Amino Acids and Peptides. Part 49.[1] 2-Amino-4-(3-pyridyl)butyric Acid and Related Peptides.

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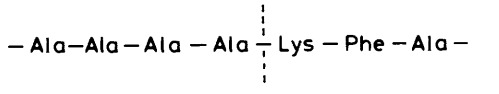
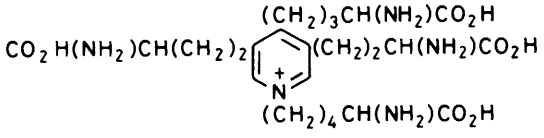
The new α-amino acid, 2-amino-4-(3-pyridyl)butyric acid (**4**) [abbreviation, Ala(3 Pm)], has been synthesized and resolved. The *N*-t-butoxycarbonyl derivative of the L-amino-acid has been used in the picolyl ester 'handle' method to synthesize Boc-Ala(3 Pm)-Ala-Ala-Ala-Lys(Z)-OPic (**8**) (Pic = 4-picolyl) and its *N*-acetyl analogue (**9**), Boc-Ala(3 Pm)-Ala-Ala-D-Ala-Lys(Z)-OPic (**10**), and Boc-Ala(3 Pm)-Ala-Ala-Val-Lys(Z)-OPic (**11**). Compounds (**8**), (**9**), and (**11**) are weak inhibitors of human leucocytic elastase.

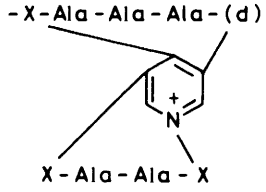
The mapping of the substrate requirements of human leucocytic elastase is of special interest because of the suggestion[2] that this enzyme may be responsible for the degradation of elastin during pulmonary emphysema; inhibitors of this enzyme could, therefore, be of pharmacological importance. Much is known of the specificity of porcine pancreatic elastase, which cleaves polyalanyl-lysine peptides such as (**1**) at the alanyl-lysine bond,[3] and such a sequence can be fitted to the active site in the enzyme model proposed by Sawyer, Shotton, and Watson.[4] The human leucocytic enzyme again favours a polyalanyl sequence but apparently prefers valine as the acyl residue at the cleavage site;[5] substitution in this position by the aza-analogues of alanine, valine, leucine, and isoleucine provides inhibitors.[6] Kasafírek, Frič, and Slabý[7] have shown that *N*-alkylamides, reported by Hassall, Johnson, and Roberts[8] to be inhibitors of porcine pancreatic elastase, inhibit human leucocytic elastase; especially effective are tetrapeptides having an *N*-terminal dicarboxylic acid, such as glutaryl-alanyl-alanyl-prolyl isobutylamide.



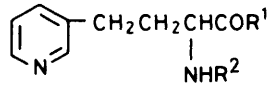
(**1**)



(**2**)



(**3**)



(**4**) R^1 = OH, R^2 = H
(**5**) R^1 = OMe, R^2 = PhCO
(**6**) R^1 = OH, R^2 = PhCO
(**7**) R^1 = OH, R^2 = Boc

X = A modified lysine residue which constitutes an arm of the pyridinium nucleus; (*d*) = desmosine plus isodesmosine.

Elastin protein chains are cross-linked by an unusual type of structure in which the side-chains of two amino acids in each chain alkylate a pyridine ring in positions 1, 3, 4, and 5 or 1, 2, 3, and 5, complete acid hydrolysis giving, for example, desmosine (**2**).[9] Foster, Rubin, Kagan, and Franzblau[10] have suggested a partial structure such as (**3**) for a cross-linkage of the elastin from bovine ligamentum nuchae, and Gerber and Anwar[11] have shown that pancreatic elastase can cleave alanyl bonds within the peptide rings. The incorporation of features of the cross-linked area of elastin might be expected to confer higher specificity on an inhibitor. Yasutake and Powers[12] have replaced alanine and proline in a model substrate by lysine and certain of its N^{ε}-derivatives, and they conclude that their results support the hypothesis that human leucocytic elastase cleaves elastin selectively near cross-linking residues. We have now synthesised the new amino-acid, 2-amino-4-(3-pyridyl)butyric acid (**4**), incorporating the 3-pyridyl feature of desmosine, and a number of protected peptides (**8**)—(**11**) in which it is combined with alanine and *C*-terminal lysine; it was hoped that the latter might provide a route to a cyclic 1,3-pyridinium peptide resembling part of the cross-linked structure, but this has not been achieved.

