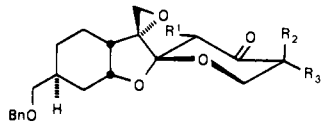
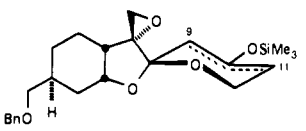


19 a: $R^1 = R^2 = H$, $R^3 = Me$
b: $R^1 = R^3 = H$, $R^2 = Me$
c: $R^1 = Me$, $R^2 = R^3 = H$



20 a: $\Delta^{10,11}$
b: $\Delta^{9,10}$

MeOH), oxidation [RuO_4–$NaIO_4$, $CH_3CN/CCl_4/H_2O$ (6:6:9)], and esterification (CH_2N_2). Following the precedent of Collum,[27] reduction of **21**[16] afforded the axial alcohol.[16,31] Finally, cinnamoylation (*t*-cinnamoylchloride, DMAP, pyr) gave (+)-phyllanthocin (**1**) identical in all respects with an authentic sample of (+)-phyllanthocin provided by Dr. Matthew Suffness (NCI).[32]

In summary, a stereochemically linear, reasonably efficient (4.8%) total synthesis of (+)-phyllanthocin has been achieved.

Acknowledgment. Support for this investigation was provided by the National Institutes of Health (Institutes of General Medical Sciences) through Grant GM 29028.

Supplementary Material Available: Characterization data for compounds **5b**, **6**, **8**, **14**, and **19a** as well as comparison NMR spectra of natural and synthetic (+)-phyllanthocin (3 pages). Ordering information is given on any current masthead page.

(31) Comparison of ester **21** with that prepared by Collum demonstrated their identity. In addition, the enantiomeric excess (Mosher ester method) was 95%.[18] We thank Professor David Collum (Cornell University) for the NMR and IR spectrum of ester **21**.

(32) We thank Dr. Matthew Suffness, Chief, Natural Products Branch NCI Developmental Therapeutics Program, for the generous sample of phyllanthoside.

Total Synthesis of (+)-Phyllanthoside

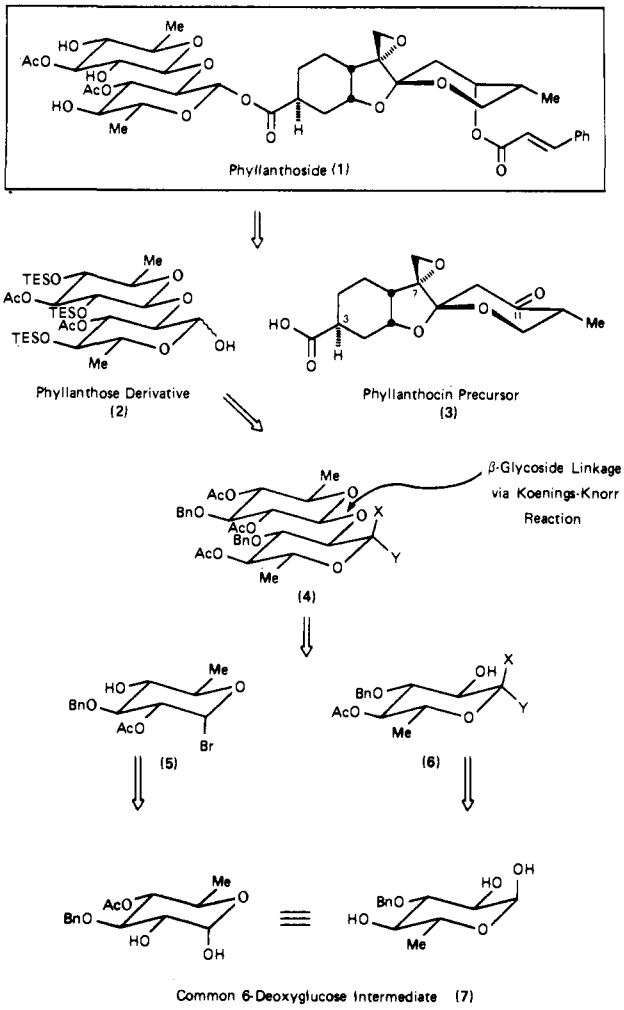
Amos B. Smith, III,*[1] and Ralph A. Rivero

