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N^{α}-ETHYLOXYCARBONYL-α-AZAORNITHINE PHENYL ESTER: A STABLE INHIBITOR OF TRYPSIN AND THROMBIN

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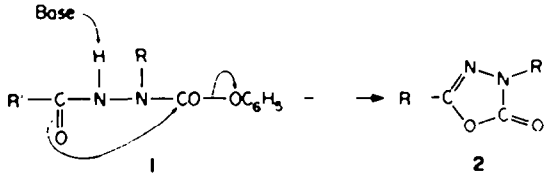
Abstract—N^{α}-Ethyloxycarbonyl-α-azaornithine phenyl ester has been synthesised and shown to be more stable at neutral pH than the N^{α}-benzoyl analogue. Consequently it is a more effective inhibitor of trypsin. N^{α}-Ethyloxycarbonyl-α-azaornithine phenyl ester also strongly inhibited thrombin but had no effect on chymotrypsin or urokinase.

It has been shown that chymotrypsin[1] and trypsin[2] can be selectively inhibited by the phenyl esters of α-azaphenylalanine and α-azaornithine derivatives respectively. The inhibition is due to the slower rate of deacylation of the aza-aminoacyl-enzyme intermediate compared with that of the corresponding derivative of a natural amino-acid.

N^{α}-Benzoyl-α-azaornithine phenyl ester was a selective inhibitor of trypsin.[2] It was, however, unstable, decomposing spontaneously at neutral pH, with liberation of phenol. In the preceding communication[3] it is reported that esters of certain acyl-α-azaamino-acids **1** undergo cyclisation giving the corresponding oxadiazolone **2**.



1 **2**

It seemed probable therefore that the lack of stability of N^{α}-benzoyl-α-azaornithine phenyl ester reported[2] was due to cyclisation to the oxadiazolone [**2**: R = $(CH_2)_3NH_2$, R' = C_6H_5].

The reaction is analogous to the formation of oxazolones from α-amino-acid derivatives. In the α-amino-acid series the cyclisation is prevented if the derivative is of the urethan type and this appears also to be the case for α-azaamino-acids.[3] We therefore set out to prepare an alkyloxycarbonyl- or aralkyloxycarbonyl-derivative of azaornithine in order to obtain a stable enzyme inhibitor and incidentally to provide support for the suggestion that the instability of the N^{α}-benzoyl-derivative is due to oxadiazolone formation.

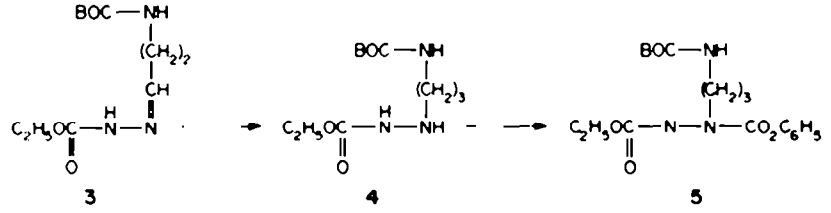
The synthetic route followed the method previously reported. At first we proposed to use the benzyloxycarbonyl-protecting group. Benzyloxycarbonyl hydrazide was condensed with Boc-3-amino propanal[3] to give the hydrazone. Attempts to reduce selectively the imino-double bond using sodium cyanoborohydride[4] were unsuccessful. Mixtures were always obtained and it was clear that reductive cleavage of the benzyloxycarbonyl group was occurring. The hydrazone **3** obtained from ethyloxycarbonyl hydrazide and Boc-3-amino propanal was reduced by catalytic hydrogenation. However even in this case yields were low and there was evidence of cleavage of the ethyloxycarbonyl group. Treatment of **4** with phenyl chloroformate in the usual way[1,2] gave N^{α}-ethyloxycarbonyl-N^{δ}-Boc-α-azaornithine phenyl ester **5**.

