Substituted Isatoic Anhydrides: Selective Inactivators of Trypsin-like Serine Proteases†,‡

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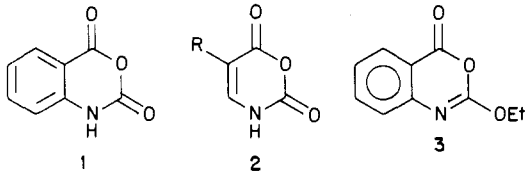
Derivatives of isatoic anhydride were prepared and tested as inhibitors of serine proteases. A number of isatoic anhydrides with positively charged substituents irreversibly inactivated several trypsin-like enzymes and preferentially inactivated trypsin over chymotrypsin. Further selectivity was obtained by introduction of an aromatic group on the N-1 position of isatoic anhydride. 7-(Aminomethyl)-1-benzylisatoic anhydride was prepared and was a selective inactivator of thrombin; thus it is possible to prepare derivatives of isatoic anhydride that are highly enzyme selective without attaching peptide recognition structures.

We have recently described the inactivation of serine proteases by isatoic anhydride (1),[1] oxaziniediones (2),[1,2] and benzoxazinones (3).[3] The mode of inactivation of



1 2 3

chymotrypsin by 1 involves the initial attack of the active-site serine onto the anhydride carbonyl group of 1 to produce an enzyme-bound carbamate. This species rapidly decarboxylates to the anthranoyl-enzyme. Because of the presence of the electron-releasing amino group, anthranoylchymotrypsin resists deacylation. We have now explored the possibility of increasing the selectivity of inactivation toward specific proteases by modification of these inhibitors, but without the attachment of polypeptides. We chose to prepare derivatives of isatoic anhydride (1) that are targeted toward thrombin. Thrombin inactivators are of interest as potential antithrombotic drugs.

Synthetic Procedures. The synthetic approach to the substituted isatoic anhydrides is illustrated in Scheme I. Commercially available **4** was converted via the Rosenmund–von Braun synthesis to nitrile **5** by heating with CuCN. As reported earlier, bromination of **5** to the benzyl bromide proved to be difficult;[4] however, when the crude bromide was heated with sodium acetate in acetic acid, a satisfactory yield of acetate **6** was obtained following chromatography on silica. Heating **6** in aqueous HBr produced acid **7**. Treatment of **7** with excess ammonia gave amino acid **8**, which was treated with di-*tert*-butyl pyrocarbonate to afford the *tert*-butyl carbamate amino acid **9**. Reduction of **9** with sodium dithionite under alkaline conditions gave the substituted anthranillic acid **10**. Reaction of **10** with phosgene gave the isatoic anhydride **11a**. Compound **11a** could be alkylated at N-1 by stirring with an alkyl halide in the presence of Na_2CO_3[5] to produce **11b** and **11c**. Final deprotection with trifluoroacetic acid gave the N-1-substituted 7-aminomethyl isatoic anhydrides **12a–c**.

Inhibition of Serine Proteases by Substituted Isatoic Anhydrides. The design of substituted isatoic anhydrides that selectively inhibited trypsin-like serine proteases was carried out in two stages. First, a substituted isatoic anhydride containing the basic aminomethyl group attached to the aromatic ring was prepared (compound **12a**). Previous studies have shown that trypsin-like enzymes are inhibited by aromatic compounds containing positively charged groups, i.e., benzamidine and phenylguanidine.[6] Results in Table I show that compounds **12a** and **12b** containing a positive charge are better inactivators for trypsin and thrombin than for chymotrypsin. For example, compound **12a** rapidly inactivates both trypsin and thrombin at 2.5 μM, whereas no detectable inactivation of chymotrypsin was measured under these conditions. In contrast, unsubstituted isatoic anhydride (1) inactivates chymotrypsin rapidly at 2.5 μM, but no inactivation of either trypsin or thrombin occurs under these conditions.

