matography of this on activity-3 neutral alumina using 20% ether/hexane elution gave 40 mg (93%) of the ketone **5** as a clear oil.

B. To 70 mg (0.15 mmol) of the silyl enol ether **15** in 0.5 mL of THF at 0 °C was added 0.17 mL of thexylborane (0.5 M solution in THF, 0.17 mmol), and the mixture was warmed to 20 °C for 18 h. Addition of 0.2 mL of 3 N aqueous sodium hydroxide solution and 0.2 mL of 30% hydrogen peroxide solution at 0 °C, stirring of the two-phase solution for 30 min, extraction of this mixture with ether, washing of the organic layer with brine, drying of it over sodium sulfate, and concentration gave a crude oil. This was purified by chromatography on activity-3 neutral alumina with 20% ether/hexane to give 50 mg (76%) of the ketone **5** as a clear oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ 3.2–3.7 (5 H, br m), 1.5–2.5 (9 H, br m), 0.90 (9 H, s), 0.89 (9 H, s), 0.10 (6 H, s), 0.07 (6 H, s); IR (neat) 3050–3600, 2750–3000, 1740, 1480, 1260, 1100, 920, 850, 790, 740 cm^{-1}; MS (70 eV), *m/e* (% intensity) 371 (M^+ – *tert*-Bu, 0.7), 353 (M^+ – *t*-Bu – H_2O, 1.9), 271 (3.6), 239 (M^+ – TBSOH – *t*-Bu, 5.3), 223 (M^+ – TBSOH – isobutylene – OH, 7.0), 131 (17.7), 91 (10.2), 89 (17.7), 75 ($OSiMe_2H^+$, 100); high-resolution MS (70 eV), *m/e* 371.2087, calcd for $C_{18}H_{35}O_4Si_2$ 371.2074, 353.1983, calcd for $C_{18}H_{33}O_3Si_2$ 353.1969, 223.1157, calcd for $C_{12}H_{19}O_2Si$ 223.1155.

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Registry No. 1, 50-55-5; **5**, 92420-94-5; **6**, 64145-56-8; **7a**, 92420-95-6; **7b**, 92420-96-7; **8a**, 92420-97-8; **8b**, 92420-98-9; **9**, 92420-99-0; **10**, 92421-00-6; **11**, 92421-01-7; **13**, 92421-02-8; **14**, 92421-03-9; Δ^6-**15**, 92421-04-0; Δ^5-**15**, 92421-05-1; propargyl alcohol, 107-19-7; propargyl benzyl ether, 4039-82-1; trimethylsilyl chloride, 75-77-4; *tert*-butyldimethylsilyl chloride, 18162-48-6.

Communications to the Editor

3-Alkoxy-7-amino-4-chloroisocoumarins: A New Class of Suicide Substrates for Serine Proteases

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Human leukocyte elastase (HLE) is a serine protease involved in a number of disease states including pulmonary emphysema. As such, there is considerable interest in the development of therapeutically useful HLE inhibitors. Previously, a number of heterocyclic structures have been shown to be suicide substrates of serine proteases.[1-3] Here we report that 3-alkoxy-7-amino-4-chloroisocoumarins are suicide substrates of HLE, porcine pancreatic elastase (PPE), and bovine chymotrypsin A_α (ChT).

Incubation of the 7-amino-4-chloroisocoumarins[4] **2** and **6**, the 7-nitro-4-chloroisocoumarins[4] **1** and **5**, 7-amino-3-methoxyisocoumarin[5] **3**, and 4-chloro-3-ethoxyisocoumarin[6] (**4**) with HLE and PPE resulted in a time-dependent loss of enzymatic activity (Table I). ChT was also inactivated by **2** and **6** with $k_{obsd}/[I]$ values of 108 M^{-1} s^{-1} ([I] = 0.1 mM) and 270 M^{-1} s^{-1} ([I] = 0.02 mM), respectively. While >99% inactivation was observed with 7-amino-4-chloroisocoumarins **2** and **6**, a maximum of 70–95% inactivation was observed with **1** and **3**–**5** under the conditions utilized. Inactivation of PPE (1.7 μM) by the 7-amino-4-chloroisocoumarin **2** (16 μM) in the presence of the reversible competitive inhibitor CF_3CO-Lys-Ala-4-methylanilide[7] (15 μM) resulted in a decrease in the inactivation rate ($k_{obsd}/[I]$ = 76 M^{-1} s^{-1}), indicating that **2** is active-site directed.