The final step was the removal of the Boc-protecting group with trifluoroacetic acid (TFA). The product from the evaporation of TFA was first thought to be the free amine as the trifluoroacetate salt. In water it gave a solution of pH 2–3 in which it was very stable giving a single, ninhydrin-positive spot on TLC with R_{f_A} 0.65. However, adjustment of the pH to 7 gave a new product with R_{f_A} 0.44. In this case no phenol was liberated at this stage (in contrast to the case of N^{α}-benzoyl-α-azaornithine phenyl ester[2]) indicating that no cyclisation involving the phenyloxycarbonyl group had taken place.

We think it probable that the product of treatment of the N^{δ}-Boc-derivative with TFA, followed by evaporation to dryness, is not the amine trifluoroacetate salt but the N^{δ}-trifluoroacetyl derivative. This appears to be stable in aqueous solution at pH 2–3 and under the conditions of TLC with solvent system A, but at neutral pH hydrolyses rapidly to the amine, R_{f_A} 0.44.

This amine was extractable from $NaHCO_3$ solution by $CHCl_3$, yielding an oil (again R_{f_A} 0.44). Retreatment of the oil with TFA gave the product with original behaviour on TLC (R_{f_A} 0.65).

A possible objection to the assignment of the first product as the trifluoroacetyl derivative is that it gave a positive response to the ninhydrin test on TLC. However the conditions of this test, requiring as it does heating the



3 **4** **5**

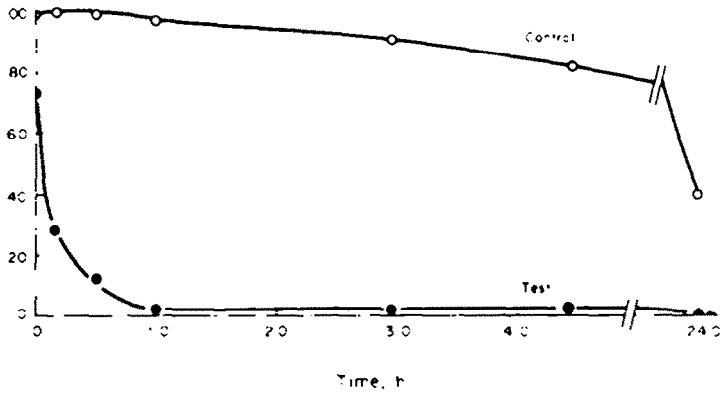


Fig. 1. Variation with time of activity of trypsin treated with N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester. Trypsin (100 μg) was treated at pH 7.6 with N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester (0.825 μmol) at 25° as described in the Experimental.

plate, are such that hydrolysis of the derivative is likely, with subsequent production of the positive response. We have observed a similar phenomenon with some *N*-Boc-derivatives.

At neutral pH, as expected, the N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester was more stable than the benzoyl derivative in that it did not undergo the rapid cyclisation with liberation of phenol. Its stability was further demonstrated by its isolation by extraction from alkaline solution as the free amine. For this reason it proved to be a more effective inhibitor of trypsin. Figure 1 shows the effect of treating trypsin with an excess of N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester. The enzymic activity was destroyed very rapidly and did not reappear even after 24 h. This contrasts strongly with the case of the N^{α}-benzoyl analogue, in which the activity had returned to approx. 35% of the original after 1 h, under virtually identical conditions.

The regeneration of activity is the result of spontaneous deacylation of the acyl-enzyme intermediate, but in the presence of excess of the stable analogue re-acylation should take place. Figure 1 therefore demonstrates the greater stability and effectiveness at pH 7.6 of the N^{α}-ethyloxycarbonyl derivative.

Interestingly the N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester had no measurable effect on the activity of chymotrypsin under the conditions described. This contrasts with the result for the N^{α}-benzoyl analogue[2] which did react, but more slowly than with trypsin.