Department of Chemistry, The Laboratory for Research on the Structure of Matter and The Monell Chemical Senses Center University of Pennsylvania Philadelphia, Pennsylvania 19104

Received June 16, 1986
Revised Manuscript Received December 12, 1986

In this paper we report the first total synthesis of (+)-phyllanthoside (**1**), a novel bisabolane glycoside isolated by Kupchan[2a] and structured by Pettit.[2b] Phyllanthoside displays selective cytotoxicity at very low concentrations toward solid tumors of the breast and colon,[3] and as such has recently been selected by NCI as a clinical candidate.[4] No reports on the synthesis and/or approaches to this biologically important glycoside have appeared. The aglycone (phyllanthocin), however, has attracted considerable attention.[5]

Scheme I. Retrosynthetic Analysis of Phyllanthoside[a]



[a] α: X = $OCH_2CH=CH_2$; Y = H. β: X = H; Y = $OCH_2CH=CH_2$.

With a viable, stereocontrolled synthesis of phyllanthocin secured,[6] the central issue became construction of a disaccharide suitable for coupling to the aglycone.[7,8] Toward this end, we designed our strategy (Scheme I) to take advantage of the well-known Koenigs–Knorr protocol[9a] to couple monosaccharides

(1) Camille and Henry Dreyfus Teacher-Scholar, 1978–1983; National Institutes of Health Career Development Award, 1980–1985; J.S. Guggenheim Fellow, 1985–1986.

(2) (a) Kupchan, S. M.; LaVoie, E. J.; Branfman, A. R.; Fei, B. Y.; Bright, W. M.; Bryan, R. F. *J. Am. Chem. Soc.* **1977**, *99*, 3199. (b) Pettit, G. R.; Cragg, G. M.; Gust, D.; Brown, P.; Schmidt, J. M. *Can. J. Chem.* **1982**, *60*, 939. Pettit, G. R.; Cragg, G. M.; Suffness, M.; Gust, D.; Boettner, F. E.; Williams, M.; Saenz-Renauld, J. A.; Brown, P.; Schmidt, J. M.; Ellis, P. *J. Org. Chem.* **1984**, *49*, 4258; Pettit, G. R.; Cragg, G. M.; Suffness, M. *J. Org. Chem.* **1985**, *50*, 5060.

(3) Powis, G.; Moore, D. J. *J. Chromatogr.* **1985**, *342*, 129. Also see: Powis, G.; Moore, D. J. *Proc. Assoc. Cancer Res.* **1985**, *26*, 354.

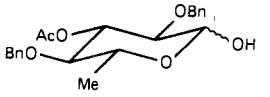
(4) This decision was made in Jan. 1986, with possible initial human studies in early 1987; personal communication from Dr. Matthew Suffness, Chief, Natural Products Branch, Developmental Therapeutics Program, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

(5) Phyllanthocin is biologically inactive. For recent syntheses of phyllanthocin, see: McGuirk, P. R.; Collum, D. B. *J. Am. Chem. Soc.* **1982**, *104*, 4496. Williams, D. R.; Sit, S.-Y. *J. Am. Chem. Soc.* **1984**, *106*, 2949. McGuirk, P. R.; Collum, D. B. *J. Org. Chem.* **1984**, *49*, 843. Burke, S. D.; Cobb, J. E.; Takeuchi, K. *J. Org. Chem.* **1985**, *50*, 3420. Dappen, M. S.; Duprē, B.; Murphy, C. J.; Martin, S. F. *Abstracts of Papers*, 192nd National Meeting of the American Chemical Society, Anaheim, CA; American Chemical Society: Washington, DC, September 1986; ORGN 291.

(6) Previous communication: Smith, A. B., III; Fukui, M. *J. Am. Chem. Soc.*, preceding paper in this issue.

