atoms were located from a difference Fourier map. The full-matrix least-squares[11] refinement using anisotropic temperature factors[12] (isotropic for H atoms) converged to an R of 0.045. The absolute configuration was determined by using the anomalous dispersion of oxygen[13] and measuring the 18 most sensitive Friedel pairs repeatedly. Differences for 15 pairs are in agreement with the enantiomer shown in Figure 1.[14] In the crystal structure tedanolide is shaped like an elongated disk (length 9.0 Å, width 5.5 Å) with the hydroxy groups at C-2 and -13 directed to the interior of the ring and the carbonyl oxygens at C-5, -11, and -15 oriented perpendicular to the plane of the ring. There is one probable intramolecular H bond [O(10)···O(9) 2.824 (4) Å] and two intermolecular H bonds [O(3)···O(7) and O(8)···O(4)].

Tedanolide is of mixed acetate–propionate biogenesis (acetate units at C-1,2 and -11,12). It differs from other macrolides in that the site of lactonization is not near the end of the carbon skeleton.[15] Tedanolide is highly cytotoxic, exhibiting an ED_{50} or 2.5×10^{-4} in KB and 1.6×10^{-5} in PS.[4] Cell-flow cytofluorometry[16] analysis revealed that tedanolide causes accumulation of cells in the S phase at concentrations as low as 0.01 μg/mL. In vivo tumor inhibition evaluation of tedanolide is in progress.

Only a few other macrolides have been isolated from marine organisms: the bryostatins,[17] aplysiatoxin,[18] debromoaplysiatoxin,[18] oscillatoxin A and brominated analogues,[19] and aplasmomycin.[20] The extremely low yield in which tedanolide is obtained indicates that it may be a metabolite of some microorganism as was found in the case of okadaic acid.[21] We are searching for such a source.

Acknowledgment. This investigation was supported by Grants CA-17256 and CA-17562 awarded by the National Cancer Institute, DHEW, and Grants NA83AA-D-00011 and NA80AA-D00084, Department of Commerce, NOAA, Office of Sea Grant. We thank Dr. Robert E. Schroeder for assistance in specimen collection, Dr. Paul Schmidt, Oklahoma Medical Research Foundation, Oklahoma City, OK, for use of a 300-MHz NMR spectrometer, Drs. K. L. Rinehart, Jr., and J. C. Cook, Jr., University of Illinois, Urbana, IL, for a high-resolution FAB mass spectrum, and Dr. C. Costello for low-resolution FAB and high-resolution EI mass spectra. We thank Dr. G. B. Boder, Lilly Research Labs, Indianapolis, IN, for providing the cell cytofluorometry data.

Registry No. Tedanolide, 92471-87-9.

Supplementary Material Available: Tables of atomic positional and thermal parameters, bond distances, bond angles, and selected torsion angles and a list of observed and calculated structure factors (7 pages). Ordering information is given on any current masthead page.

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An Organic Chemical Model of the Acyl-α-chymotrypsin Intermediate

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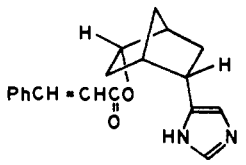
Chymotrypsin is a large (MW 24 800) and complicated (245 amino acids) enzyme. For many years we have been working on an organic model of it. This is feasible because the constituents of the active site are only three,[1] and their stereochemistry (distances and angles with respect to one another) is known through X-ray analysis.[2]

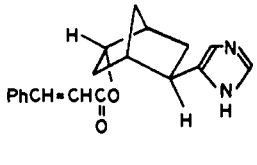
Several models of enzymes have already been synthesized, mainly based on cyclomayloses. These include models of chymotrypsin,[3,4,8] ribonuclease,[5] carbonic anhydrase,[6] and transaminase.[7] Previous models of chymotrypsin synthesized either by us[3,4] or by others[8] have suffered from the fact that they have used the binding ability of the cycloamyloses but have not incorporated all three known catalytic groups of chymotrypsin. Chymotrypsin is the archetype of about 20 serine proteases and is thus an important enzyme. All of the serine proteases proceed through an acyl-enzyme intermediate.

We started on models of the acyl-enzyme intermediate (summarized in Table I) since its reactions do not have to consider binding. We first attached two of the three known constituents of the active site to a completely rigid backbone whose stereochemistry could be regulated easily.