3-(3-Pyridyl)propionaldehyde, previously obtained by ozonolysis of 5-(3-pyridyl)pent-2-ene,[13] was more conveniently prepared by the oxidation of 3-(3-pyridyl)propan-1-ol by lead tetra-acetate in pyridine, and the crude aldehyde was converted directly into the amino nitrile and thence into the racemic amino acid (**4**). This was resolved by asymmetric hydrolysis of the methyl ester of the benzoyl derivative (**5**) by α-chymotrypsin; acid hydrolysis of the acid so formed gave L-2-amino-4-(3-pyridyl)butyric acid, which was converted into the N^{α}-t-butoxycarbonyl derivative by the use of di-t-butyl dicarbonate.[14] Acid hydrolysis of the residual ester from the resolution and analogous acylation of the resulting D-amino-acid gave the t-butoxycarbonyl D-derivative.

R-Ala(3 Pm)-Ala-Ala-Ala-Lys(Z)-OPic

(**8**) R = Boc; (**9**) R = Ac

Boc-Ala(3 Pm)-Ala-Ala-D-Ala-Lys(Z)-OPic

(**10**)

Boc-Ala(3 Pm)-Ala-Ala-Val-Lys(Z)-OPic

(**11**)

Ac-Ala-Ala-Ala-Lys(R^1)-R^2

(**12**) R^1 = Z, R^2 = OPic; (**13**) R^1 = H, R^2 = OH

We have synthesized the following protected peptides of the new amino acid * by the picolyl ester 'handle' method:[15-19] N^{α}-t-butoxycarbonyl-β-(3-pyridylmethyl)alanyl-alanyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyl-lysine 4-picolyl ester (**8**), and the corresponding N^{α}-acetyl derivative (**9**); N^{α}-t-butoxycarbonyl-β-(3-pyridylmethyl)alanyl-alanyl-alanyl-D-alanyl-N^{ε}-benzyloxycarbonyl-lysine 4-picolyl ester (**10**); N^{α}-t-butoxycarbonyl-β-(3-pyridylmethyl)alanyl-alanyl-alanyl-valyl-N^{ε}-benzyloxycarbonyl-lysine 4-picolyl ester (**11**); and, for comparison, we have prepared acetyl-alanyl-alanyl-alanyl-N^{ε}-benzyloxycarbonyl-lysine 4-picolyl ester (**12**) and the deprotected acetyl peptide (**13**). We include here the preparation of the starting material for the synthesis of *C*-terminal lysine peptides by the picolyl ester method, N^{α}-t-butoxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysine 4-picolyl ester.

The syntheses were stepwise, using t-butoxycarbonyl for α-amino protection, and coupling was by dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. Isolation of the coupled products normally made use of the picolyl 'handle' and was by the citric acid procedure[18,19] when the product was soluble in ethyl acetate or in dichloromethane and otherwise by the Amberlyst cation-exchange procedure.[18,19]

The Appendix describes the determination of the inhibition constants of protected peptides containing 2-amino-4-(3-pyridyl)butyric acid residues, using succinyl-L-Ala-L-Pro-L-Ala-4-nitroanilide as substrate for porcine pancreatic elastase and *O*-methylsuccinyl-L-Ala-L-Ala-L-Pro-L-Val-4-nitroanilide as substrate for human leucocytic elastase; elastatinal[20] was included in the experiments for comparison. Table 2 gives the inhibition constants for Boc-Ala(3 Pm)-Ala-Ala-Ala-Lys(Z)-OPic (**8**), Ac-Ala(3 Pm)-Ala-Ala-Ala-Lys(Z)-OPic (**9**), and Boc-Ala(3 Pm)-Ala-Ala-Val-Lys(Z)-OPic (**11**); protected peptides (**10**) and (**13**) were insufficiently soluble under the conditions of the assay. It is seen that none of the compounds is an effective inhibitor of porcine pancreatic elastase; all three are somewhat more active against human leucocytic elastase although none is as effective as elastatinal.

Experimental

General directions are as detailed in Part 46.[17] Additional solvents used in t.l.c. were (proportions are by volume): (A2), butanol–acetic acid–water, 10:1:3; (G1), ethyl acetate (3 vol.) and pyridine–acetic acid–water, 20:6:11, (2 vol.); (G6), ethyl acetate (1 vol.) and pyridine–acetic acid–water, 20:6:11, (3 vol.); (J), acetonitrile–water, 3:1; (K), butan-2-ol–3% aqueous ammonium hydroxide, 3:1; (R2), ethyl acetate–acetic acid–water, 5:2:1. Ether refers to diethyl ether.