Second, additional modifications of **12a** were made in order to prepare selective inhibitors for thrombin. The inhibitory properties toward thrombin of a series of ester and amide derivatives of N^{α}-substituted arginine has been reported.[7] Many of the reported compounds contain an aromatic substituent on the N^{α} group of arginine. Since this position is somewhat isosteric with N-1 of isatoic anhydride, we attached aromatic substituents to N-1 with the expectation that such derivatives would be selective inactivators of thrombin. The data in Table I show that incorporation of an aromatic residue increases selectivity toward thrombin. The most effective thrombin inhibitor in the series was the *N*-1-benzyl derivative **12b**. Compound **12b**, at 2.5 μM, inactivated thrombin with a half-time of 2.6 min. No detectable inactivation of trypsin, chymotrypsin, or plasmin was observed under these conditions.

The reactivation of thrombin inactivated with **12b** was studied. Thrombin was incubated with 10 μM **12b** until no activity remained (ca. 5 min). Excess inactivator was removed by dialyzing the solution for several hours at 4 °C. The dialyzed solution was kept at 25 °C, and the return of enzymatic activity was followed by periodically assaying small aliquots of the mixture. No activity was detected after 1 h at 25 °C. After 13 h, 30% of the activity had returned. These results demonstrate that thrombin inactivation by **12b** is long-lasting but not completely irreversible.

Compound **12b** was also tested as an anticoagulant. The presence of 500 μM **12b** in serum increased the partial thromboplastin time by 2.6-fold. If **12b** is preincubated

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Scheme I

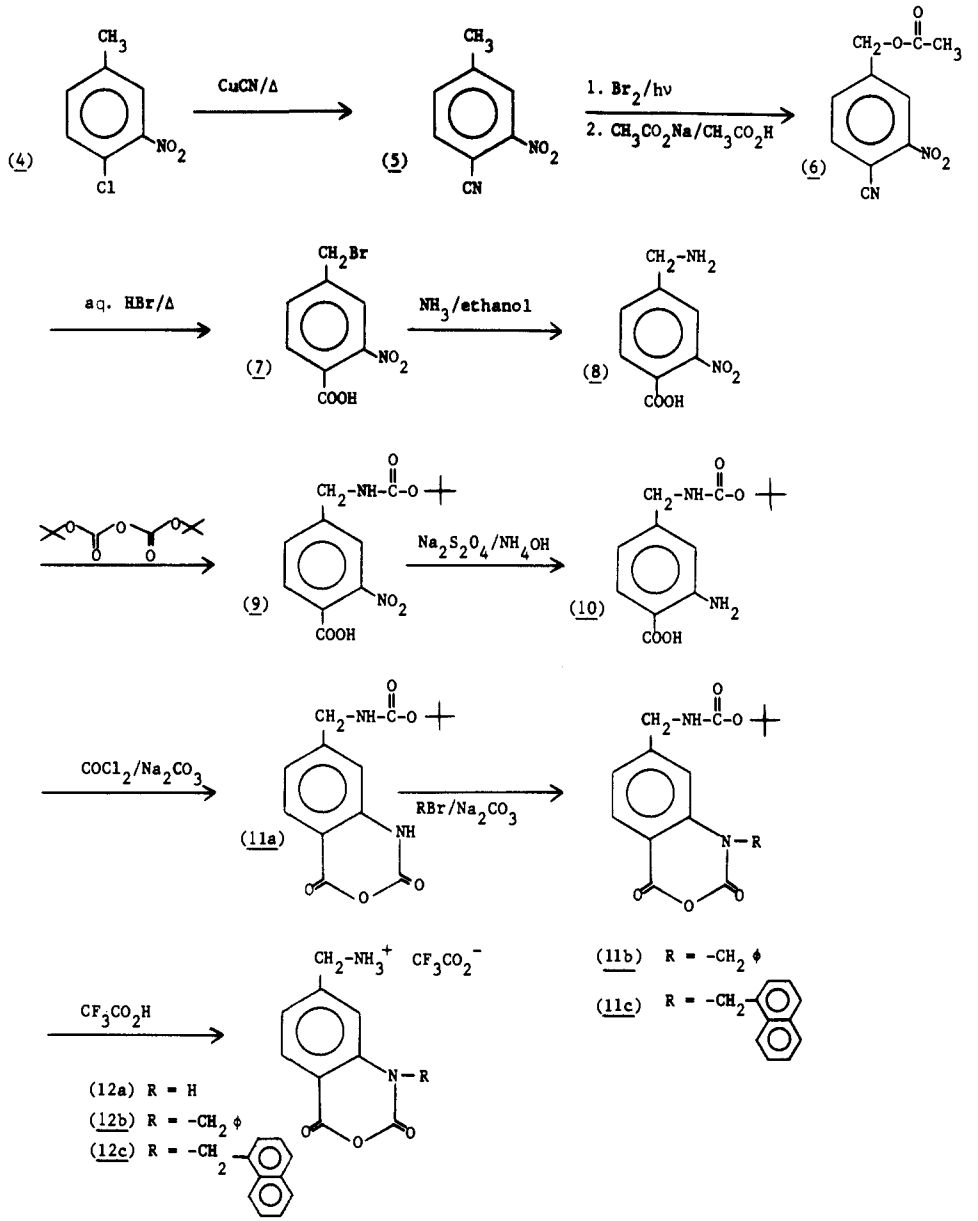
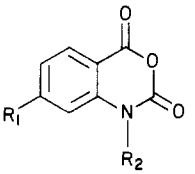
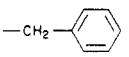
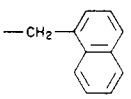