(7) While seemingly straightforward, several structural-reactivity features of the disaccharide unit, in conjunction with the structure of the aglycone, rendered a direct coupling approach inoperative. Not the least problematic was construction of two β-glycoside bonds, one an unusual $1\rightarrow 2\beta$ glycoside linkage the other the β-glycosidic ester. In addition, we were faced with the known propensity of the acetates in phyllanthoside to undergo facile migration.[2b]

(8) Initial model studies suggested that formation of the β-glycosidic ester would not present a serious problem. Specifically, reaction of 2 equiv of monosaccharide i, shown by NMR to be a 2:1 (β/α) mixture of anomers, with



i

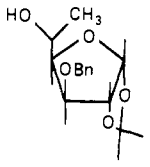
the acid chloride derived from **3** afforded an 8:1 mixture of glycosides (β/α). The favorable selectivity presumably derives from the enhanced reactivity of the equatoral (i.e., β) anomer. We recognized that success with disaccharide **3** would directly depend on a similar favorable anomeric ratio (vide infra).

(9) (a) Koenigs, W.; Knorr, E. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 957. (b) Helferich, B.; Weis, K. *Chem. Ber.* **1956**, *89*, 314.

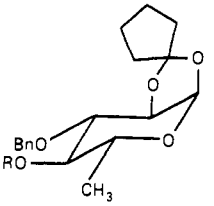
0002-7863/87/1509-1272$01.50/0

5 and **6**, thereby assuring β-glycoside formation. In addition, we selected the benzyl protecting group for mono- and disaccharide construction. While this choice would assure positional integrity of the acetates, union with a fully endowed aglycone was precluded, given the anticipated competitive hydrogenation of the cinnamoyl group.[10] Instead, we envisioned coupling dissacharide **2** to acid **3**. This tactic would require a benzyl–acetyl exchange latter in the sequence (i.e., **4** → **2**). Reduction of the C(11) carbonyl, cinnamoylation, and removal of the TES groups would then afford phyllanthoside (**1**).

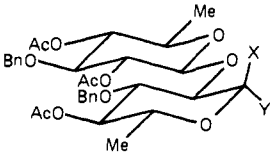
Synthesis of disaccharide **2** began with monosaccharide **8**.[12] Hydrolysis of the ketal [2 N H_2SO_4–THF (2:2), 94%] afforded **7**,[11a] the requisite common monosaccharide for both **5** and **6**. Subsequent acetylation (Ac_2O, catalytic DMAP, pyr, 85%) provided the corresponding triacetate, which upon treatment with HBr (30% in HOAc)[14] led to **5**[11a] in 85% yield.



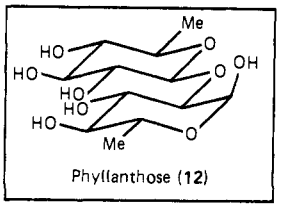
8



9: R = H
10: R = Ac



11: X = OH; Y = H
13a: X = OCH=CHCH$_3$; Y = H

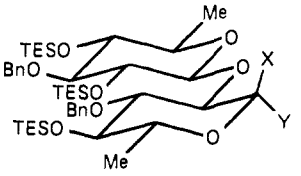


Phyllanthose (**12**)

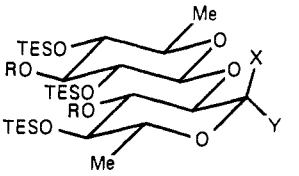
Monosaccharide **6** in turn was prepared from **7**. We opted for cyclopentylidene **9**[11] (1,1-dimethoxycyclopentane, catalytic CSA, THF, 68%),[15] which was acetylated (Ac_2O, cat DMAP, pyr, $\simeq$100%) and then treated with excess allyl alcohol (catalytic CSA, benzene at reflux) to afford a 1:1 mixture (96% yield) of monosaccharides **6**α[11] and **6**β,[11] separable by flash chromatography.[16]

Coupling of **5** and **6** was accomplished, exploiting the Helferich modification[9b] of the Koenigs–Knorr process. That is, reaction of **5** with **6**α and **6**β in the presence of $Hg(CN)_2$ [2 equiv, benzene–$MeNO_2$ (1:1)] led to **4**α[11] and **4**β[11] in 68% and 84% yield, respectively.

