss in nucleophilic susceptibility.

Table II. Subtilisin-Catalyzed Hydrolysis of 2,2,2-Trifluoro-*p*-nitroacetanilide at pH 8.42[a]

$10^4[S]_0$, *M*	10^2[Inh],[b] *M*	10^5[E], *M*	Rate constant, sec^{-1}	k_2/K_s, M^{-1} sec^{-1}	K_I(Inh), *M*
1.33	0	4.82	1.193×10^{-3}	17.7	
1.42	0	0	3.39×10^{-4}		
1.42	1.53	0	3.40×10^{-4}		
1.33	1.436	4.82	6.73×10^{-4}		5.34×10^{-2}

[a] I = 0.1 *M* Tris buffer, 0.62% acetonitrile, 25°. Kinetics were observed at 390 nm. [b] The inhibitor is *N*-benzoyl-L-arginine.

While these substrates are structurally analogous to the nonspecific subtilisin substrate *p*-nitrophenyl acetate[3] it seemed useful to determine whether catalysis of these highly activated compounds is dependent on the active site serine hydroxyl. Therefore, the rate of hydrolysis was also determined in the presence of phenylmethanesulfonyl fluoride (PMSF)[4] inhibited subtilisin (Table I). In another experiment the inhibitory effect of *N*-benzoyl-L-arginine was also determined (Table II).[5]

The results obtained are unexpected in light of the pretransition state protonation theory of chymotryptic anilide hydrolysis[6,7] but are consistent with the view that anilide hydrolysis proceeds through an initial tetrahedral intermediate[8-10] followed by rate-determining breakdown to give products. This latter view is strengthened by the observation that chymotryptic hydrolytic constants toward specific amides are apparently independent of any electronic effect.[8] Since highly specific subtilisin ester substrates also show no electronic effect on acylation[9] while less specific substrates show an electronic effect comparable to that in chymotrypsin,[11-13] it is evident that nonspecific amides may also show an electronic effect that is absent in specific amides.

In any case, such an electronic effect is the only log-

Acknowledgment. This work was supported by a National Institutes of Health predoctoral fellowship to M. P. and Grant RO1-HLO-5726 of the NIH to M. L. B.

(3) L. Polgar and M. L. Bender, *Biochemistry*, **6**, 610 (1967).
(4) C. S. Wright, R. A. Alden, and J. Kraut, *Nature (London)*, **221**, 235 (1969), and references therein.
(5) Ethyl *N*-benzoyl-L-argininate is a subtilisin substrate with a K_m of 1×10^{-2} *M*: A. N. Glazer, *J. Biol. Chem.*, **241**, 635 (1966).
(6) T. Inagami, A. Patchornik, and S. S. York, *J. Biochem. (Tokyo)*, **65**, 809 (1969).
(7) J. H. Wang and L. Parker, *Proc. Nat. Acad. Sci. U. S.*, **58**, 2451 (1967).
(8) M. Philipp, R. M. Pollack, and M. L. Bender, *Proc. Nat. Acad. Sci. U. S.*, in press.
(9) A. R. Fersht, *J. Amer. Chem. Soc.*, **94**, 293 (1972).
(10) M. Caplow, *ibid.*, **91**, 3639 (1969).
(11) M. Philipp, Doctoral Dissertation, Northwestern University, Evanston, Ill., 1971.
(12) M. L. Bender and K. Nakamura, *J. Amer. Chem. Soc.*, **84**, 2577 (1962).
(13) Chymotrypsin also demonstrates ester hydrolysis Hammett ρ values that vary with substrate specificity, but the effect is not nearly so pronounced as in subtilisin; see (a) R. E. Williams and M. L. Bender, *Can. J. Biochem.*, **49**, 210 (1971); (b) A. Williams, *Biochemistry*, **9**, 3383 (1970); (c) C. D. Hubbard and J. F. Kirsch, *ibid.*, **11**, 2483 (1972).
(14) K. Morihara, T. Oka, and H. Tsuzuki, *Arch. Biochem. Biophys.*, **138**, 515 (1970).
(15) It is interesting that this activity is comparable to that shown toward the nonspecific activated ester *p*-nitrophenyl acetate (2).
(16) P. M. Mader, *J. Amer. Chem. Soc.*, **87**, 3191 (1965).

M. L. Bender,* M. Philipp
Division of Biochemistry, Department of Chemistry
Northwestern University, Evanston, Illinois 60201

Received December 4, 1972

A New Rhodium(I)–Porphyrin Complex. II.[1] Synthesis and Oxidative Alkylation

Sir:

A new type of metalloporphyrin with two metal ions bonded to a porphyrin has been reported for Re and Rh complexes.[1,2] The crystallographic study of its rhenium(I) complex has shown that each Re ion is bonded to three nitrogen atoms and that two Re atoms are bonded to one porphyrin on opposite sides of the plane of the porphyrin.[3] Recently James and Stynes have reported on the synthesis of the rhodium(I)–porphyrin complex formulated as $H[Rh(porphyrin)] \cdot 2H_2O$ and the reactivity of lower valent metal porphyrins.[4] A rhodium porphyrin in various oxidation states may provide an interesting model to investigate the reaction behavior of vitamin B_{12} as well as the other models.[5,6]

We wish to report a new rhodium(I)–porphyrin

(1) For the previous paper in this series, see Z. Yoshida, H. Ogoshi, T. Omura, E. Watanabe, and T. Kurosaki, *Tetrahedron Lett.*, 1077 (1972).
(2) D. Ostfeld, M. Tsutsui, C. P. Hrung, and D. C. Conway, *J. Amer. Chem. Soc.*, **93**, 2548 (1971).
(3) D. Cullen, E. Meyer, T. S. Srivastava, and M. Tsutsui, *ibid.*, **94**, 7603 (1972).
(4) B. R. James and D. V. Stynes, *ibid.*, **94**, 6225 (1972).
(5) J. H. Weber and G. N. Schrauzer, *ibid.*, **92**, 726 (1970).
(6) J. Kwiatek, "Transition Metals in Homogeneous Catalysis," G. N. Schrauzer, Ed., Marcel Dekker, New York, N. Y., 1971, p 31.