Table I. $T_{1/2}$ for Inactivation of Serine Proteases by Substituted Isatoic Anhydrides



			$T_{1/2}$ for inactivation,[a] min										
			[I], μM: chymotrypsin			[I], μM: trypsin			[I], μM: thrombin			[I], μM: plasmin	
no.	R_1	R_2	2.5	25	125	2.5	25	125	2.5	25	125	2.5	25
1	H	H	2.7	0.8	0.5	NI[b]	3.9	2.0	NI	CK[c]	11.5		
12a	$CH_2NH_3^+$	H	NI	CK	1.5	1.3	0.7	<0.1	3.4	2.6	<0.1		
12b	$CH_2NH_3^+$		NI	NI	CK	NI	CK	10	2.6	0.7	<0.1	NI	15
12c	$CH_2NH_3^+$		CK	2.3	0.5	NI	9.5	6.2	7	1.0	0.1		

[a] Enzyme and inhibitor were preincubated for varying amounts of time in buffer and then diluted 100-fold into buffer containing excess assay substrate. Assays were conducted as described in Experimental Procedures. [b] No inactivation was observed within 1 h. [c] Complex inactivation kinetics was observed (see text).

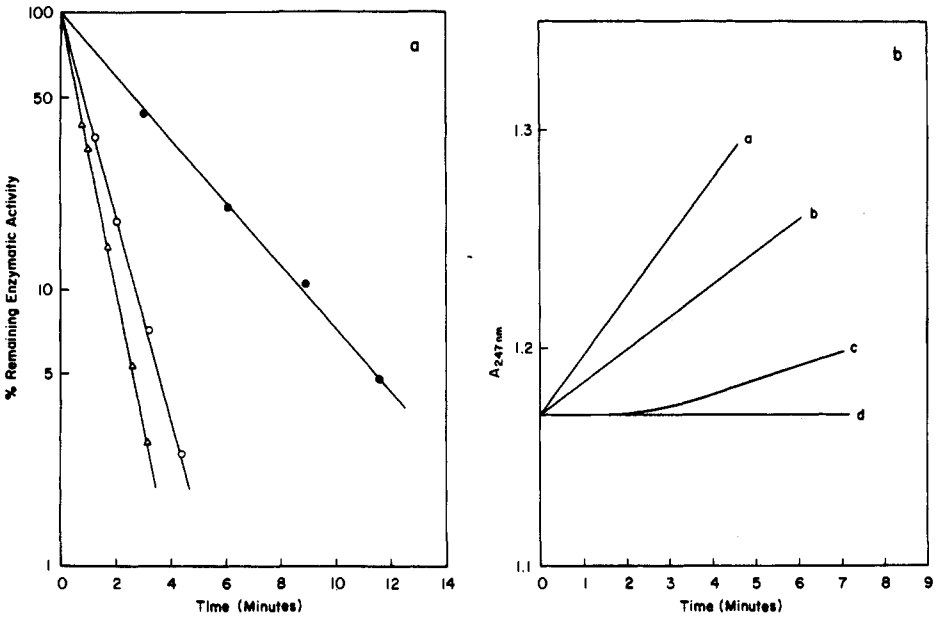


Figure 1. (a) Inactivation of thrombin by 12b. A solution of thrombin (100 NIH units/mL) in 50 mM sodium citrate, pH 6.0, 0.15 M NaCl, was preincubated with various concentrations of **12b**. Aliquots were removed periodically and diluted 50-fold into buffer containing assay substrate (see Experimental Procedures). The semilog plot shows the percent remaining enzymatic activity vs. the time of preincubation with 2.5 μM (●), 5 μM (○), and 19 μM (▲) inhibitor. (b) Inactivation of trypsin by (12b). A solution of trypsin (5 μM) in 20 mM potassium phosphate, pH 7.0, was preincubated with 25 μM **12b**. Aliquots were removed periodically and diluted into buffer containing assay substrate (see Experimental Procedures). The figure shows the reaction progress curve for the hydrolysis of tosylarginine methyl ester after addition of native enzyme (a); enzyme precubated with **12b** for 2 min (b), 15 min (c), and 30 min (d).

in serum for several minutes prior to the initiation of coagulation, no effect is seen. These results suggest that although **12b** is an active anticoagulant, it may be degraded upon prolonged incubation in serum. The stability of **12b** in serum was estimated by mixing **12b** in buffer with an aliquot of serum and following the changes in the visible spectrum in the 300–350-nm region. Hydrolysis of **12b** to the substituted anthranilic acid produces a decrease in absorbance at 319 nm. Alternately, the stability of **12b** in serum can be measured by preincubation of **12b** in serum and periodically testing the mixture for its ability to inactivate pure thrombin. Both methods demonstrate that **12b** is rapidly degraded in serum with a half-time of less than 1 min. The degradation of **12b** in serum appears to be enzyme catalyzed. The degrading activity is destroyed by heat treatment at 100 °C. Furthermore, no activity was found in the ultrafiltrate when serum was fractionated with use of a 10000 molecular weight cut-off membrane. The enzymatic activity may also be metal ion dependent since the degradation of **12b** is completely inhibited by treatment with EDTA.