We next turned to the preparation of disaccharide **2**; required here was a benzyl–acetyl exchange. Toward this end, isomerization of the olefinic bond[19] in **4**α (Pd/C, MeOH) led in 76% to enol ether **13**α,[11,20] contaminated with 20% of **11**, the latter removed by flash chromatography. Saponification (K_2CO_3, MeOH) followed by protection of the resultant triol (TESCl, catalytic DMAP, DMF, imide)[21] led to **14**α[11] (85%, two steps), which in turn was subjected to ozonolysis (O_3, CH_2Cl_2; Ph_3P) to afford formate ester **15**α in 90% yield. While ester **15**α[11] was sufficiently



14a: X = OCH=CHCH$_3$; Y = H
15a: X = OCHO; Y = H



16a: X = OCHO; Y = H; R = H
17a: X = OCHO; Y = H; R = Ac

stable to isolate and characterize, selective benzyl-acetyl exchange (**15**α → **17**α) was not without problem. Eventually conditions were devised; these were: hydrogenation (Pd/C, EtOAc) followed by acetylation of **16**α[11] employing pyrrolidinopyridine[22] as catalyst (2 equiv of Ac_2O); the yield for the two steps ranged from 68% to 79%. Careful saponification of the formate ester in **17**α[11a] (MeOH, catalytic NEt_3; 99%) then afforded lactol **2**,[11] the requisite disaccharide to complete phyllanthoside.[23]

By far the most challenging step in the phyllanthoside venture proved to be coupling of **2** with acid **3**.[7] Given the unfavorable 2:1 (α/β) anomeric ratio (NMR), simple condensation of **2** with the acid chloride of **3**, not surprisingly, provided the α-anomer (ca. 5:1, 92%).[24] It thus appeared that any coupling process employing *2* as the nucleophile would lead to the α-glycoside as the major product. On the other hand, if we employed acid **3** as the nucleophile to displace an activated anomeric hydroxyl, we conjectured that the β-glycoside would predominate.[25] The Mitsonobu protocol[26] appeared ideal.[27] With this in mind, condensation of **2** with **3** (2 equiv) employing DIAD and PPh_3 (1.5 equiv of each, THF) led to a 2:1 mixture of **18**[11] and the corresponding α-anomer (55%), which was readily separable by preparative HPLC.

Having completed the phyllanthoside skeleton, there remained only reduction of the C(10) carbonyl, cinnamoylation, and removal of the TES groups. Toward this end, stereoselective axial reduction of ketone **18** ($NaBH_4$, MeOH; 85%),[28] cinnamoylation (*trans*-cinnamoyl chloride, catalytic 4-PP,[18] Et_3N, pyr; 91%), and removal

(10) Mc Guirk, P. R.; Collum, D. B. *J. Org. Chem.* **1984**, *49*, 843.

(11) (a) The structure assigned to each new compound is in accord with its infrared and 250-MHz ^{1}H NMR spectra, as well as its high-resolution mass spectrometry. (b) In addition, an analytical sample of this new compound, obtained by recrystallization or chromatography (LC or TLC) gave satisfactory C and H combustion analysis within 0.4%.

(12) Monosaccharide **8** was available in four steps (54% yield) from 1,2:5,6-diisopropylideneglucofuranose;[13] see: Arrick, R. E.; Baker, D. C.; Horton, D. *Carbohydr. Res.* **1973**, *26*, 441.

(13) Aldrich Chemical Co., Milwaukee, WI 53233.

(14) Finan, P. A.; Warren, C. D. *J. Chem. Soc.* **1962**, 3089.

(15) van Heeswijk, W. A. R.; Goedhart, J. B.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1977**, *58*, 337. In our hands, the corresponding 1,2-cyclopentylideneglucofuranose was formed to the extent of ca. 15%.