The new stable analogue was also tested for its ability to inactivate two other proteolytic enzymes, both of which function by "active-site serine" mechanisms and show specificity for basic side-chain amino-acid residues in their substrates. Ethyloxycarbonyl-α-azaornithine phenyl ester (7.5×10^{-4} M) caused rapid and complete loss of activity of thrombin with respect to both its action against the synthetic substrate benzoyl-L-arginine ethyl ester (BAEE) and its ability to cause clotting of fibrinogen. The same concentration of the azaornithine ester, however, had no apparent effect on the ability of urokinase to catalyse the hydrolysis of the peptide substrate N^{α}-acetylglycyl-L-lysine methyl ester.

EXPERIMENTAL

Trypsin (EC 3.4.21.4) (2 × crystallised; ethanol precipitate, essentially salt free), chymotrypsin (EC 3.4.21.1) [3 × crystallised; prepared free of autolysis products and contaminants by the method of Yapel *et al.*[4]] and *N*-α-benzoyl-L-arginine ethyl ester were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Human serum albumin for protein standard curves, thrombin (fibrinogenase, EC 3.4.21.5) and *N*-acetylglycyl-L-lysine methyl ester were obtained from Koch-Light Laboratories Ltd. Urokinase (EC 3.4.99.26) and fibrinogen were provided by Abbott Laboratories, North Chicago, Illinois, U.S.A. TLC was carried out by using Merck "Kieselgel G nach Stahl" and the following solvent systems. (a) Butanol–acetic acid–water (4:1:5 by vol); (b) hexane–ethylacetate (1:1, by vol); (c), hexane–ethylacetate (2:3); (d) ethylacetate–ether (1:3); (e) ethylacetate–chloroform (6:1); (f) ethylacetate–chloroform (1:3); components were detected by using iodine vapour, ninhydrin or the chlorine spray of Pan and Dutcher.[5]

Proteinase activities of trypsin and chymotrypsin preparations were determined by the casein method of Laskowski,[6] modified to allow the concentration of hydrolysis products in the supernatant after precipitation by trichloroacetic acid to be determined by using the Folin-Ciocalteu reagent in a manner similar to that described by Anson.[7] It was established for the enzyme assays that over the range required the method was linear with respect to both time and enzyme concentration. Protein concentrations were determined by the method of Lowry *et al.*[8]

N-t-Butyloxycarbonyl-3-aminopropanal benzyloxycarbonyl hydrazone. Benzyloxycarbonyl hydrazide[10] (1.51 g; 9.1 mmol) was added to *N*-t-butyloxycarbonyl-3-aminopropanal[2] (1.57 g; 9.08 mmol) in ethanol (20 cm^3) and the solution stirred at room temperature for 24 h and then evaporated to give a light brown syrup. The product was dissolved in chloroform, which was then washed with 0.5 M citric acid and water, dried ($MgSO_4$) and evaporated to give a residue which was treated with ether to give a white solid (1.95 g; 67.0%). Recrystallization was achieved from chloroform–ether. M.p. 115–6°. (Found: C, 59.5; H, 7.2; N, 13.4. $C_{16}H_{23}N_3O_4$ requires: C, 59.8; H, 7.2; N, 13.1%). TLC: Single iodine positive spot (R_f 0.5). No aldehyde remained (R_f 0.4; positive to chlorine and iodine). IR ν_{max} (Nujol mull): 1670 and 1740 (C=O), 3260 and 3300 cm^{-1} (N–H). NMR (chloroform-*d*): τ 1.4 (singlet; 1 proton: =N–NH), 2.7 (multiplet; 5 protons: aromatic), 4.8 (singlet; 2 protons: –O–CH_2), 5.0 (broad triplet; 1 proton: –CO–NH^{α}), 6.7 (quartet; 2 protons: –NH^{α}–CH_2^{β}–CH_2^{γ}–, $J_{\alpha,\beta} = J_{\beta,\gamma} = 8$ Hz), 7.6 (quartet; 2 protons: –CH_2^{γ}–CH_2^{β}–CH^{α}=, $J_{\beta,\alpha} = J_{\gamma,\beta} = 8$ Hz), and 8.6 (singlet; 9 protons: t-butyl).

