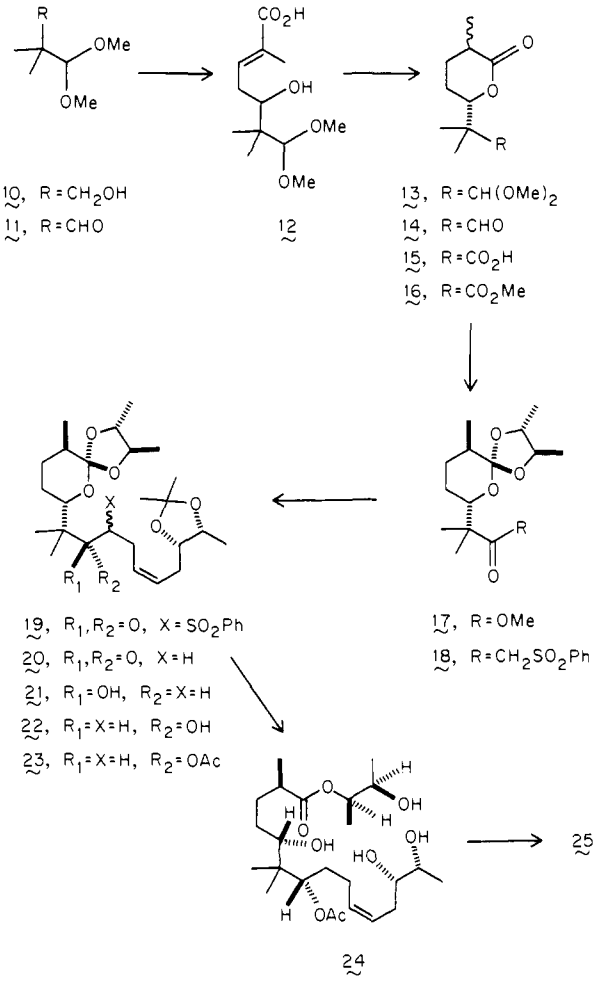
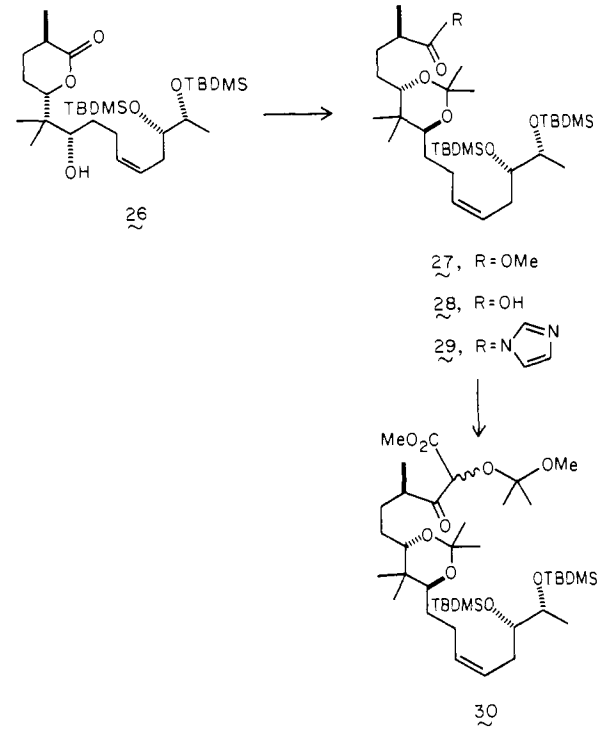
THF-H_2O (10:1), 75 °C, 1 h) to furnish **20** as an oil ($[\alpha]^{23}_D$ +3.5°) in 75% yield from **18**. Reduction of this ketone ($NaBH_4$, MeOH, 25 °C) gave a mixture of **21** and **22** (98%; 2:1 respectively), the acetates of which (Ac_2O, pyridine, DMAP) were easily separated. The 7*S* acetate **23** underwent hydrolysis (*p*-TsOH, THF-H_2O (4:1), 56 °C, 12 h) to **24**, which was saponified (NaOH, THF-H_2O, 25 °C, 3 h) and acidified (5% aqueous HCl) to provide **4** (91% from **23**), identical with the degradation product from boromycin.[3] For a rigorous comparison with naturally derived material, synthetic **4** was converted to triacetate **25** (68%, $[\alpha]^{20}_D$ +14.2°; Ac_2O, pyridine, DMAP), which was spectroscopically identical with the substance ($[\alpha]^{20}_D$ +17.6°)[6b] obtained from **1**.