(16) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(17) To establish rigorously the integrity of the β-glycoside linkage, both anomers were individually converted to phyllanthose (**12**), the parent β-disaccharide previously obtained by Pettit[3] during structural elucidation studies. Toward this end, treatment of **4**α and **4**β with $PdCl_2$[18] (1.5 equiv, HOAc, NaOAc, H_2O, room temperature, 18 h) yielded lactol **11**[11] [mp 171–172 °C; hexane–Et_2O (5:1); 76%]. Subsequent saponification of the acetates (NaOMe, MeOH, room temperature, 18 h) followed by removal of the benzyl groups [H_2, Pd/C, EtOH–AcOH (5:1), room temperature, 2 h] gave phyllanthose (**12**); the yield for the two steps was 87%. To confirm the structure of phyllanthose, the peracetylated derivative was prepared (Ac_2O, pyr, catalytic DMAP, room temperature, 3 h) and shown to be identical in all respects [i.e., ^{1}H NMR, IR, MS, and mp 225–226 °C; lit.[4b] 224–226 °C; $[\alpha]^{22}$ 64.1° (*c* 0.8, $CHCl_3$); Lit.[4b] $[\alpha]^{24}$ 71.1° (*c* 0.83, $CHCl_3$)] with an authentic sample of phyllanthose peracetate kindly provided by Professor Pettit (Arizona State University).

(18) Ogawa, T.; Nakabayashi, S.; Kitajima, T. *Carbohydr. Res.* **1983**, *114*, 225.

(19) Boss, R.; Scheffold, R. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 558.

(20) The olefin configuration in enol ether **13**α was 4:1 (cis/trans).

(21) Hart, T. W.; Metcalfe, D. A.; Scheinmann, F. *J. Chem. Soc., Chem. Commun.* **1979**, 156.

(22) Hassner, A.; Krepski, L. R.; Alexanian, V. *Tetrahedron* **1978**, *34*, 2069.

(23) Disaccharide **4**β, as well as the mixture, was independently converted to **2** via the same reaction sequence; yields in all steps were comparable.

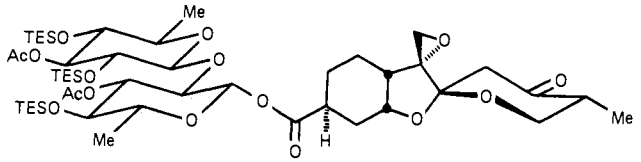
(24) (a) Bugianesi, R.; Shen, T. Y. *Carbohydr. Res.* **1971**, *19*, 179. (b) Kornhauser, A.; Keglevic, D. *Carbohydr. Res.* **1969**, *11*, 407. (c) Ogawa, T.; Nozaki, M.; Matsui, M. *Carbohydr. Res.* **1978**, *60*, C7.

(25) This assumes that displacement takes place via an SN_2 process with no anomer equilibration upon activation.

(26) Mitsunobu, O. *Synthesis* **1981**, 1.

(27) To the best of our knowledge, this approach has not been exploited for β-glycosides. However, activation of anomeric hydroxyl groups via the Mitsunobu protocol has been exploited for the synthesis of nuclosides, disaccharides, glycosyl fluorides, phenolic glycosides, and glycosides of aliphatic alcohols; see, for example: Szarek, W. A.; Depew, C.; Jarrell, H. C.; Jones, J. K. N. *J. Chem. Soc., Chem. Commun.* **1975**, 648. Schorkhuber, W.; Zbiral, E. *Ann. Chem.* **1980**, 1455. Kunz, H.; Sager, W. *Helv. Chem. Acta* **1985**, *68*, 283. Grynkiewicz, G. *Carbohydr. Res.* **1977**, *53*, C11. Garegg, P. J.; Inverson, T.; Norberg, T. *Carbohydr. Res.* **1979**, *73*, 313. Szarek, W. A.; Jarrell, H. D.; Jones, J. K. N. *Carbohydr. Res.* **1977**, *57*, C13. Grynkiewicz, G.; Zamojski, A. *Synth. Commun.* **1978**, *8*, 491.

(28) The stereoselectivity was 6:1 (axial/equitorial); the equitorial alcohol was removed by flash chromatography [hexane–EtOAc (5:1)].