DL-2-*Amino*-4-(3-*pyridyl*)*butyric Acid* (**4**).—Redistilled 3-(3-pyridyl)propanol (25.9 g, 0.189 mol) in dried pyridine (930 ml) and lead tetra-acetate (85 g, 0.192 mol) reacted during 16 h at room temperature. The solvent was removed, the residue was dissolved in water (250 ml) and the solution was saturated with potassium carbonate. The upper phase of aldehyde was collected and the aqueous phase was filtered; precipitate and filtrate were extracted with chloroform (400 ml) and the organic solutions were combined and dried (K_2CO_3). Evaporation and fractional distillation of the residue gave 3-(3-pyridyl)-propionaldehyde (6.9 g, 27%), b.p. 84—86 °C at 0.3 mmHg. Characterisation was made by conversion into the *semicarbazone*, m.p. 143 °C (Found: C, 56.35; H, 6.15; N, 29.2. $C_{19}H_{12}N_4O$ requires C, 56.24; H, 6.29; N, 29.15%); the thiosemicarbazone, m.p. 177—178 °C (Found: C, 51.85; H, 5.8; N, 27.1; S, 15.3. Calc. for $C_9H_{12}N_4S$: C, 51.90; H, 5.81; N, 26.90; S, 15.39%) (lit.,[13] m.p. 164—165 °C), and the 2,4-*dinitrophenylhydrazone*, m.p. 203—204 °C (Found: 53.35; H, 4.25; N, 22.4. $C_{14}H_{13}N_5O_4$ requires C, 53.33; H, 4.16; N, 22.21%). The aldehyde was immediately converted into the amino nitrile by the following general procedure. To a solution of the aldehyde (24 g, 0.178 mol) in ether (6.5 ml) were added a solution of ammonium chloride (31.68 g, 0.592 mol) in water (108 ml) at 0—4 °C and an ice-cold solution of sodium cyanide (26.64 g, 0.544 mol) in water (72 ml). The reaction mixture was stirred for 4 h and then warmed to 50 °C; after a further 7.5 h the mixture was allowed to cool and was extracted with chloroform (1500 ml). Evaporation of the extracts gave the crude aminonitrile (17 g) as an oil, which was hydrolysed by 12M-hydrochloric acid during 64 h at room temperature and then 30 h at reflux temperature. The solution was evaporated with repeated addition of water and the residual amino acid was taken up on a column of Dowex 50W-X8 resin (NH_4^+ form) and chloride was washed off by water. The amino acid was eluted with 2M-ammonium hydroxide; evaporation left a residue which was triturated with warm ethanol–ether, giving DL-2-*amino*-4-(3-*pyridyl*)*butyric acid* (**4**) as a white solid which was recrystallised from ethanol–water (overall yield from the aldehyde, 41%); R_f 0.42 (G6), 0.17 (K), and 0.03 (A2) (Found: C, 57.0; H, 7.05; N, 14.9. $C_9H_{12}N_2O_2 \cdot 0.5\,H_2O$ requires C, 57.13; H, 6.92; N, 14.80%).

DL-2-*Benzamido*-4-(3-*pyridyl*)*butyric Acid* (**6**).—DL-2-Amino-4-(3-pyridyl)-butyric acid reacted with 4-nitrophenyl benzoate (1.2 equiv.) and redistilled 1,1,3,3-tetramethylguanidine (1.2 equiv.) in dried dimethylformamide during 48 h at room temperature. The solvent was evaporated and addition of an equal volume of glacial acetic acid to the residual oil caused crystallisation; a large volume of ethyl acetate was added and *benzoyl derivative* (**6**), m.p. 210 °C, R_f 0.23 (K) and 0.17 (R2) was collected (yield, 91%) (Found: C, 67.45; H, 5.5; N, 9.85. $C_{16}H_{16}N_2O_3$ requires C, 67.59; H, 5.67; N, 9.85%).

Methyl DL-2-*Benzamido*-4-(3-*pyridyl*)*butyrate* (**5**).—The benzamido acid (**6**) (4.0 g, 13.4 mmol) was suspended in dried methanol (16 ml) and the solution was cooled to −10 °C. Thionyl chloride (1.35 ml, 18.51 mmol) was added dropwise and after 20 min the temperature was allowed to rise. After a further 1 h the solution was evaporated, saturated aqueous sodium hydrogen carbonate was added to give pH 8.2 and the precipitated product was extracted into chloroform, giving the *methyl ester* (**5**) (97%), m.p. 116—120 °C, R_f 0.52 (R2) (Found: C, 68.6; H, 6.4; N, 9.3. $C_{17}H_{18}N_2O_3$ requires C, 68.44; H, 6.08; N, 9.39%).