Since **12b** is rapidly degraded in serum, it is not useful as an anticoagulant. By varying the N-1 substituent on isatoic anhydride, it may be possible to prepare derivatives of isatoic anhydride that selectively inactivate thrombin and are more stable to enzymatic degradation.

Kinetics of Inactivation. Preincubation of enzyme and inhibitor in buffer followed by 100-fold dilution into buffer containing excess assay substrate resulted in a time-dependent loss of enzymatic activity. For most of the experiments reported (those compounds in Table I for which half-lifes are reported), semilog plots of the fraction of remaining enzymatic activity vs. time were linear. For example, results of the inactivation of thrombin by **12b** are shown in Figure 1a. The rate of inactivation was found to saturate as the inhibitor concentration was increased (Figure 1a). This behavior is consistent with the model shown below in which the enzyme and inhibitor first interact to produce a Michaelis complex (E·I) followed by an irreversible inactivation to produce (E – I).

$$E + I \rightleftharpoons E{\cdot}I \rightarrow E - I$$

In some of the experiments given in Table I, more complex inhibition kinetics were observed. For example, data for the inhibition of trypsin by **12b** is shown in Figure 1b. Trypsin (5 μM) was incubated with 25 μM **12b** in 20 mM potassium phosphate, and aliquots were periodically withdrawn and diluted 100-fold into buffer containing excess assay substrate. Preincubation of enzyme and inhibitor for a short time (ca. 2 min) leads to a partial loss of tryptic activity (curve b, Figure 1b). Dilution of the mixture after a longer preincubation (ca. 15 min) produced curve c (Figure 1b). In curve c, the initial velocity was zero; however, a small fraction of the enzymatic activity returned over several minutes. No enzymatic activity remained if the preincubation was carried out for long times (ca. 30 min) as shown in curve d (Figure 1b). These results are consistent with the presence of both an irreversibly inactivated enzyme species and a reversibly inactivated form. The reversibly inactivated enzyme forms slowly since curved reaction progress plots (curve c, Figure 1) are observed only after long preincubation times. Eventually, all of the enzyme becomes irreversibly inactivated. These results are consistent with the model shown below:

$$I^*{\cdot}E \rightleftharpoons I + E + I \rightleftharpoons E{\cdot}I \longrightarrow E{-}I$$

This model is similar to the one shown above except that it contains an additional reaction in which E and I slowly react in a reversible manner to produce E·I*. The chemical nature of this slowly reversible process is not known.