Table I. Inactivation of Serine Proteases by Substituted 3-Alkoxyisocoumarins[a]

enzyme		inhibitor concentration, μM	$k_{obsd}/[I]$, M^{-1} s^{-1}	$k_{reactivation}$, s^{-1}
HL elastase	**1**	15	>2600[b]	0.5 × 10^{-3}
	2	5	10000	0[c]
	3	112	200	
	4	0.6	43000[d]	0.2 × 10^{-3}[e]
	5	34	>2800[b]	0.33 × 10^{-3}
	6	4.4	9500	0[c]
PP elastase	**1**	40	>600[b]	0.5 × 10^{-3}
	2	16	1000	0[c]
	3	91	18	
	4	37	1400	0.16 × 10^{-3}
	5	30	>1300[b]	1.3 × 10^{-3}
	6	19	700	0[c]

[a] Unless otherwise noted, enzyme (0.4–2.0 μM) was incubated with inhibitor in 0.25–0.6 mL of 0.1 M Hepes, 0.5 M NaCl, pH 7.5, 8–12% Me_2SO at 25 °C. Aliquots (10–50 μL) were withdrawn at various times and the residual enzymatic activity measured as previously described.[3] The k_{obsd} values were calculated from plots of ln v/v_0 vs. time with r > 0.99. [b] Inactivation was extremely rapid and the $k_{obsd}/[I]$ values are based on residual enzymatic activity at 0.25 min. [c] Less than 0.5% activity regained after standing 100 h at 25 °C. Controls retained >90% enzymatic activity over this time period. [d] Inactivation rate measured using the progress curve method[10] with 0.171 mM MeO-Suc-Ala-Ala-Pro-Val-4-nitroanilide and 8 nM HLE. [e] [I] = 0.013 mM.

Loss of the isocoumarin ring chromophore of 7-amino-4-chloro-3-ethoxyisocoumarin (**6**) (0.030 mM, ϵ_{385} = 3330 M^{-1} cm^{-1}; spontaneous hydrolysis, 5.9 × 10^{-5} s^{-1}) occurred concurrently with inactivation of PPE (6.4 μM, $k_{obsd}/[I]$ = 940 M^{-1} s^{-1}) and ChT (12.6 μM, $k_{obsd}/[I]$ = 200 M^{-1} s^{-1}). A reaction stoichiometry of 1.03 ± 0.07 and 1.31 ± 0.03 equiv of **6** with PPE and ChT, respectively, was calculated from the absorbance change (385 nm). The inactivated enzymes showed no new bands in the UV–visible spectrum before or after dialysis (0.1 M phosphate pH 6.8 buffer, 48 h, 4 °C). The reaction of 4-chloro-3-ethoxyisocoumarin (**4**) (0.066 mM) with ChT (0.050 mM) was monitored by the absorbance decrease at 350 nm (ϵ = 2920 M^{-1} cm^{-1}) and 0.97 equiv of **4** are required for total (>99%) inactivation. PPE (7 μM) hydrolyzed 7-amino-3-methoxyisocoumarin (**3**) (0.054 mM, ϵ_{385} = 4300 M^{-1} cm^{-1}; spontaneous hydrolysis, 3 × 10^{-5} s^{-1}) with a pre-steady-state rate constant of 0.75 × 10^{-3} s^{-1} (burst = 0.66 equiv) and a steady-state rate constant of 0.084 × 10^{-3} s^{-1}.

The reaction of PPE (8.9 μM) with 7-amino-4-chloro-3-methoxyisocoumarin (**2**) (0.031 mM, 0.1 M phosphate buffer,

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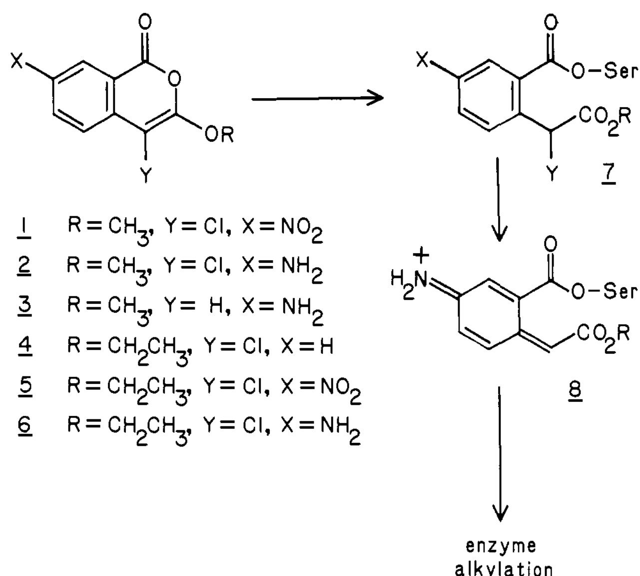

Figure 1. Proposed pathway for inactivation of serine proteases by substituted 3-alkoxyisocoumarins.