Thus we synthesized *exo*-imidazolyl-*endo*-hydroxyl- and *endo*-imidazolyl-*endo*-hydroxylnorbornane, which we converted to the corresponding cinnamates, *endo,endo*-(**1**) and *exo,endo*-5-[4(5)-imidazolyl]bicyclo[2.2.1]hept-2-yl *trans*-cinnamate (**2**).[9]



1



2

We found that the imidazolyl group in the endo,endo compound, **1**, but not in the exo,endo compound, **2**, participated in the hydrolysis of the ester linkage across the bicyclic ring system. This was apparent from the kinetics of hydrolysis, which showed dependence on the ionization of the imidazolyl group in the endo,endo compound but not in the exo,endo compound.[10]

We then found that the participation by the imidazolyl group in the endo–endo compound **1** was due to a base catalysis by the imidazolyl group since a k_{H_2O}/k_{D_2O} effect of 3.0 occurs.[10] This was mechanistically interesting since the deacylation of cinnamoyl–chymtrypsin proceeds by imidazole acting as a base with a k_{H_2O}/k_{D_2O} effect of 2.5[11] and since imidazole in all other

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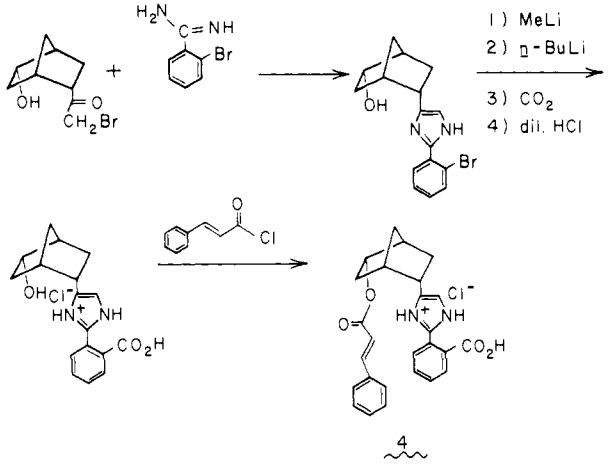
Table I. Steps in the Development of a Model for an Artifical Acyl-Enzyme

	development	hydrolysis of	rate constant,[a] s^{-1}	rel rate constant	ref
(1)	2-hydroxyl and 5-imidazolyl group regiochemistry in the norbornane ring system confirmed	**1**	4.5×19^{-8} [a]	1	10, 13
(2)	endo,endo stereochemistry of the hydroxyl and imidazolyl groups	**1**	4.5×10^{-5}	1	13
(3)	the function of the imidazolyl group is a general base[b]				
(4)	carboxylate (benzoate) ion is necessary	**1** + 0.5 M benzoate	8.6×10^{-5}	1917	13 + present work
(5)	intramolecular benzoate ion is necessary	**4**	6.9×10^{-3} [c,d]	154000	present work

[a] this rate constant corresponds to that for the rate of hydrolysis of norbornane cinnamate without an imidazolyl group. If extrapolation to 60 °C was required, it was done using the data of ref 11. [b] $k_{H_2O}/k_{D_2O} = 3.0$. [c] the purity of this compound dictates that the rate constant be only good to ±5%. The others are better. [d] the rate constant of deacylation of cinnamoylchymotrypsin at pH 7.9 and 60 °C in water is 1.3×10^{-1} s^{-1}, which is 2888888 faster than a "normal" cinnamate or 18 faster than our artificial acyl-enzyme.

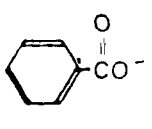
Scheme I. Synthesis of 4 by the Condensation of 2-Bromobenzamide with *endo, endo*-5-(Bromoacetyl)-2-hydroxybicyclo[2.2.2]heptane Followed by Lithium Exchange of the Bromine Atom, Carboxylation of the Lithium Derivative, and Cinnamoylation of the Hydroxyl Group



intramolecular situations acts as a nucleophile,[12] which would give a k_{H_2O}/k_{D_2O} effect of 1.0.

We further found that the addition of benzoate ion (**3**) (the third component of the imagined active site) produced a 2500-fold increase in the hydrolytic rate at a concentration of 0.5 M in 0.42 mol fraction dioxane–water as solvent, used to simulate the apolar nature of the chymotrypsin active site. This was satisfying from both a mechanistic and kinetic viewpoint since we were using the constituents of the active site of the enzyme to produce a rate of reaction which was calculated to be within a factor of 4 of the rate of cinnamoyl-chymotrypsin deacylation. But this was accomplished by using the *assumption* that an intramolecular catalyst (as in an enzyme) equals 10 M of an intermolecular catalyst.[13,14]

We now report the synthesis and reactivity of the intramolecular analogue of this system, *endo,endo*-5-[2-(2-carboxylphenyl)-4-(5)-imidazolyl]bicyclo[2.2.1]hept-2-yl *trans*-cinnamate (**4**). It



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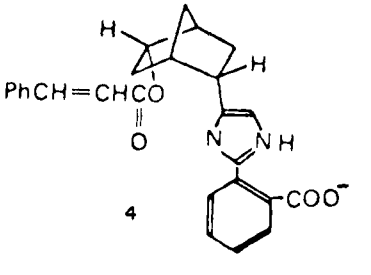