18

of the TES groups ($HOAc:H_2O:THF \simeq 100\%$) gave (+)-phyllanthoside (**1**) identical in all respects [NMR, IR, MS, and TLC] with an authentic sample provided by Dr. Matthew Suffness (NCI).[29]

In summary, the first total synthesis of (+)-phyllanthoside has been achieved. The central features of the strategy were the Koenigs-Knorr protocol followed by benzyl-acetyl interchange to construct disaccharide **2** and then a Mitsonobu coupling to **3**. While the lack of high stereoselectivity in the Mitsonobu reaction is somewhat disappointing, it detracts little from the overall efficiency of the synthesis in that all other reactions proceed in good to excellent yield.

Acknowledgment. Support for this investigation was provided by the National Institutes of Health (Institute of General Medical Sciences) through Grant GM 29028.

Supplementary Material Available: Characterization data for compounds **2**, **4α**, **5**, **6**, and **18** as well as comparison NMR spectra of natural and synthetic (+)-phyllanthoside (3 pages). Ordering information is given on any current masthead page.

(29) We are grateful to Dr. Matthew Suffness (National Cancer Institute, NIH) for the generous sample of natural (+)-phyllanthoside as well as his encouragement during the course of this work.

Photochemical Activation of Acylated α-Thrombin

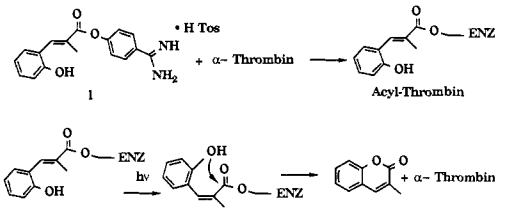
A. D. Turner,[†] S. V. Pizzo,[‡] G. W. Rozakis,[§] and N. A. Porter*[†]

Departments of Chemistry, Pathology and Biochemistry and Ophthalmology, Duke University Durham, North Carolina 27706

Received October 20, 1986

The control of enzyme catalytic activity by deactivation or inhibition is an important strategy in biochemistry and medicinal chemistry. Many approaches to enzyme inhibition involve specific acylation or alkylation of crucial nucleophilic centers (-OH, $-NR_2$, -SH) in enzyme active sites.[1,2] For example, acylation of the serine active-site hydroxyl of a serine proteinase renders the enzyme inactive.[3] Restoration of catalytic activity of such inhibited enzymes has received much less attention. Photochemical reactivation or restoration[4] of enzyme activity offers the posibility of efficiently and rapidly converting an inactive enzyme into an enzyme with full catalytic capacity and general approaches toward this goal may have widespread application. We have chosen the serine proteinase enzymes of the blood coagulation pathway for study since control of their activity is a major strategy in medicinal chemistry. In the present report, we describe the first photochemical reactivation of irreversibly inhibited human α-thrombin.

Scheme I



Compound **1** was chosen for study as a potential photoactive enzyme inhibitor. On the basis of previous study of analogous compounds,[5] we reasoned that **1** would efficiently acylate the active-site serine of α-thrombin and that this acylated, inactive enzyme would be stable in the absence of light. The structure of **1** further suggests that photoisomerization of the acyl-enzyme would lead to rapid enzyme deacylation (activation) by the internal ortho-hydroxyl nucleophile, as shown in Scheme I. Compound **1** was synthesized by Wittig coupling of salicylaldehyde and (carbethoxyethylidene)triphenylphosphorane, followed by base hydrolysis of the ethyl ester and DCC coupling to *p*-amidinophenol.[6]