Experimental Procedures

Bovine α-chymotrypsin (59 units/mg, type II), bovine trypsin (10000 units/mg, type I), bovine thrombin (2000 NIH units/mg), and human plasmin (3–6 units/mg) were purchased from Sigma. Isatoic anhydride was purchased from Aldrich. Chymotrypsin was assayed in 20 mM potassium phosphate, pH 7.0, with ben-

zoyl-L-tyrosine ethyl ester.[8] Trypsin was assayed in 20 mM potassium phosphate, pH 7.0, with tosyl-L-arginine ethyl ester.[8] Thrombin and plasmin were assayed in 0.1 M Tris-(hydroxymethyl)aminomethane, pH 8.0, with thiobenzyl (benzyloxycarbonyl)-L-lysinate in the presence of 5,5'-dithiobis(2-nitrobenzoic acid).[9] All spectrophotometric assays were conducted at 25 °C with a Perkin-Elmer Lambda-3 UV-vis instrument.

The stability of **12b** in serum was determined by addition of heparinized bovine serum (10–100 μL) to a 1-mL solution of **12b** (500 μM) in 20 mM potassium phosphate, pH 7.0. The loss of **12b** was monitored by the decrease in absorbance at 319 nm. The stability of **12b** in serum was also estimated by a biological procedure. Thus, **12b** (500 μM) was incubated in 950 μL of 20 mM potassium phosphate, pH 7.0, containing 50 μL of heparinized bovine serum. At various times, 100-μL aliquots were mixed with 400 μL of CH_3CN, and the precipitated protein was removed by centrifugation. The supernatant (1 μL) was mixed with 20 μL of thrombin (100 NIH units/mL), and the mixture was incubated for 10 min at room temperature. An aliquot of the solution was then assayed for thrombin activity. Control experiments demonstrated that no inactivation of thrombin was observed if **12b** was omitted from the procedure. Partial thromplastin times were determined as described.[10]

Syntheses. Proton nuclear magnetic resonance spectra (90 MHz) were recorded on a Varian Model EM-390 spectrometer. All chemical shift values are reported as δ units (ppm) relative to tetramethylsilane. Column chromatography and thin-layer chromatography were performed with use of silica gel.

4-Methyl-2-nitrobenzonitrile (5). 4-Chloro-3-nitrotoluene (100 g, 0.58 mol; Aldrich) and anhydrous cuprous cyanide (60 g, 0.67 mol) were dissolved in dimethylacetamide (150 mL) and refluxed for 4.5 h with stirring. The solution was protected from moisture with a $CaSO_4$ dry tube. The mixture was poured into ice–water (1 L), and the brown precipitate was collected by filtration and pressed dry. The moist solid was suspended in 600 mL of CH_2Cl_2 and stirred vigorously for 30 min. Insolubles were removed by filtration. The organic filtrate was separated from a residual water layer and dried ($MgSO_4$). The dried solution was stirred for 10 min with Norit (5 g) and alumina (grade 1, 50 g). The mixture was filtered and concentrated in vacuo to give a dark oil. The oil was mixed with ca. 750 mL of ether, and the mixture was gently boiled for 10 min. The ether layer was decanted from the residual dark oil and concentrated to give yellow crystals. Recrystallization from ca. 200 mL of ether gave 20–25 g of pure product: mp 96–97 °C.[4]

4-Cyano-3-nitrobenzyl Acetate (6). The following is a modification of the procedure reported previously.[4] 4-Methyl-2-nitrobenzonitrile (10 g, 61.6 mmol) was heated at 125 °C with stirring. Bromine (10.4, 65.3 mmol) was added in small portions over 7 h from an addition funnel protected with a $CaSO_4$ dry tube. During the addition, the reaction was illuminated with a 250-W tungsten lamp. After a total of 9.5 h, the reaction was allowed to cool and the dark oil was triturated with ether (3 × 100 mL). The combined ether layers were concentrated in vacuo, giving a dark oil (16 g). NMR analysis indicated that significant amounts of starting material and dibrominated product were present. The crude oil was mixed with anhydrous sodium acetate (16 g) and acetic acid (150 mL). The mixture was refluxed for 2 h with moisture protection from a $CaSO_4$ dry tube. The cooled mixture was poured into ice–water, extracted with ether (two times), and dried ($MgSO_4$), giving an orange oil. The crude acetate, obtained from two runs as described above, was purified on silica (7.2 × 28 cm) eluting with 10% ethyl acetate in hexane (0–5.5 L) followed by 20% ethyl acetate in hexane (5.5–9 L). The dibromide and starting material eluted first (2.5–5 L) followed by the acetate (6.5–8.5 L). The yield of product was 9.5 g (34%): mp 64–65 °C; NMR ($CDCl_3$) δ 2.20 (s, 3 H), 4.92 (br s, 2 H), 7.75–7.98 (dd, 2 H), 8.38 (br s, 1 H).