With the configuration at the five chiral centers in this segment authenticated, attention was turned to its homologation in order to complete the C(1)-C(17) perimeter of **2**. After protection of **4** as its bis(*tert*-butyldimethylsilyl) ether **26** (excess TBDMSCl, imidazole, DMF, 48 h), the latter was treated with 2,2-dimethoxypropane (*p*-TsOH, C_6H_6-MeOH) to give a quantitative yield of **27**. This ester was saponified (20% aqueous NaOH, MeOH, followed by 2% HCl, 0 °C), and the derived carboxylic acid **28** was converted to **29** (carbonyldiimidazole, THF). Acylation of the enolate of methyl methoxyisopropylglycolate[21] (LDA, THF, -78 °C, 10 min) with **29** afforded **30** as a C(2) epimeric mixture in 35% overall yield from **27**.[22] Stereochemical inhomogeneity at this stage is probably of no consequence, since it has been demonstrated in the synthesis of aplasmomycin that borate formation from the macrocyclic tetraol is accompanied by epimerization at C(2) to the natural *R* configuration.[7]



The synthesis of **30** permits access to a fully functionalized subunit of **2** with rigorously defined stereochemistry and also opens a prospective route to the second half of boromycin.

Acknowledgment. We are grateful to Bernard G. Sheldon, Paul R. Johnson, and Jeffrey Fitzner for experimental assistance. Financial support was provided by the National Institutes of Health (Grant AI 10964).

(21) Prepared by exposing a mixture of methyl glycolate and 2-methoxypropene to the vapor of $POCl_3$ (**Caution**: exotherm).

(22) For a recent account of the elegant approaches by Hanessian to the two halves of boromycin, see: Hanessian, S.; Delorme, D.; Tyler, P. C.; Demailly, G.; Chapleur, Y. In "Current Trends in Organic Synthesis"; Nozaki, H., Ed.; Pergamon Press: Oxford, U.K., 1983; p 205.

New Mechanism-Based Serine Protease Inhibitors: Inhibition of Human Leukocyte Elastase, Porcine Pancreatic Elastase, Human Leukocyte Cathepsin G, and Chymotrypsin by 3-Chloroisocoumarin and 3,3-Dichlorophthalide

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Mechanism-based irreversible inhibitors, which have been reported for porcine pancreatic (PP) elastase and bovine pancreatic chymotrypsin A_α, include halo enol lactones and 6-chloropyrones.[1] Human leukocyte (HL) elastase and cathepsin G are related serine proteases which are involved in the connective tissue destruction that occurs in emphysema and various inflammatory diseases. Both enzymes are inhibited reversibly by heterocyclic structures such as benzoxazinones[2] and benzisothiazolinones,[3] and this

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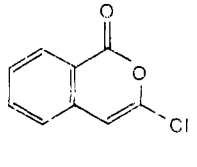
Table I. Inactivation of Serine Proteases by 3-Chloroisocoumarin (**1**) and 3,3-Dichlorophthalide (**2**)

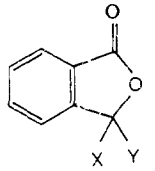
enzyme	inhibitor	k_{obsd}/I M^{-1} s^{-1}	$t_{1/2}$ (inactivation), s	$t_{1/2}$ (reactivation), h
HL elastase	1	3900[a]	42	*b*
	2	N.D.[c]	<25[d]	1.5
PP elastase	1	512[e]	277	19
	2	N.D.[c]	<25[f]	0.4
chymotrypsin A_α	1	163[g]	88	1.1
	2	N.D.[c]	<25[h]	1.0