L-2-*Benzamido*-4-(3-*pyridyl*)*butyric Acid*.—Methyl DL-2-benzamido-4-(3-pyridyl)butyrate (4.1 g, 13.74 mmol) in methanol and 0.2M-ammonium acetate buffer of pH 7.8 (1 270 ml) was treated with a solution of α-chymotrypsin (25 mg) in buffer (10 ml) during 3 h at 37 °C, the pH being maintained at 7.8 by an autotitrator. The pH was then reduced to 5.3 by addition of acetic acid and the solution was evaporated to dryness. The solid residue was collected and dissolved in 2M-acetic acid; addition of concentrated aqueous ammonium hydroxide to pH 8.5—9.0 precipitated methyl D-2-benzamido-4-(3-pyridyl)butyrate which was collected (Solid A, see below) and the filtrate was extracted with ethyl acetate. The aqueous filtrate and washings were combined and repeatedly evaporated with addition of water to remove ammonium acetate. The residue was triturated with water, then dried and crystallised from methanol, giving L-*benzamido acid* (94% of theory), m.p.

* We use the trivial name, β-(3-pyridylmethyl)alanine for (**4**), by analogy with β-cyanoalanine, [Ala(CN)], and hence the abbreviation Ala(3 Pm). Pic = 4-picolyl. Amino acid residues are of the L-configuration unless otherwise stated.

Table 1. Synthesis of protected peptides

Compd.[a]	Amino component[b] (mmol)	Acylating[c] component (mmol)	Isolation[d]	Yield (%)	$[\alpha]_D^{20}$[e] (°)	R_f (t.l.c.)	Found (%) (Required) C	H	N	Formula
(14) Boc-Ala-Lys(Z)-OPic	Boc-Lys(Z)-OPic (25.3)	Boc-Ala (32.9)	C	97[f]	−13	0.33 (E4); 0.77 (P2)	61.75 (61.98)	7.1 (7.06)	10.35 (10.32)	$C_{27}H_{38}N_4O_7$
(15) Boc-Ala-Ala-Lys(Z)-OPic	Compd. **(14)** (18.3)	Boc-Ala (24.65)	C	86.5	−19	0.26 (E4); 0.71 (P2)	58.8 (58.94)	7.1 (7.18)	11.35 (11.09)	$C_{31}H_{43}N_5O_8 \cdot H_2O$
(16) Boc-Ala-Ala-Ala-Lys(Z)-OPic	Compd. **(15)** (14.25)	Boc-Ala (23.5)	g	81	−21	0.24 (E4); 0.59 (P2)	58.25 (58.11)	6.75 (7.17)	12.1 (11.60)	$C_{34}H_{48}N_6O_9 \cdot H_2O$
(8) Boc-Ala(3 Pm)-Ala-Ala-Ala-Lys(Z)-OPic	Compd. **(16)** (0.61)	Boc-Ala(3 Pm) (0.82)	A[h]	71[i]	−16	0.20 (E4); 0.56 (P2)	60.25 (60.34)	6.9 (6.95)	13.0 (13.09)	$C_{43}H_{58}N_8O_{10} \cdot 0.5H_2O$
(17) Boc-D-Ala-Lys(*Z*)-OPic	Boc-Lys(*Z*)-OPic (1.02)	Boc-D-Ala (1.53)	C	87	−4	0.35(E4);0.75(P2)	60.95 (60.97)	6.9 (7.13)	10.1 (10.16)	$C_{28}H_{38}N_4O_7 \cdot 0.5\ H_2O$
(18) Boc-Ala-D-Ala-Lys(Z)-OPic	Compd. **(17)** (0.73)	Boc-Ala (0.865)	A	88	−12	0.29 (E4); 0.69 (P2)	59.15 (58.94)	7.05 (7.18)	11.15 (11.08)	$C_{31}H_{43}N_5O_8 \cdot H_2O$
(19) Boc-Ala-Ala-D-Ala-Lys(Z)-OPic	Compd. **(18)** (0.25)	Boc-Ala (0.35)	C	90[j]	−10	0.26 (E4); 0.61 (P2)	58.4 (58.11)	7.0 (7.17)	11.9 (11.96)	$C_{34}H_{48}N_6O_9 \cdot H_2O$
(10) Boc-Ala(3 Pm)-Ala-Ala-D-Ala-Lys(Z)-OPic	Compd. **(19)** (0.154)	Boc-Ala(3 Pm) (0.207)	C[h]	91[k,l]	−10	0.15 (E4); 0.35 (P2)	59.85 (59.71)	7.15 (6.99)	12.9 (12.95)	$C_{43}H_{58}N_8O_{10} \cdot H_2O$
(20) Boc-Val-Lys(Z)-OPic	Boc-Lys(Z)-OPic (1.04)	Boc-Val (1.67)	C	69[m]	−9	0.33 (E4); 0.89 (R2)	62.15 (62.14)	7.3 (7.48)	9.55 (9.53)	$C_{30}H_{42}N_4O_7 \cdot 0.5\ H_2O$
(21) Boc-Ala-Val-Lys(Z)-OPic	Compd. **(20)** (0.56)	Boc-Ala (0.696)	A	78[n]	−16.5	0.32 (E4); 0.63 (J)	61.75 (61.76)	7.25 (7.38)	10.7 (10.91)	$C_{33}H_{47}N_5O_8$
(22) Boc-Ala-Ala-Val-Lys(Z)-OPic	Compd. **(21)** (0.156)	Boc-Ala (0.203)	*o,p*	100	−20	0.36 (E4); 0.80 (G1)	60.4 (60.66)	7.5 (7.35)	11.65 (11.79)	$C_{36}H_{52}N_6O_9$
(11) Boc-Ala(3 Pm)-Ala-Ala-Val-Lys(Z)-OPic	Compd. **(22)** (0.16)	Boc-Ala(3 Pm) (0.203)	C[k]	90[q]	−13	0.06 (E4); 0.58 (J)	61.35 (61.14)	7.05 (7.18)	12.90 (12.68)	$C_{45}H_{62}N_8O_{10} \cdot 0.5\ H_2O$