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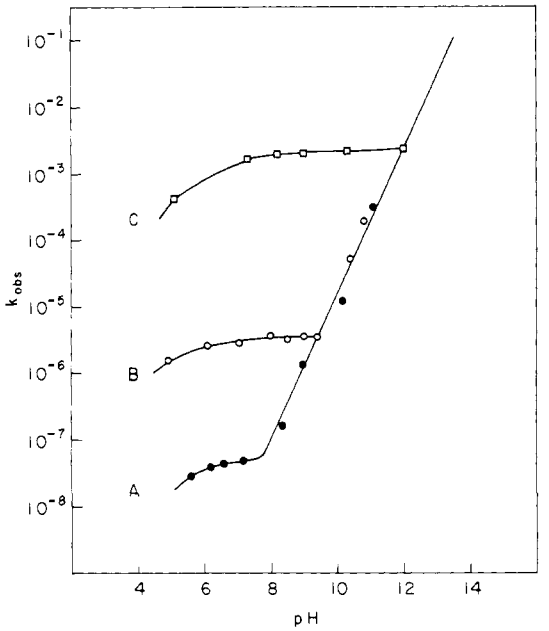

Figure 1. Hydrolysis of norbornane *trans*-cinnamate models, pH 7.9, 60 °C. A, hydrolysis of **1**, black circles; B, hydrolysis of **1** + 0.5 M benzoate ion, open circles; C, hydrolysis of *endo,endo*-5-[2-(2-carboxyphenyl)-4-(5)-imidazolyl]bicyclo[2.2.1]hept-2-yl *trans*-cinnamate (**4**), open squares.

was synthesized from *endo,endo*-5-acetyl-2-hydroxybicyclo-[2.2.1]heptane by first converting it to *endo,endo*-5-(bromoacetyl)-2-hydroxybicyclo[2.2.1]heptane, then condensing it with 2-bromobenzamidine to the corresponding imidazole derivative, carboxylating the 2-bromophenylimidazole to give the corresponding carboxylate ion, and finally cinnamoylating the hydroxyl group on the norbornane ring, according to Scheme I, thereby producing **4**.[15]

We have determined the rate of hydrolysis of the endo,endo compound containing intramolecular benzoate ion **4** and shown it to be equal to $^1/_{18}$ the rate of deacylation of *trans*-cinnamoyl-α-chymotrypsin (Table I). Furthermore, we find that an increasing dioxane concentration in the solvent increases the reaction rate as seen before.[13]

As shown in Figure 1 and Table I, there is a large increase in the rate constant for the hydrolysis of **4** over that of hydrolysis of "normal" cinnamates. Although this acceleration of 154000, in the mixed solvent system, is 18-fold less than that of the real acyl-enzyme in water, if one suggests a differential solvating system for the active site of the artificial enzyme, as exists in the real

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(17) Highlights of the NMR, IR, and mass spectra of **4** follow: IR (KBr) ν_{max} 3400–2200, 1700, 1630, 1570 cm^{-1}; NMR (270 MHz, $CDCl_3$) δ 8.38 (2 H, s), 7.66 (1 H, d, J = 16 Hz), 7.51–7.36 (10 H, m), 6.41 (1 H, d, J = 16 Hz), 5.12 (1 H, m), 3.25 (1 H, m), 2.85–1.10 (8 H, m); mass spectrum (70 eV); m/z 411 (M − 17), 384 (M − 44), 131 (base).

enzyme, it is possible that both the real and artificial acyl-enzymes would have the same rate of deacylation.

Thus we have synthesized an organic chemical model of an acyl-enzyme intermediate of MW 428 whose hydrolysis rate is approximately equivalent to a real acyl-enzyme intermediate of MW 25 100 (Table I). This achievement indicates that out initial assumptions of a three amino acid active site and the intermediacy of an acyl-enzyme are correct for the chymotrypsin structure and mechanism.

The molecular weight ratio of 58.25 for the real over the artificial acyl-enzyme, which we have achieved, can be explained in part by the fact that we do not utilize any amino acids in our synthesis, which immediately makes it nonutilizable for biosynthesis including transmission through the genetic chain from one generation to the next and also reduces the water solubility greatly. Since all life takes place in water, it reduces the utility of the artificial acyl-enzyme to nonlife processes.

The mechanism by which this large rate acceleration occurs will be discussed in the full paper.