[a] HL elastase (0.7 μM) was incubated in 450 μL of buffer (0.1 M Hepes, 0.5 M NaCl, 10% Me_2SO, pH 7.5 at 25 °C) containing 13 μM inhibitor. At various time intervals, 50 μL aliquots were withdrawn and assayed with MeO-Suc-Ala-Ala-Pro-Val-NA (171 μM, buffered as above) as a substrate (MeO-Suc = $CH_3OCO(CH_2)_2CO$; NA = 4-nitroanilide). [b] No activity was regained after 6 h. [c] Not determined. [d] Conditions as given in *a* except [I] = 9 μM and [E] = 0.09 μM. [e] PP elastase (Sigma, 0.07 μM) was incubated as in *a* with [I] = 4.8 μM. Residual activity was measured with Suc-Ala-Ala-Ala-NA (1.63 mM) as a substrate. [f] Conditions as given in *a* except [I] = 69 μM and [E] = 1.8 μM. Residual activity was measured with MeO-Suc-Ala-Ala-Pro-Val-NA (266 μM) as a substrate. [g] Chymotrypsin A_α (Sigma, 1.3 μM) was incubated as in *a* with 48 μM inhibitor. Residual activity was measured with Suc-Phe-Pro-Phe-NA (515 μM) as a substrate. [h] Chymotrypsin A_α (1.9 μM) was incubated as in *a* with 23 μM inhibitor and residual activity measured with Suc-Phe-Pro-Phe-NA (220 μM) as a substrate.

suggested that heterocycles containing masked reactive functionalities might act as mechanism-based irreversible inhibitors for HL elastase and cathepsin G. Therefore we prepared 3-chloroisocoumarin (3-chloro-1*H*-2-benzopyran-1-one)[4] (**1**) and 3,3-dichlorophthalide[5] (**2**) and have found them to be potent inhibitors of several serine proteases.



1

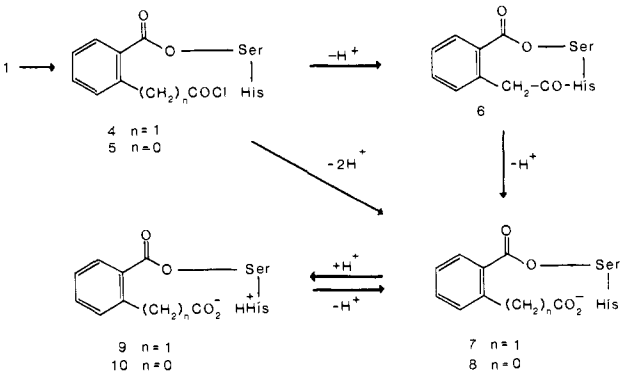


2 X = Y = Cl
X = Cl, Y = H

Incubation of **1** and **2** with HL elastase, PP elastase, and chymotrypsin A_α resulted in a rapid time-dependent inhibition of enzyme activity (Table I). While human leukocyte cathepsin G was inhibited by **2** ($t_{1/2}$ <25 s, [I] = 52 μM), neither **1** or **2** inhibited trypsin or papain. In all cases the inhibition rate was dependent upon inhibitor concentration:[6] reaction of **1** with HL elastase, K_I = 55 μM, k_2 = 0.59 s^{-1} (HLE, 78 nM; substrate MeO-Suc-Ala-Ala-Pro-Val-NA, 171 μM; **1**, 7–35 μM); PP elastase, K_I = 65 μM, k_2 = 0.22 s^{-1} (PPE, 8.7 nM; substrate Suc-Ala-Ala-Ala-NA, 1.63 mM; **1**, 11–55 μM). Rates of inhibition of HL and PP elastase were decreased dramatically when the reversible inhibitors 2-(pentafluoropropyl)-4*H*-3,1-benzoxazin-4-one[2] (at 54 μM, k_{obsd}/I = 9 M^{-1} s^{-1}) and CF_3CO-Lys-Ala-$NHC_6H_4CH_3$[7] (at 22 μM, k_{obsd}/I = 20 M^{-1} s^{-1}) were added, respectively, to the incubation solutions, indicating that the inhibitors are active-site directed.

HL and PP elastase inhibited by **1** were quite stable to reactivation upon standing, while cathepsin G, chymotrysin, and all the enzymes inhibited with **2** regained activity upon standing (Table I). The presence of labile acyl moieties was indicated by the rapid reactivation (<4 min) of HL and PP elastase inactivated by **1** when buffered hydrazine (1–3 mM) was added.

