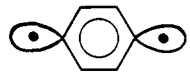
benzaldehyde, and ethylbenzene. That these products are formed in the film itself is a reasonable deduction in view of the limited success that was achieved in scavenging them with oxygen.[15] Since the absorption of the light pulse followed by bond breaking is probably the fastest of the successive steps in the formation of these products, there is an interesting prospect that radicals such as **2** may be transiently present in significant concentration in the



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PET film. This opens up interesting possibilities for the use of the far-UV laser for the synthesis of exotic radical species in frozen media.

Registry No. 1, 25038-59-9.

(15) In homogeneous gas-phase reaction, as little as 0.01 mol % of O_2 is capable of inhibiting a radical chain process. See: Benson, S. W. "The Foundations of Chemical Kinetics"; McGraw-Hill: New York, 1960; p 111. As a referee has pointed out, the present system cannot be termed homogeneous, and the pressure of oxygen at the site of photodecomposition may be far less than in the surrounding atmosphere. This would, in turn, require a modification of the statement that "the products are formed in the film itself".

A New Class of Serine Protease Inactivators Based on Isatoic Anhydride

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The development of suicide inactivators of serine proteases appears feasible by two general approaches. The first employs a substrate that, during the normal enzymatic process, gives rise to a reactive intermediate. This intermediate then reacts with functional groups at the active site to afford a covalent enzyme–inactivator complex. A number of compounds of this type have been reported previously.[1] The second approach utilizes a substrate that leads to a relatively stable acyl-enzyme. The studies of Caplow and Jencks[2] suggest that *p*-aminobenzoyl-α-chymotrypsin is sufficiently stable to effectively inactivate the enzyme (calculated $t_{1/2}$ for hydrolysis approximately 23 h). In agreement with this, Haugland and Stryer[3] have shown that the *p*-nitrophenyl ester of anthranilic acid gives rise to an extremely stable anthraniloyl-α-chymotrypsin. Since the same factors that cause slow deacylation of the enzyme will also decrease the rate of acylation, an efficient inactivator requires that the electron-donating properties of the amine are masked prior to acylation and become unmasked subsequent to formation of the acyl-enzyme. This suggested to us that isatoic anhydride (**1**) incorporates the essential features of this second approach. Reaction of **1** with α-chymotrypsin, as shown in Scheme I, should afford anthraniloyl-α-chymotrypsin.[4]

Scheme I. Hypothetic Scheme for Inactivation of α-Chymotrypsin by Isatoic Anhydride

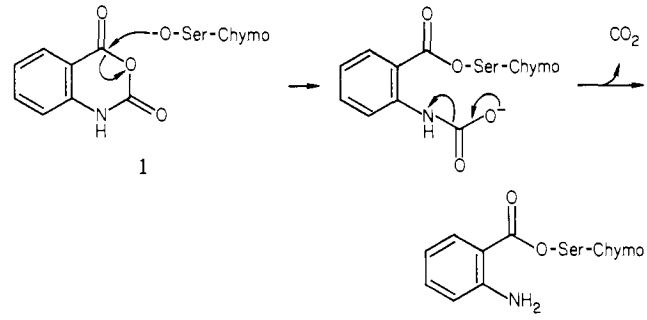

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Table I. $t_{1/2}$ (min) for Inactivation by Isatoic Anhydride

enzyme	concn of Isatoic Anhydride in preincubation mixture, μM 12.5	125	1250
α-chymotrypsin[a]	1.2	0.5	<0.25
porcine pancreatic elastase[b]		<0.25	<0.25
α-lytic protease[c]	2.0	<0.25	<0.25
trypsin[c]	no inactivation	2.0	<1.0
papain[c]	48	2.0	<0.25

[a] α-Chymotrypsin (three times crystallized, Sigma, 50 μL of 160 μM in 1 mM HCl) was incubated in 925 μL of buffer (0.1 M potassium phosphate, pH 7.5) with 25 μL of inhibitor in 10% Me_2SO/CH_3CN. At various times, 25-μL aliquots were withdrawn, and activity was measured spectrophotometrically by adding to 925 μL of buffer and 50 μL of 11.5 mM benzoyltyrosine ethyl ester in CH_3CN. [b] Pancreatic elastase (two times crystallized, Sigma, 200 μL of 500 μM) was incubated at 25 °C in 775 μL of buffer with 25 μL of inhibitor in Me_2SO, 50-μL aliquots were withdrawn, and activity was assayed by adding to 900 μL of buffer and 50 μL of *N*-acetyl-Ala-Pro-Ala-*p*-nitroanilide (50 mM) in buffer. [c] Similar spectrophotometric assays were carried out with the remaining enzymes. The enzyme concentration was 8 μM in the incubation mixture. Buffers and substrates (with assay concentration) for the enzymes are as follows: α-Lytic protease (gift from Professor W. Bachovchin, Tufts University, School of Medicine), 0.1 M potassium phosphate, pH 7.5, *N*-acetyl-Ala-Pro-Ala-*p*-nitroanilide (2.5 mM); trypsin (three times crystallized, Worthington), 0.04 M Tris, 0.01 M $CaCl_2$, pH 8.1, *p*-toluenesulfonyl-L-arginine methyl ester (1.0 mM); papain (two times crystallized, Sigma), 0.05 M Tris, 0.005 M L-cysteine, 0.02 M Na_2EDTA, pH 7.5, α-*N*-Benzoyl-DL-arginine-*p*-nitroanilide (1.0 mM).