4-Carboxy-3-nitrobenzyl Bromide (7). Acetate **6** (9.5 g) was refluxed for 6 h in 80 mL of 48% aqueous HBr. The crystals that formed after cooling the reaction mixture on ice were collected, washed with water, and air-dried briefly. The crystals were dissolved in $CHCl_3$ (some material does not dissolve), filtered, and dried ($MgSO_4$). Concentration in vacuo gave the pure bromide (6.9 g, 66%): mp 134–136 °C; NMR ($CDCl_3$) δ 4.53 (s, 2 H), 7.65–7.95 (m, 3 H).

4-Carboxy-3-nitrobenzylamine (8). Bromide **7** (2.0 g) in 5 mL of ethanol was added dropwise over 30 min with stirring to a solution of ca. 13 g of NH_3 in 200 mL of 90% aqueous ethanol. After standing overnight at room temperature, the mixture was concentrated in vacuo. The solid was suspended in a small amount of water, to solubilize the ammonium bromide, and filtered. The product was dried in vacuo over P_2O_5, giving (1.1 g, 68%) of the amino acid: NMR (D_2O) δ 4.21 (s, 2 H), 7.7–7.9 (dd, 2 H), 8.1 (br s, 1 H). The product was used in the next step without further purification.

***N*-(*tert*-Butoxycarbonyl)-4-carboxy-3-nitrobenzylamine (9).** Amino acid **8** (0.71 g, 3.6 mmol) was dissolved in dioxane–water (2:1, 11 mL) and 1 N NaOH (3.6 mL). The mixture was stirred in an ice bath while di-*tert*-butyl dicarbonate (0.9 g, 4.1 mmol; Aldrich) was added in one portion. After the mixture was stirred for 30 min at room temperature, the dioxane was removed in vacuo. The aqueous mixture was extracted with ethyl acetate (two times), and the organic layers were discarded. The aqueous layer was cooled on ice, covered with ethyl acetate, and acidified to pH 1–2 by addition of an aqueous solution of $NaHSO_4$. The layers were separated, and the aqueous layer was extracted with ethyl acetate (two times). The combined organic layers were washed with water (one time), dried ($MgSO_4$), and concentrated in vacuo to give 0.83 g (78%) of product as a light-orange oil: NMR ($CDCl_3$) δ 1.50 (s, 9 H), 4.45 (br d, 2 H), 7.55–8.0 (m, 3 H). The product was used in the next step without further purification.

***N*-(*tert*-Butoxycarbonyl)-3-amino-4-carboxybenzylamine (10).** Compound **9** (0.83 g, 2.8 mmol) was dissolved in water (9 mL) and aqueous ammonium hydroxide (30%, 1 mL). Sodium dithionite (1.3 g) was added, and the mixture was stirred for 10 min at room temperature. The ammonia was removed in vacuo. The mixture was cooled on ice and acidified by addition of acetic acid. The precipitate was collected, washed with water, and dried in vacuo over P_2O_5. The yield was 0.25 g (34%): mp 175–178 °C dec; NMR (Me_2SO-d_6) δ 1.50 (s, 9 H), 4.20 (d, 2 H), 6.50 (dd, 1 H), 6.75 (d, 1 H), 7.70 (d, 1 H).