[a] All compounds are new. [b] The amino-component was prepared by the action of trifluoroacetic acid on the stated-t-bu'oxycarbonyl derivative.[17] [c] Coupling was by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole.[17] [d] C = citric acid method; A = Amberlyst method, using ethyl acetate–ether (1:1 by vol.) as solvent for the application of the product to the resin. [e] Optical rotations were measured in Me_2NCHO (*c* 1). [f] M.p. 83—85 °C. [g] The product was precipitated by ether from the washed ethyl acetate extract and was t.l.c. pure without use of citric acid; additional material was obtained from the mother liquids by use of the Amberlyst method. M.p. 90—92 °C. Found: Lys, 0.94; Ala, 3.00. [h] The solvent for the preliminary wash of the product was dichloromethane containing 12% butanol. [i] Found: Lys + Ala(3 Pm), 2.12; Ala, 3.00. [j] Crystallised from dichloromethane–light petroleum; m.p. 105—107 °C. [k] The solvent for the preliminary wash of the product and for extraction from aqueous citric acid was dichloromethane containing 10% n-butanol. [l] M.p. 146—148 °C. Found: Lys + Ala(3 Pm), 1.89; Ala, 3.00. [m] M.p. 127—128 °C. [n] M.p. 134 °C. [o] The solvent for the preliminary wash of the product was dichloromethane. [p] Trituration of the residue from evaporation of the dichloromethane solution of the washed product with pentane gave chromatographically pure product of m.p. 149—151 °C. Found: Lys, 0.95; Ala, 2.00; Val, 1.06. [q] M.p. 178—181 °C. Found: Lys + Ala(3 Pm), 1.93; Ala, 1.99; Val, 1.00.

Table 2.

Compd.	K_i(mM) PPE	HLE
(**8**) Boc-Ala(3 Pm)-Ala-Ala-Ala-Lys(Z)-OPic	1.11	0.032
(**9**) Ac-Ala(3 Pm)-Ala-Ala-Ala-Lys(Z)-OPic	0.80	0.043
(**11**) Boc-Ala(3 Pm)-Ala-Ala-Val-Lys(Z)-OPic	1.04	0.095
Elastatinal	0.00 074	0.012

PPE = porcine pancreatic elastase; HLE = human leucocytic elastase.

216—219 °C, R_f 0.73 (G6), 0.33 (J), 0.23 (K), and 0.17 (R2), $[\alpha]_D^{20}$ −18.9° (*c* 1 in M-hydrochloric acid) (Found: C, 67.45; H, 5.65; N, 9.8. $C_{16}H_{16}N_2O_3$ requires C, 67.59; H, 5.67; N, 9.85%).