Preincubation of isatoic anhydride (12.5 μM) with α-chymotrypsin (8 μM) resulted in loss of 50% of the activity in approximately 1.2 min and complete inactivation in less than 6 min. At subequivalent levels of **1**, residual activity approximating the difference between enzyme and inactivator concentrations was detected (e.g., 5 μM isatoic anhydride, 8 μM α-chymotrypsin, 38% residual activity). These results suggest that the inactivation is stoichiometric. Dialysis of the inactivated enzyme against buffer (0.1 M potassium phosphate, pH 6.8, 48 h, 4 °C) resulted in the recovery of 3–5% of the enzyme activity, commensurate with the formation of a stable, covalent enzyme adduct.

The absorption spectrum of the inactivated enzyme following dialysis displayed maxima at 340 and 280 nm. With extinction coefficients of 4.0×10^3 and 5.0×10^4 cm^2/mmol, respectively,[3] a stoichiometry of one anthraniloyl moiety per α-chymotrypsin was determined. Fluorescence emission spectra of the inactivated enzyme following dialysis were found to be identical with the observations of Haugland and Stryer.[3] Taken together, these

(1) (a) Bechet, J.-J.; DuPaix, A.; Yon, J.; Wakselman, M.; Robert, J.-C.; Vilkas, M. *Eur. J. Biochem.* **1973**, *35*, 527–539. (b) Wakselman, M.; Hamon, J. F.; Vilkas, M. *Tetrahedron* **1974**, *30*, 4069–4079. (c) White, E. H.; Roswell, D. F.; Politzer, I. R.; Branchini, B. R. *J. Am. Chem. Soc.* **1975**, *97*, 2290–2291. (d) Nicolle, J. P.; Hamon, J. F.; Wakselman, M. *Bull. Soc. Chim. Fr.* **1977**, 83–88. (e) Bechet, J.-J.; DuPaix, A.; Blagoeva, I. *Biochimie* **1977**, *59*, 231–239. (f) Bechet, J.-J.; DuPaix, A.; Roucous, C.; Bonamy, A.-M. *Ibid.* **1977**, *59*, 241–246. (g) Vilkas, M. In "Enzyme-Activated Irreversible Inhibitors"; Seiler, N., Jung, M. J., Koch-Weser, J., Eds.; Elsevier/North Holland, Amsterdam; New York, 1978, pp 323–335. (h) Chakravarty, P. K.; Krafft, G. A.; Katzenellenbogen, S. A. *J. Biol. Chem.* **1982**, *257*, 610–612.

(2) Caplow, M.; Jencks, W. P. *Biochemistry* **1962**, *1*, 883–893.

(3) Haugland, R. P.; Stryer, L. *Conform. Biopolym., Pap. Int. Symp.* **1967**, *1*, 321–335.

(4) A similar strategy has been considered: Kaiser, E. T. "Bayer Symposium V"; Fritz, H., Tschesche, H., Green, L. J., Truscheit, E., Eds.; Springer Verlag: New York, 1974; pp 523–530. The *N*-*tert*-butyl-β-lactam of anthranilic acid was found to inactivate α-chymotrypsin, affording an anthraniloyl-chymotrypsin. Related compounds were found to be extremely unstable and, therefore, of limited biological utility.

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Table II. $t_{1/2}$ (min) for Inactivation by 3*H*-1,3-Oxazine-2,6-diones

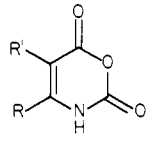
compd	concn, μM	enzyme[a] α-chymotrypsin	enzyme[a] pancreatic elastase
2a	1250	3.5	2.0
	125	85	27
2b	1250	>90	>120
	125	ND[b]	ND[b]
2c	1250	2.3	<1.0
	125	27.5	8.5

[a] Enzymes were incubated and assayed as described in Table I. [b] ND = experiment not done.

results indicate that the species arising from the incubation of α-chymotrypsin with isatoic anhydride or *p*-nitrophenyl anthranilate are equivalent and provide support for the mechanism depicted in Scheme I.

The specificity of **1** for serine and thiol proteases is illustrated in Table I. Incubation of 1250 μM isatoic anhydride with yeast aldehyde dehydrogenase, acetylcholinesterase, creatine kinase, carboxy peptidase A, yeast alcohol dehydrogenase, and pig liver esterase resulted in $t_{1/2}$ for inactivation >25 min. With pancreatic elastase, α-lytic protease, and trypsin, activity returned during the time course of the experiment (<2 h). Only with α-chymotrypsin was the inactivation found to be rapid, stoichiometric, and essentially irreversible.