Treatment of N-t-butyloxycarbonyl-3-aminopropanalbenzyloxycarbonylhydrazone with sodium cyanoborohydride. Sodium cyanoborohydride (0.223 g) was dissolved in purified methanol (30 cm^3). The stirred solution was adjusted to pH 3.0 with HCl-saturated methanol, and *N*-t-butyloxycarbonyl-3-aminopropanal benzyloxycarbonylhydrazone (0.5 g) in purified methanol (25 cm^3) was added in one portion. The pH changed immediately to alkaline and was adjusted again to pH 3.0 by HCl-saturated methanol. The reaction was followed by TLC; three iodine-positive spots were observed. The reaction was

stopped after 2.5 h and the solvent evaporated down, water was added to the residue, and the aqueous layer was extracted with chloroform (4 × 75 cm³). The organic layers were combined and washed with water, dried ($MgSO_4$) and evaporated to give a brown oil (0.443 g). The product was chromatographed on silica gel (70 g in 20 mm diameter column) eluting with ethyl acetate–hexane (1:1 v/v) (150 cm³) followed by ethyl acetate–hexane (3:2 v/v) (450 cm³). The eluate was monitored by TLC and fractions containing the major products were pooled and evaporated to give the following components.

The fractions with R_{f_c} 0.75 gave a white solid (0.082 g) which was recrystallized from chloroform–hexane, m.p. 132–3°. IR ν_{max} (Nujol mull): 1710 (C=O), 3280 and 3560 cm⁻¹ (N–H). The fractions with R_{f_c} 0.6 gave a syrup (0.131 g) with similar IR. The fractions with R_{f_c} 0.5 gave a white solid (0.197 g), m.p. 115–6° which corresponds to the starting material.

N-t-Butyloxycarbonyl-3-aminopropanal ethyloxycarbonyl hydrazone. Ethyloxycarbonyl hydrazide[11] (0.87 g; 8.36 mmol) in ethanol (10 cm³) was added to *N*-t-butyloxycarbonyl-3-amino propanal[2] (1.44 g; 8.32 mmol) in ethanol (20 cm³). The solution was stirred at room temperature for 20 h, and the solvent was then removed under vacuum to give a brown oil which solidified after a short time. The solid was dissolved in chloroform which was washed with 0.5 M citric acid (3 × 40 cm³), water (2 × 40 cm³), dried ($MgSO_4$) and evaporated. TLC: the product showed three iodine positive spots. R_{f_g} 0.36 (faint, hydrazide), R_{f_b} 0.55 (major) and R_{f_r} 0.8 (faint). The mixture on washing with ether gave *N* - t - butyloxycarbonyl - 3 - aminopropanal ethyloxycarbonyl hydrazone (0.97 g; 44.9%), which was recrystallized from ethyl acetate. M.p. 132–3°. (Found: C, 51.3; H, 8.1; N, 16.4. $C_{11}H_{21}N_3O_4$ requires: C, 51.0; H, 8.1; N, 16.1%). IR ν_{max} (Nujol mull): 1675 and 1740 (C=O), 3420 and 3320 cm⁻¹ (N–H). NMR (chloroform-*d*): τ 1.4 (singlet; 1 proton: –N–NH), 2.7 (triplet; 1 proton: –CH=N), 4.95 (broad triplet, 1 proton: CO-NH), 5.75 (quartet, 2 protons: $\underline{CH_2}$–CH_3), 6.7 (quartet; 2 protons: –NH^{ω}–$\underline{CH_2}^{\gamma}$–$CH_2^{\beta}$–, $J_{\omega,\gamma} = J_{\gamma,\beta} = 7$ Hz), 7.5 (quartet, 2 protons: –CH_2^{γ}–$\underline{CH_2}^{\beta}$–CH^{α} $J_{\beta,\alpha} = J_{\beta,\alpha'} = 7$ Hz), 8.7 (triplet, 3 protons: –CH_2–CH_3) and 8.6 (singlet; 9 protons *t*-butyl).