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Exciplex Ionic Dissociation in Nonpolar Solvent Induced by Multipolar Salt Complexes

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Exciplex intermediates play a pervasive role in organic photochemistry.[1] In relatively nonpolar solvents, radiative relaxation of the exciplex often occurs in competition with chemical reactions. In more polar solvents, dissociation of the exciplex to radical ions is the dominant reaction when it is permitted energetically. Salt effects are often used to control or probe organic reactions.[2] However, only quite recently have salts been employed in the investigation of exciplexes.[3,4] Herein we report the results of a study of the interactions between tetra-*n*-butylammonium tetrafluoroborate (R_4NBF_4) and the exciplex formed from singlet excited pyrene and 1,4-dicyanobenzene, (Py-DCNB)*[1], in dimethoxyethane (DME) solution. The findings reveal that (Py-DCNB)*[1] is quenched by monomeric, dipolar R_4NBF_4 without formation of ions but that reaction of the exciplex with higher aggregates $(R_4NBF_4)_n$ leads to efficient formation of pyrene radical cation ($Py^{+}\cdot$) and dicyanobenzene radical anion ($DCNB^{-}\cdot$). This phenomenon is useful both for controlling the chemical reactions of exciplexes and for analyzing putative electron-transfer reactions.

The fluorescence of pyrene in DME is rapidly and irreversibly quenched by addition of DCNB, and simultaneously a new, broad emission centered at 450 nm due to (Py-DCNB)*[1] appears in the spectrum.[5] The fluorescence of pyrene is unaffected by addition

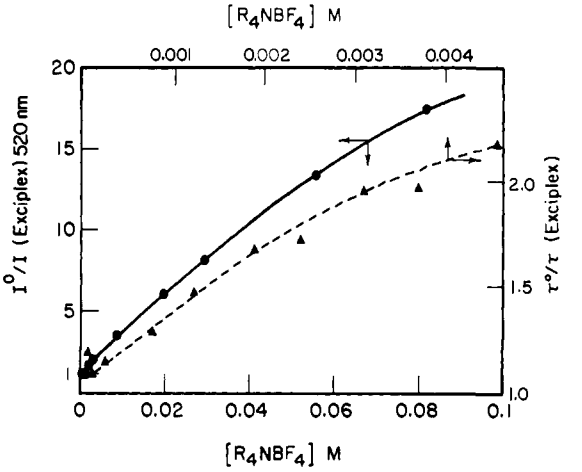

Figure 1. Stern-Volmer analysis of the quenching of the pyrene-dicyanobenzene exciplex in dimethoxyethane by tetra-*n*-butylammonium tetrafluoroborate. Circles are for intensity quenching and are plotted according to the scales shown on the left and bottom axes. Triangles are for lifetime quenching and are plotted according to scales shown on the right and top axes.

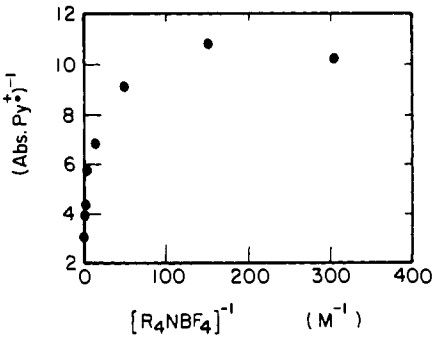

Figure 2. Double-reciprocal plot of the relative yield of pyrene radical cation against the concentration of tetra-*n*-butylammonium tetrafluoroborate in dimethoxyethane.

of R_4NBF_4, but the emission of the exciplex is quenched by this salt. The maximum of the exciplex emission spectrum is not changed noticeably by the salt. In contrast to observations reported for related systems,[3] Stern-Volmer analysis of the exciplex quenching by the salt reveals a distinctly nonlinear relationship.[6] In effect, the salt is a less effective quencher at higher concentrations than it is at the lower concentrations. Precisely the same nonlinear behavior is observed when the lifetime of the exciplex is monitored. These results are displayed on Figure 1.

Excitation of a DME solution of pyrene containing 0.02 M DCNB with the output of a nitrogen laser (15 ns, 337 nm, 7 mJ, absorbed exclusively by the pyrene)[7] permits analysis of these reactions by transient absorption spectroscopy. In the absence of added salt this experiment reveals, not unexpectedly, that the radical ions are not formed in detectable amount from dissociation of the exciplex in this relatively nonpolar solvent. However, when the solution also contains 0.05 M R_4NBF_4 the absorbance of $Py^{+}\cdot$ is readily observed, and its yield (determined from the absorbance change at 445 nm)[8] is virtually the same as that obtained from the dissociation of this exciplex in acetonitrile solution.[5] The yield of $Py^{+}\cdot$ is dependent on the salt concentration in a curious, nonlinear way (Figure 2). The amount of $Py^{+}\cdot$ formed when the salt concentration is less than ca. 5×10^{-2} M is small even though

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