Scheme I. Proposed Pathway for the Inactivation of Serine Proteases by 3-Chloroisocoumarin (**1**) and 3,3-Dichlorophthalide (**2**)



Hydrolysis of **1** to 2-carboxyphenylacetic acid in the presence and absence of enzyme could be monitored by an absorbance decrease at 325 nm (ε = 3500 M^{-1} cm^{-1}, 0.1 M Hepes, 0.5 M NaCl, [**1**] = 0.107 mM, 5% Me_2SO, pH 7.5 at 25 °C). The pseudo-first-order hydrolysis rate increased from 0.088 × 10^{-3} to 0.42 × 10^{-3} and 2.0 × 10^{-3} s^{-1} upon addition of 12 μM and 38 μM PP elastase, respectively. Inhibition of HL and PP elastase by increasing ratios of **1** (e.g., 80% inactivation of HL elastase was observed at I/E = 28) suggested >15 turnovers/inactivation for HL elastase and >4 for PP elastase. The reaction of **1** with chymotrypsin A_γ is almost stoichiometric since 1.0 equiv of **1** resulted in 90% inhibition.

Reaction of **1** (250 μM) with chymotrypsin A_γ (250 μM) in aqueous solution (250 μM NaCl, 10% Me_2SO, pH 7.5) utilizing a pH stat resulted in the rapid release of 0.92 equiv of proton after 6 min at which time the residual enzymatic activity was 9.8%. Similar experiments with **2** (244 μM) and chymotrypsin A_γ (244 μM) resulted in the release of 2.7 equiv of protons after 15 min at which time the enzymatic activity was 8%. Further incubation resulted in the release of 3.1 total protons with **1** (theoretical 3) after 115 min. At pH 8.5 under identical conditions, **1** reacted with chymotrypsin A_γ to release 0.95 proton within 3 min and slowly released the additional protons over the course of 1 h.[8]

3-Chlorophthalide (**3**)[9] did not inhibit any of the enzymes tested (I/E > 190) but was hydrolyzed by chymotrypsin A_γ as monitored by an increase in absorbance at 295 nm (ε = 1600 M^{-1} s^{-1}). The hydrolysis rate constant of **3** (29 μM, 0.1 M Hepes, 0.5 M NaCl, 10% Me_2SO, pH 7.5) increased from 1.1 × 10^{-3} to 2.7 × 10^{-3} s^{-1} upon the addition of chymotrypsin A_γ (27 μM).

The above results are consistent with Scheme I where **1** reacts with the active-site serine of the protease forming the acyl enzyme **4** and generating an acid chloride or its corresponding ketene.[10] Formation of the diacylated product **6** then occurs by reaction at histidine-57, the most likely nucleophile in the active site of most serine proteases (Met-192 in chymotrypsin and Gln-192 in PP elastase are less likely possibilities). The stabilized acyl enzyme **9** can be ruled out since only one proton is released at both pH 7.5 and 8.5, and at high pH one would expect **9** to release an additional proton to give **7**. The finding that 3 protons are released upon inactivation of chymotrypsin A_γ by **2** indicates formation of the monoacylated product **8**. The observation that **3** reacts with chymotrypsin but does not inhibit points to the requirement for

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(10) The rapid loss of the 325-nm chromophore indicates that initial attack does not take place at C-3.

the formation of an acid chloride or carboxylic acid. And the observation that **10** is not formed from **2** would also argue against **9** being responsible for inhibition of serine proteases by **1**.

Evidence presented here indicates that **1** and **2** are mechanism-based irreversible inhibitors of serine proteases. These are the first demonstrated examples of enzyme-activated inhibitors of HL elastase and cathepsin G. These enzymes have been noted to be major contributors to elastin destruction observed in emphysema.[11] These inhibitors and similar structures may have considerable pharmacologic potential as inhibitors in vivo. Studies leading to a clearer understanding of these inhibition processes 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. We are grateful to Dr. Jim Travis at the University of Georgia for supplying the leukocyte proteases.