These results suggested that the simpler 3*H*-1,3-oxazine-2,6-dione ring system (**2**) could also lead to a stable acyl-enzyme.



2a, R = R′ = H
b, R = CH_3; R′ = H
c, R = H; R′ = CH_3

Through modification of the molecule, enhanced selectivity for other serine or thiol proteases might be achieved. Studies in several laboratories have indicated a preference for L-alanine at the p_1 subsite of pancreatic elastase,[5] suggesting that the desired selectivity of action toward pancreatic elastase might be achieved by incorporating a methyl group at C-4 (**2b**) or C-5 (**2c**).

Compounds **2a** and **2b** were prepared from maleic and citraconic anhydride, respectively, by the method of Warren et al.[6] The 5-methyl-3*H*-1,3-oxazine-2,6-dione (**2c**) was prepared by treating citraconimide[7] with sodium hypochlorite as described by Bobek and co-workers.[8]

Table II shows the results obtained with these compounds. These compounds inactivate α-chymotrypsin in a time-dependent process. Inactivation does not proceed as rapidly as with isatoic anhydride, reflecting the enzyme's preference for aromatic residues. Pancreatic elastase was also inactivated. There was no detectable recovery of activity during the course of the experiments, unlike the results with isatoic anhydride. Upon dialysis (0.1 M potassium phosphate, pH 7.5, 48 h, 4 °C), a maximum of 30% recovery of activity with a half-life of approximately 60 h was noted. These results suggest that pancreatic elastase forms a covalent intermediate with **2a**, which hydrolyzes slowly.

A comparison of the results with **2a** and **2c** confirms our hypothesis that the presence of a methyl group enhances the inactivation of pancreatic elastase. The results with **2b** indicate that the orientation of this methyl group, presumably into the hydrophobic pocket, is crucial.

Studies are currently under way to confirm the mechanism of action of these compounds and to expand their specificity to other serine and/or thiol proteases.

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Registry No. 2a, 34314-63-1; **2b**, 51440-82-5; **2c**, 51255-10-8; serine protease, 37259-58-8; isatoic anhydride, 118-48-9; α-chymotrypsin, 9004-07-3; elastase, 9004-06-2; α-lytic protease, 37288-76-9; trypsin, 9002-07-7; papain, 9001-73-4.

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Electronic Absorption Spectra of Polarity-Polarizability Indicators in the Gas Phase

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Most "polarity-polarizability indicators" (PPI) used in solvatochromic studies are polar species,[1-3] sparingly soluble in low polarity-polarizability solvents, and endowed with very small vapor pressures at room temperature. As a consequence, the quantitative analysis of solvent effects on their electronic absorption spectra has long been hampered by the lack of data for these solvents and for the gas phase. This information is of crucial importance in order to unravel the respective contributions from polarity and polarizability effects.[4] Since Brady and Carr[5] have recently succeeded to obtain the UV-visible spectra of a number of PPI's (particularly those used to construct the π* scale of solvent polarity-polarizability[6]) in several perfluorinated solvents, we have decided to meet the most significant challenge left: the determination of gas-phase data.

Here, we report that we have obtained the electronic absorption spectra of the following compounds[7] in the gas phase: 4-nitroanisole (**4**), *N*,*N*-diethyl-4-nitroaniline (**6**), ethyl 4-(dimethylamino)benzoate (**9**), 4-nitroaniline (**14**), ethyl 4-aminobenzoate (**20**), 3-nitroaniline (**28**), 4-nitrophenol (**1b**), and *N*,*N*-dimethyl-3-nitroaniline (**28b**). This was conveniently done by heating a few crystals of these materials at temperatures ranging from 65 to 92 °C in 1-cm silica cells placed in the sample holder of the spectrophotometer.[8] We have also determined the spectra of these compounds in cyclohexane solutions at several temper-

(1) The important solvatochromism of the PPI's is associated with the high polarity of the ground and/or the excited states.[2,3]

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(3) Bayliss, N. S.; Mac-Rae, E. G. *J. Phys. Chem.* **1954**, *58*, 1002; **1957**, *61*, 562.

(4) Polarizability effects on both the ground and the excited states play an important role, either as dipole-induced dipole interactions or as London's dispersion forces. See, e.g.: Hirschfelder, J. O.; Curtiss, C. F.; Bird, R. B. "The Molecular Theory of Gases and Liquids"; Wiley: New York, particularly Chapters 12 and 13.

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(7) All compounds, except **1b** and **28b**, are numbered as in ref 6.

(8) The measurements were carried out with a Cary 219 spectrophotometer. The slit width was 0.5 nm. The apparatus was cooled by means of a double-loop water circulation at ca. 20 °C while the temperature of the cell holder was kept constant by means of a water circulation provided by a Lauda LS15 ultrathermostat. In all cases, matched 1-cm silica cells fitted with Teflon stoppers were used. In the gas-phase experiments, the cells were allowed to warm for periods of about 1 h before the spectrum was recorded. We also established that the same spectra were obtained when the temperature of the sample was either increased or decreased.

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