pH 6.8, 10% Me_2SO) was also monitored by the decrease in fluorescence emission at 510 nm (ϵ_{ex} = 400 nm), and the rate of ring opening (18 × $10^{-3}s^{-1}$) was identical with the inactivation rate (17 × 10^{-3} s^{-1}) under these contions. The inactivation stoichiometry (1.06 ± 0.02) was similar to that determined spectrophotometrically. No new emission bands (ϵ_{ex} = 400 nm) were observed in the fluoroscence spectra of inactivated PPE (8.9 μM) after dialysis against phosphate buffer (pH 6.8) for 24 h at 4 °C. Inactivation of ChT (0.27 mM) by **6** (0.33 mM) resulted in <1% release of EtOH.[8]

HLE and PPE inactivated by the 7-amino-4-chloroisocoumarins **2** and **6** regained <0.5% enzymatic activity after standing for 100 h, while these enzymes inactivated by the 7-nitro derivatives **1** and **5** and the 4-chloro-3-ethoxyisocoumarin (**4**) regained >85% activity ($t_{1/2}$ < 72 min) upon standing at 25 °C (Table I). In addition, HLE and PPE inactivated by **6** regained <0.5% enzymatic activity after dialysis for 48 h (0.1 M phosphate pH 6.8 buffer, 4 °C) and upon further standing for 48 h at 25 °C, which is evidence for irreversible inactivation. Addition of buffered hydroxylamine (0.46 M) to HLE and PPE inactivated by **6** (0.016 mM) resulted in 34-42% reactivation ($t_{1/2}$ = 5.7-7.2 h), while addition of buffered hydroxylamine (0.26 mM) to these enzymes inactivated by 7-amino-3-methoxyisocoumarin (**3**) (0.09-0.11 mM) resulted in rapid and complete reactivation ($t_{1/2}$ = 12 and 9.6 min, respectively).

These results are consistent with Figure 1 where the 3-alkoxy-7-amino-4-chloroisocoumarins **2** and **6** react with the active-site serine of serine proteases to give the acyl enzyme **7** which decomposes to the acyl-*p*-quinonemethide imine **8**. An irreversibly inactivated enzyme could then result by reaction of the acyl-*p*-quinonemethide imine **8** with an active-site nucleophile.[9] The requirement of both the 7-amino and 4-chloro substituents for irreversible inactivation is demonstrated by the finding that the 7-nitro-4-chloroisocoumarins **1** and **5**, 4-chloroisocoumarin **4**, and 7-aminoisocoumarin (**3**) react to give **7** but reactivate rapidly either upon standing or upon addition of hydroxylamine. The 3-alkoxy-7-amino-4-chloroisocoumarins **2** and **6** are some of the most potent HLE inactivators yet reported, represent a new class of suicide substrates for serine proteases, and may be useful in the prevention of proteolysis in vitro and in vivo. Studies leading to further proof of the proposed mechanism and extention to other serine proteases are now in progress.

Acknowledgment. This work was supported by grants from the National Institutes of Health (HL 29307) and from the Council for Tobacco Research.

(8) EtOH release measured upon dilution (2×) of inactivated enzyme into liver alcohol dehydrogenase (1 μM) and NAD^+ (1.5 mM) in 0.1 M phosphate pH 7.8 buffer. Alcohol dehydrogenase was measured by monitoring the increase in absorbance of NADH at 340 nm (ϵ = 6220 M^{-1} cm^{-1}) and <2 μM ethanol could have been detected.

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The Kharasch Reagent. Regioselective Generation of Dienol Ethers from Enones

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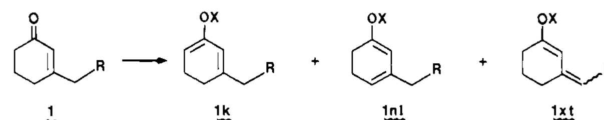
After a hiatus of over 40 years, we have uncovered the identity of the Kharasch reagent.[1] Stoichiometric use of this reagent now makes possible, for the first time, the regioselective preparation of "thermodynamic" trimethylsilyl dienol ethers from cyclic enones.

Deprotonation of a cyclic enone (e.g., **1**) can potentially give three regioisomeric dienolates: the cross-conjugated ("kinetic") isomer **1k**, the through-conjugated ("thermodynamic") endocyclic isomer **1nt**, and through-conjugated ("thermodynamic") exocyclic isomer **1xt**. The synthetic utility of dienolates **1k**, **1nt**, and **1xt**



has been recognized for some time.[2-4] However, exploitation of this utility requires methods for the regiospecific preparation of each of the three possible dienolates. Unfortunately, prior to this work only enolates of type **1k** have been generally accessible from the corresponding enone (LDA/THF/-78 °C).[2c,5,6]

One of the rare examples of formation of a through-conjugated dienolate from the corresponding enone was reported by Kharasch.[1] In a modified version of this reaction, treatment of **2** with

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