***N*-(*tert*-Butoxycarbonyl)-7-(aminomethyl)isatoic Anhydride (11a).** Compound **10** (100 mg, 0.38 mmol) and Na_2CO_3 (45 mg, 0.38 mmol) were dissolved in water (5 mL). Phosgene was bubbled through the solution with stirring for 1 min. The mixture was stirred for an additional 15 min. The solid was collected by filtration, washed with water, and dried in vacuo over P_2O_5, giving 80 mg (60%) of product. The product was triturated twice with warm ethyl acetate and recrystallized from acetone: mp 221–223 °C d; TLC, R_f 0.29, 5% methanol in $CHCl_3$; MS, *m/e* 292 (M^+); NMR (CD_3CN) δ 1.55 (s, 9 H), 4.35 (br s, 2 H), 7.15 (br s, 1 H), 7.25 (br d, 1 H), 8.0 (d, 1 H). Anal. ($C_{14}H_{16}N_2O_5$) H, N; C: calcd, 57.50; found, 56.61.

***N*-(*tert*-Butoxycarbonyl)-4-(aminomethyl)-1-benzylisatoic Anhydride (11b).** Compound **11a** (50 mg, 0.17 mmol) and Na_2CO_3 (20 mg, 0.19 mmol) were dissolved in dimethylacetamide (0.34 mL). The mixture was stirred for 30 min followed by the addition of the benzyl bromide (0.19 mmol). The reaction was stirred for 18 h at room temperature. The mixture was diluted 5-fold with water, and the precipitated product was pelleted by centrifugation, washed with water, and recentrifuged. The pellet was dried in vacuo over P_2O_5. The solid was triturated with petroleum ether (pb 20–40 °C, 2 × 2.5 mL) and air-dried, giving 42 mg (65%) of product. The product was purified by chromatography on silica gel using 20% ethyl acetate in $CHCl_3$: mp 148–149 °C; TLC, R_f 0.20, 20% ethyl acetate in $CHCl_3$; MS, *m/e* 382 (M^+); NMR ($CDCl_3$) δ 1.50 (s, 9 H), 4.32 (d, 2 H), 4.90 (br d, 1 H), 5.25 (s, 2 H), 7.05–7.50 (m, 7 H, includes 7.31, s), 8.10 (d, 1 H). Anal. ($C_{21}H_{22}N_2O_5$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-7-(aminomethyl)-1-(α-methylnaphthyl)isatoic Anhydride (11c).** The title compound was prepared in 60% yield in a manner similar to **12a**, using 2-(bromomethyl)naphthalene (Aldrich). The product was purified by chromatography on silica gel using 20% ethyl acetate in $CHCl_3$: mp 165–166 °C; TLC, R_f 0.20, 20% ethyl acetate in $CHCl_3$; MS, *m/e* 432 (M^+); NMR ($CDCl_3$) δ 1.45 (s, 9 H), 4.30 (d, 2 H), 5.45

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(s, 2 H), 7.05–8.20 (m, 10 H). Anal. ($C_{25}H_{24}N_2O_5$) H, N; C: calcd, 69.43; found, 68.85.

Removal of *N*-*tert*-Butoxycarbonyl Group (12a–c). The *N*-(*tert*-butoxycarbonyl) derivatives (11a–c, 10–20 mg) were dissolved in trifluoroacetic acid (1 mL). After the mixture was allowed to stand for 30 min at room temperature, the trifluoroacetic acid was removed by passing a stream of N_2 over the solution. The residue was triturated with ether and the ether was discarded. The solid was dissolved in 1 mM aqueous HCl for use in the enzyme inhibition studies: NMR (D_2O) for **12a**: δ 4.40 (s, 2 H), 7.35 (s, 1 H), 7.45 (d, 1 H), 8.20 (d, 1 H). NMR (CD_3CN/D_2O) for **12b**: δ 4.35 (s, 2 H), 5.30 (s, 2 H), 7.15 (s, 1 H), 7.30 (d, 1 H), 7.40 (s, 5 H), 8.30 (d, 2 H); NMR (CD_3CN/D_2O) for **12c**: δ 4.40 (s, 2 H), 5.25 (s, 2 H), 7.10–8.25 (m, 10 H).

The compounds gave a single spot on TLC and were detected by UV absorption and ninhydrin. The solvents used were as follows: (A) 1-butanol–CH_3COOH–H_2O, 4:1:5; (B) EtOH–H_2O, 7:3. **12a**: R_f 0.54 solvent A, R_f 0.64 solvent B. **12b**: R_f 0.38 solvent A, R_f 0.75 solvent B. **12c**: R_f 0.59 solvent A, R_f 0.83 solvent B.