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Chemiluminescence from Hyponitrite Esters. Excited Triplet States from Dismutation of Geminate Alkoxyl Radical Pairs[1]

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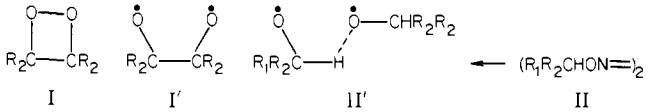
Numerous studies have focussed attention on chemiluminescence from excited states produced by thermal decomposition of dioxetanes.[2-5] There is general agreement[6-8] that a bialkoxyl 1,4-biradical intermediate (I′) is consistent with the production



I I′ II′ II

a, $R_1 = CH_3$, R_2 = Ph; b, $R_1R_2 = (CH_3)_2CH$; c, $R_1R_2 = (CH_2)_5$; d, $R_1 = H_1$, R_2 = Ph

of these excited states, and spin inversion in the intermediate to a triplet biradical is a convenient way to rationalize the high triplet yields commonly realized from these compounds. We wish to present preliminary results of a study of the quantum yields arising from alkoxyl radical pairs, in which the assumed transition state (II′) exhibits a formal similarity to I′. In the latter, one σ bond is subsequently lost and two π bonds are formed, while in the former, one σ bond is lost with a gain of one π and one σ bond. The exothermic self-reaction of alkoxyl pairs provide one way to assess the relative importance of cyclic structures and ring strain for efficient generation of excited states from oxygenated precursors.[9,10]

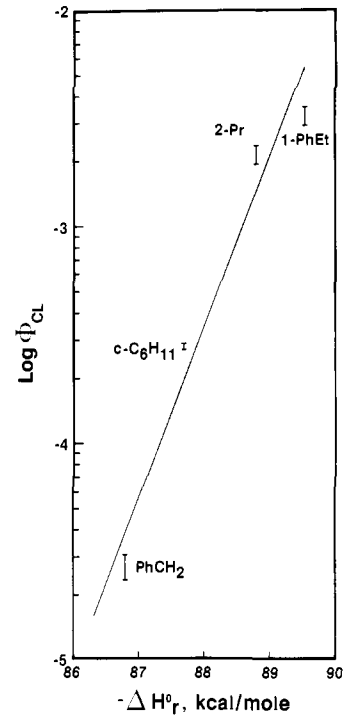


Figure 1. Quantum yields from alkyl hyponitrites in *tert*-butylbenzene as a function of enthalpy for disproportionation to ground-state products. The ordinate values were obtained as described in the text at 43.7 °C.

The chemiluminescence intensity from 5.0×10^{-3} to 5.0×10^{-4} M solutions of hyponitrites (HN; IIa-d) or dioxetane (D; I (R = CH_3)) was measured as a function of 7.0×10^{-4}-3.0×10^{-3} M 9,10-dibromoanthracene (DBA, triplet acceptor) or 9,10-diphenylanthracene (DPA, singlet acceptor).[3,11] The data for every case showed linear relationships ($r > 0.98$) when $1/(d(h\nu)/dt)$ was plotted against $1/[\text{acceptor}]$, but the ratios of intercept to slope[12] ($=k_f/(k_d + k_0[^3O_2])$) for the hyponitrite solutions were about 1000-5000 for both aromatic sensitizers. This suggested that the enhanced chemiluminescence from the DPA-sensitized solutions arose from an inefficient triplet-singlet energy-transfer process and that the same, relatively long-lived triplet precursor was responsible for exciting both aromatic fluorescers. In subsequent experiments, addition of piperylene[8] was shown to strongly quench the emission from solutions of DPA and IIa, but not from DPA and dioxetane. Extrapolation to infinite diene concentration gave limiting values of singlet emission that were comparable to the background signal. The singlet yield was 3.7×10^{-6} at the upper limit from 1-phenylethyl hyponitrite at 48.6 °C, corresponding to $^3T/^1S > 1500$.

The intercepts of the Stern-Volmer plots gave values of I_∞^{-1}, from which triplet quantum yields were calculated from the relation in eq 1, in which k's are the first-order rate constants for

$$\Phi_{HN} = (k_D[D]\Phi_D/k_{HN}[HN])(I_\infty^{HN}/I_\infty^{D}) \quad (1)$$

decomposition, and the bracketed terms refer to initial concentrations. The values of k were obtained from the decay of chemiluminescence from the same solutions over long time periods, which follows the relation $I_t = I_0e^{-kt}$ or by extrapolation of k's obtained at elevated temperatures.[13a] The value of Φ_D was taken as 0.31.[14]

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