In the absence of light, the *p*-amidinophenyl ester of *o*-hydroxy-α-methylcinnamic acid **1** irreversibly inhibits human α-thrombin, presumably by acylation of the active site serine hydroxyl. This acyl-enzyme is identified in the scheme as "acyl-thrombin". The substrate H-D-Phe-Pip-Arg-*p*-nitroanilide hydrochloride (S-2238) was employed to assess enzyme activity.[7] As portrayed in Figure 1, 2-100 μM concentrations of inhibitor **1** reduces enzyme activity by 80-97% in less than 1 h. Photolysis (through Pyrex, using a medium-pressure mercury xenon lamp) of enzyme aliquots containing 80- and 400-fold excess inhibitor resulted in 100% reactivation of the enzyme (see Figure 1). This complete reactivation required 12 min for the experiment with 80 equiv of inhibitor, while photochemical reactivation of the enzyme inhibited 400-fold required nearly an hour.[8] Thrombin clotting times of these thrombin samples were >120 s (essentially incapable of clotting) before photolysis and 20 s after irradiation (essentially complete reactivation). A thrombin sample reactivated photochemically to 40% activity had a clotting time of 38 s. Photolysis for 1 h of a thrombin sample containing 800-fold excess of compound **1** (97 μM) regenerated approximately 65% of the original activity. In each case, unphotolyzed solutions of enzyme inactivated with these inhibitor concentrations demonstrated less than 5% activity. Percent reactivation was based on the average of five controls, $A_{405\ nm} = 2.846 \pm 0.025$. Control enzyme solutions were treated with identical concentrations of ethanol containing no inhibitor, and these solutions were also subjected to identical periods of photolysis (30 min or less). No difference was detected between photolyzed and unphotolyzed controls.

[†] Department of Chemistry.
[‡] Department of Pathology and Biochemistry.
[§] Department of Ophthalmology.

(1) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman: San Francisco, 1979.
(2) Bender, M. L. *Mechanisms of Homogeneous Catalysis from Protons to Proteins*; wiley: New York, 1971.
(3) Barrett, A. J.; McDonald, J. K. *Mammalian Proteases*; Elsevier: New York, 1980.
(4) We thank Dr. D. Sternbach of the Glaxo Corp. for a helpful discussion in the early stages of this research.
(5) Turner, A. D.; Monroe, D. M.; Roberts, H. R.; Porter, N. A.; Pizzo, S. V. *Biochemistry* **1986**, *25*, 4929.
(6) Mp 216-217 °C after recrystallization from methanol; IR (KBr) 3350 (amidine N—H), 3000-3250 (amidine NH_2, Ar C—H), 1725 (ester C=O), 1680 (amidine C=N), 1610 (amidine N—H), 1585, 1490 (amidine N—H), 1455 (CH_3), 1210—1000 (S=O), 760, 690 cm^{-1} (S-O); 300-MHz ^1H (C-D_3OD) δ 2.2 (s, 3 H), 2.4 (s, 3 H), 6.8-7.0 (m, 2 H), 7.2-7.3 (d, 3 H), 7.35-7.4 (d, 1 H, J = 10 Hz), 7.4-7.5 (d, 2 H, J = 12.5 Hz), 7.65-7.75 (d, 2 H, J = 10 Hz), 7.85-7.95 (d, 2 H, J = 12.5 Hz), 8.15 (s, 1 H); ^{13}C NMR (Me_2SO) δ 166.3, 165.0 (C=O, amidine C), 156.3, 155.1 (phenolic C's), 137.5 (C=CCH_3), 20.8, 14.3 (CH_3's), 144.7, 138.4, 130.7, 130.0, 129.9, 128.4, 125.7, 125.5, 125.4, 122.8, 121.8, 118.9, 115.7; MS (EI) 296 (M - tosylate), 281 (M - tosylate, OH) 161 (100, M - tosylate, amidine). Anal. Calcd for $C_{24}H_{24}N_2O_6S$: C, 61.52; H, 5.16. Found: C, 61.25; H, 5.23. X-ray crystal structure; UV λ_{max} (EtOH) 322, 274 nm.
(7) Svendsen, L.; Stocker, K. *New Methods with Chromogenic Substrates*; de Gruyter: New York, 1977; p 23.
(8) Photolysis of **1** in ethanol leads to quantitative formation of 3-methylcoumarin and *p*-amidinophenol. Experiments are in progress to prepare radiolabeled **1** to substantiate the intermediacy of an "acyl thrombin" and to determine the products of the acyl-thrombin photochemistry. α-Thrombin loses some activity upon extensive photolysis and this may be the cause of reduced activity after photolysis of the solution with 800-fold excess **1**.

0002-7863/87/1509-1274$01.50/0