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Registry No. 4, 89-60-1; 5, 26830-95-5; 6, 90771-66-7; 7, 100466-27-1; 8, 100466-28-2; 9, 100466-29-3; 10, 100466-30-6; 11a, 100466-31-7; 11b, 100466-32-8; 11c, 100466-33-9; 12a, 100466-34-0; 12b, 100466-35-1; 12c, 100466-36-2; 4-(bromomethyl)-2-nitrobenzonitrile, 100466-37-3; di-*tert*-butyl dicarbonate, 24424-99-5; benzyl bromide, 100-39-0; 1-naphthylmethyl bromide, 3163-27-7; isotoic anhydride, 118-48-9; serine protease, 37259-58-8; chymotrypsin, 9004-07-3; trypsin, 9002-07-7; thrombin, 9002-04-4; plasmin, 9001-90-5.

Absolute Configuration of (–)-5-Benzoyl-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]pyrrole-1-carboxylic Acid, the Active Enantiomer of Ketorolac[1]

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The (–)-*S* isomer of 5-benzoyl-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]pyrrole-1-carboxylic acid (1) is about 60 times more potent than the (+)-*R* isomer in the carrageenan edema test and ca. 230 times more active than the (+)-*R* isomer in the mouse phenylquinone writhing assay.

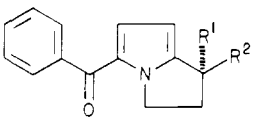
Ketorolac (1), a new α-substituted arylacetic acid with potent cyclooxygenase inhibitory activity, is a powerful antiinflammatory and analgesic agent in animal models.[3] In humans, it is essentially equivalent to morphine sulfate, on a weight basis, for the relief of moderate to severe postoperative pain.[4] This paper describes the resolution of ketorolac and the determination of the absolute configuration of the enantiomers.

Chemistry. The resolution of ketorolac was effected as follows. Crystallization of the cinchonidine salt from ethyl acetate gave the easily purifiable, less soluble salt of the (–) enantiomer. The more soluble salt was not readily obtained in optically pure form by recrystallization. Therefore, the crude, material from evaporation of the mother liquor was decomposed and the partially resolved (+) acid was converted into the cinchonine salt, which was diastereoisomerically pure after two crystallizations from ethyl acetate. Decomposition of the above salts with dilute sulfuric acid gave pure (–)- and (+)-ketorolac.[5]

The absolute configuration of the enantiomers was determined by a single-crystal X-ray analysis of the amide **2b** of (+)-(R)-1-(1-naphthyl)ethylamine and (+)-ketorolac. Inasmuch as the absolute configuration of the chiral center in the amine is known, that at C(1) in (+)-ketorolac was deduced to be *R* by internal reference.[6] Thus (–)-ketorolac must have the *S* absolute stereochemistry.

Results and Discussion

The antiinflammatory and analgesic activities of (+), (–), and racemic ketorolac were determined by using the carrageenan rat paw edema and mouse phenylquinone writhing assays, respectively. This side-by-side comparison shows (Table I) that essentially all of the pharmacological



(+)-(*R*)-1, R^1 = COOH; R^2 = H
(–)-(*S*)-1, R^1 = H; R^2 = COOH

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[∥] Syntex Research, Institute of Organic Chemistry.

(1) Contribution No. 698 from the Syntex Institute of Organic Chemistry.
(2) Syntex Research Postdoctoral Fellow, 1983–1984.
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(5) Esterification of ketorolac with (–)-α-phenethyl alcohol (trifluoroacetic anhydride/triethylamine, benzene, 0–5 °C) gave a mixture of diastereoisomeric esters that was separable by HPLC [9 mm × 50 cm Lichrosorb SI 60 (10 μm), using hexane–ethyl acetate (96:4) at 1000 psig]. Cleavage of each diastereoisomerically pure ester, with 45% trifluoroacetic acid in benzene at room temperature (1.5–2 h), gave (+)- and (–)-ketorolac, both of which possessed rotations ca. 20° lower than the enantiomerically pure acids obtained by the classical procedure. It is evident that partial racemization had occurred during transesterification.
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