N-Ethyloxycarbonyl-N'-(3-t-butyloxycarbonylamido)-propylhydrazine

Palladium catalyst (10% on charcoal; 0.5 g) was carefully added in small portions to dry methanol (50 cm³), followed by a solution of pure *N*-t-butyloxycarbonyl-3-aminopropanal ethyloxycarbonylhydrazone (1.28 g) in methanol (40 cm³). The mixture was shaken under hydrogen at room temperature and atmospheric pressure for 24 h, and then filtered; the filtrate was evaporated down to give an oil (1.12 g). TLC: several iodine positive spots were present; R_{f_g} 0.25 (faint), R_{f_g} 0.4 (intense), R_{f_b} 0.45 (intense) and R_{f_r} 0.75 (intense). The crude material was purified by column chromatography using silica gel (70 g, in 20 mm diameter column) eluting with chloroform–ethyl acetate (1:4 v/v). The eluate was monitored by TLC and fractions containing the component (R_{f_r} 0.75) were pooled and evaporated to give an oil which was crystallized from ether–hexane to give a white solid (0.5 g). M.p. 73–5°. The IR and NMR spectra and the elemental analysis were not consistent with the proposed structure. The fractions containing the component (R_{f_r} 0.45) were pooled and evaporated to give an oil which was crystallized from ether–petrol to give *N* - ethyloxycarbonyl - *N'* - (3 - butyloxycarbonylamido) - propylhydrazine (0.26 g; 26.0%), m.p. 47–48°. (Found: C, 50.8; H, 8.2; N, 15.9. $C_{11}H_{21}N_3O_4$ requires: C, 50.6; H, 8.8; N, 16.1%). IR ν_{max} (Nujol mull): 1700 (C=O), 3320 cm⁻¹ (N–H). NMR (chloroform-*d*): τ 3.2 (singlet; 1 proton: N–NH–CO), 4.9 (broad triplet; 1 proton: CO–NH), 5.9 (quartet; 2 protons: $\underline{CH_2}$–CH_3), 6.8 (quartet; 2 protons: NH^{ω}–$\underline{CH_2}^{\gamma}$–$CH_2^{\beta}$, $J_{\omega,\gamma} = J_{\gamma,\beta} = 7.0$ Hz), 7.1 (triplet; 2 protons: CH_2^{β}–$\underline{CH_2}^{\alpha}$–NH*, $J_{\beta,\alpha} = 7.0$ Hz, $J_{\alpha,*} = 0$ Hz), 7.4 (singlet; 1 proton: –CH_2^{α}–NH*), 8.35 (quintet; 2 protons: –CH_2^{γ}–$\underline{CH_2}^{\beta}$–CH_2^{α}), 8.75 (triplet; 3 protons: –CH_2–$\underline{CH_3}$) and 8.55 (singlet; 9 protons: t-butyl).

N^{ω}-t-Butyloxycarbonyl-N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester

Phenyl chloroformate (0.078 g; 0.5 mmol) in ethyl acetate (10 cm³) was added over 20 min to a stirred ice-cooled solution of *N* - ethyloxycarbonyl - *N'* - (3 - t - butyloxycarbonylamido) - propylhydrazine (chromatographically pure; 0.13 g; 0.5 mmol) and triethylamine (0.052 g; 0.5 mmol) in ethyl acetate (15 cm³). Triethylamine hydrochloride precipitated from the solution. The mixture was stirred at 0° for 2 h and overnight at room temperature, then water (20 cm³) was added to dissolve the precipitate. The organic layer was separated, washed with 0.1 M sodium carbonate solution (3 × 25 cm³), 0.5 M citric acid solution (2 × 25 cm³) and water (2 × 25 cm³), dried ($MgSO_4$) and evaporated to give an oil (0.17 g). R_{f_y} 0.25 (faint; starting material), R_{f_y} 0.58 (major) and R_{f_r} 0.8 (faint).

The crude material was chromatographed on silica gel (40 g in a 20 mm diameter column) eluting with ethyl acetate–chloroform (1:3 v/v). The eluate was monitored by TLC and fractions containing the major component (R_{f_y} 0.58) were pooled and evaporated to give an oil (0.132 g; 69.5%) of N^{α} - t - butyloxycarbonyl - N^{ω} - ethyloxycarbonyl - α - azaornithine phenyl ester. IR ν_{max} (CH_2Cl_2): 1730 (C=O), 3400 and 3440 cm⁻¹ (N–H). NMR (chloroform-*d*): τ 2.8 (broad singlet; 1 proton: N–NH), 2.8 (multiplet; 5 protons: aromatic), 5.0 (broad triplet; 1 proton: CO–NH), 5.8 (quartet; 2 protons: $\underline{CH_2}$–CH_3), 6.35 (triplet; 2 protons; CH_2^{γ}–$\underline{CH_2}^{\beta}$–N^{ω}–, $J_{\beta,\gamma} = 6.8$ Hz, $J_{\beta,\omega} = 0$ Hz), 6.8 (quartet; 2 protons: NH^{ω}–$\underline{CH_2}^{\gamma}$–$CH_2^{\beta}$–, $J_{\omega,\gamma} = J_{\gamma,\beta} = 7$ Hz), 8.2 (quartet; 2 protons: –CH_2^{γ}–$\underline{CH_2}^{\beta}$–CH_2^{α}–), 8.75 (triplet; 3 protons: –CH_2–$\underline{CH_3}$) and 8.55 (singlet; 9 protons: t-butyl).

N^{ω}-Ethyloxycarbonyl-α-azaornithine phenyl ester

A solution of N^{α} - t - butyloxycarbonyl - N^{ω} - ethyloxycarbonyl - α - azaornithine phenyl ester (chromatographically pure; 27 mg) in trifluoroacetic acid (2.0 cm³) was left at room temp. for 60 min. Excess trifluoroacetic acid was evaporated in vacuo to give an oil assumed to be N^{α} - trifluoroacetyl - N^{ω} - ethyloxycarbonyl - α - azaornithine phenyl ester. TLC: an aqueous solution, approximately pH 2.0 gave a single spot (R_{f_A} 0.65; positive to ninhydrin). Adjusting the pH of the solution to approx. 7 gave two new spots (R_{f_A} 0.56 (minor) and R_{f_A} 0.44); both ninhydrin positive. No phenol was detected. The ester was characterized as its picrate, a yellow solid prepared from aqueous solution, m.p. 136–8°. (Found: C, 45.0; H, 4.4; N, 16.6. $C_{19}H_{22}N_8O_{11}$ requires: C, 44.7; H, 4.3; N, 16.5%).

N^{ω}-Ethoxycarbonyl-α-azaornithine phenyl ester (free base)

Trifluoroacetic acid (2.0 cm³) was added to a solution of N^{α} - t - butyloxycarbonyl - N^{ω} - ethoxycarbonyl - α - azaornithine phenyl ester (35 mg) and the mixture was allowed to stand for 60 min, and then evaporated to dryness leaving N^{α} - trifluoroacetyl - N^{ω} - ethyloxycarbonyl - α - azaornithine phenyl ester as an oil. The product was dissolved in water and the pH was adjusted to above pH 8.0 with 1.0 M sodium hydrogen carbonate, and extracted with chloroform. The extract was dried ($MgSO_4$) and evaporated to give an oil (22 mg). TLC: single spot (R_{f_A} 0.44; positive to ninhydrin). NMR (chloroform-*d*): τ 2.85 (multiplet; 5 protons: aromatic), 4.4 (broad singlet; 1 proton: N–NH), 5.8 (quartet; 2 protons: $\underline{CH_2}$–CH_3), 6.45 (triplet; 2 protons: CH_2^{γ}–$\underline{CH_2}^{\beta}$–N), 6.75 (triplet; 2 protons: N–$\underline{CH_2}^{\beta}$–CH_2^{γ}), 8.0 (quartet; 2 protons: –CH_2^{γ}–$\underline{CH_2}^{\beta}$–CH_2^{α}) and 8.75 (triplet; 3 protons: –CH_2–$\underline{CH_3}$).

The reaction of N^{ω}-ethyloxycarbonyl-α-azaornithine phenyl ester with trypsin

An aqueous solution of N^{ω}-ethyloxycarbonyl-α-azaornithine phenyl ester (8.25 mM; 0.1 cm³) was added to a solution of trypsin (100 μg/cm³, 1.0 cm³) in 0.05 M phosphate buffer, pH 7.6 at 25°. The mixture was maintained at 25° and aliquots (0.05 cm³) were removed at intervals (0–24 h) and assayed for proteolytic activity by the casein method using an incubation time of 5 min at a temp. of 25°.

A control experiment was conducted in which buffer only was added. The results are shown in Fig. 1.

The reaction of N^{ω}-ethyloxycarbonyl-α-azaornithine phenyl ester with chymotrypsin

To a solution of α-chymotrypsin (135 μg/cm³; 1.0 cm³) in 0.05 M phosphate buffer, pH 7.6 at 25° was added an aqueous

solution of N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester (7.5 mM; 0.1 cm³). The mixture was maintained at 25° and aliquots (0.05 cm³) were removed at intervals and assayed for proteolytic activity by the casein method using an incubation time of 10 min at temp. 25°.

A control experiment was carried out in which buffer alone was added to the enzyme solution.

Another experiment was also performed in which 0.05 M phosphate buffer pH 6.2 was used. No loss of activity was detected in either case.

The reaction of N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester with thrombin

(a) To a solution of N^{α}-benzoyl-L-arginine ethyl ester hydrochloride (1 mg/cm³; 2.0 cm³) in water was added a mixture of thrombin solution (100 N.I.H. units/10 cm³; 0.1 cm³) in 0.05 M phosphate buffer, pH 7.6 and an aqueous solution of N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester (7.5 mM; 0.1 cm³). The mixture was titrated automatically against 0.02 M sodium hydroxide at 37° using a Radiometer autoburette ABU11, Autotitrator TTT2 and Titrigraph system. A control experiment was performed in which the analogue solution was replaced by water.

The analogue caused complete loss of activity against this substrate.

(b) *Clotting of fibrinogen (based on the method of Laki*[11]). Thrombin solution (100 N.I.H. units/10 cm³; 0.2 cm³) in 0.05 M phosphate buffer pH 7.6 was diluted with (0.2 cm³) using the same buffer.

To a solution of fibrinogen (1 mg/cm³; 1.0 cm³) in 0.05 M phosphate buffer was added a diluted thrombin solution (0.1 cm³), the mixture was shaken and incubated at 37°. After a short time clotting appeared and the time recorded. The average of several incubations gave a clotting time of 40 sec.

Another experiment was carried out in which the buffer was replaced by an aqueous solution of N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester (7.5 mM; 0.2 cm³). No clotting was observed, even after 30 min.

The reaction of N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester with urokinase

To a solution of *N*-acetylglycyl-L-lysine methyl ester acetate (5 mg/cm³; 2.0 cm³) in water was added a mixture of urokinase solution (0.3 mg/cm³; 0.1 cm³) in 0.05 M phosphate buffer pH 7.6 and an aqueous solution of N^{α}-ethyloxycarbonyl-α-azaornithine phenyl ester (7.5 mM; 0.1 cm³). The mixture was titrated automatically against 0.02 M sodium hydroxide at 37° as described above.

A control experiment was conducted in which the analogue solution was replaced by water. No loss of activity was caused by the analogue.

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