Methyl D-2-*Benzamido*-4-(3-*pyridyl*)*butyrate*.—The solid A and the residue from evaporation of the ethyl acetate extracts (above) gave a product which was recrystallised from tetrahydrofuran, giving D-*methyl ester* (96% of calc.), m.p. 141—142 °C, $[\alpha]_D^{20}$ +15.2° (*c* 0.99 in Me_2NCHO) (Found: C, 68.3; H, 6.1; N, 9.45. $C_{17}H_{18}N_2O_3$ requires C, 68.44; H, 6.08; N, 9.39%).

D-2-*Benzamido*-4-(3-*pyridyl*)*butyric Acid*.—The D-methyl ester in methanol was hydrolysed by M-sodium hydroxide (1.16 equiv.) during 2.5 h, giving D-*benzamido acid* (63%), m.p. 217—218 °C, $[\alpha]_D^{20}$ +18.7° (*c* 1 in M-hydrochloric acid) (Found: C, 67.45; H, 5.5; N, 9.85).

L-2-*Amino*-4-(3-*pyridyl*)*butyric Acid*.—The L-benzoyl derivative (**6**) was hydrolysed by 6M-hydrochloric acid at reflux temperature during 3 h. The benzoic acid was removed by filtration and after repeated evaporation of water the residue was triturated with hot acetone and then with hot benzene. The crude amino acid hydrochloride was converted into the free base by Amberlite IR-120 resin (NH_4^+ form) and elution with 2M-ammonium hydroxide gave L-*amino acid*, m.p. 239—242 °C, R_f 0.42 (G6) and 0.17 (K) (Found: C, 58.65; H, 6.5; N, 15.2. $C_9H_{12}N_2O_2 \cdot 0.5H_2O$ requires C, 58.52; H, 6.82; N, 15.16%).

t-*Butoxycarbonyl*-L-2-*amino*-4-(3-*pyridyl*)*butyric Acid*.—The L-amino acid (**4**) was acylated by the method of Moroder *et al.*[14] using di-t-butyl dicarbonate in dimethylformamide and ethyl diisopropylamine as base. After evaporation of the solvent, the pH was brought to 3.5—4.0 with acetic acid and the precipitate was collected; evaporation of the filtrate and washings yielded more product, and the combined solids were recrystallised from ethanol, giving t-*butoxycarbonyl derivative* (**7**) (71%), m.p. 185—187 °C, R_f 0.32 (R2) $[\alpha]_D^{26}$ −7.2° (*c* 1 in Me_2NCHO) (Found: C, 59.65; H, 7.05; N, 10.1. $C_{14}H_{20}N_2O_4$ requires C, 59.99; H, 7.19; N, 9.99%). The analogous D-*derivative* had m.p. 187—188 °C, $[\alpha]_D^{20}$ +6.8° (*c* 1 in Me_2NCHO) (Found: C, 59.75; H, 7.25; N, 9.95%) and the DL-*derivative* m.p. 187—188 °C (Found: C, 60.25; H, 7.25; N, 9.8%).

N^{α}-t-*Butoxycarbonyl*-N^{ε}-*benzyloxycarbonyl*-L-*lysine* 4-*Picolyl Ester*.—This was prepared from N^{α}-t-butoxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysine[21] and 4-picolyl alcohol by the standard method[22] (yield, 97%) but it could not be crystallised; the *ester* had R_f 0.44 (E4) and 0.59 (P2), $[\alpha]_D^{20}$ −18.2° (*c* 1 in Me_2NCHO) (Found: C, 63.55; H, 7.2; N, 8.95. $C_{25}H_{33}N_3O_6$ requires C, 63.68; H, 7.05; N, 8.91%).

General Procedures for the Synthesis of Protected Peptides.—These followed closely those described in Part 46[17] under this heading. Details of the coupling reactions and the isolation procedures are shown in Table 1. For isolation by the citric acid method, the reaction mixture was evaporated; the residue was dissolved in ethyl acetate, stirred at 0 °C for 0.5—1 h, and then filtered. The filtrate was evaporated and the residue was dissolved normally in ethyl acetate and washed (aqueous sodium hydrogen carbonate, water, and brine). The solution was diluted with ether and exhaustively extracted with equal volumes of 0.7 M-citric acid (completion confirmed by t.l.c.). The combined extracts were washed with ether and made alkaline by solid sodium hydrogen carbonate and the product was extracted into ethyl acetate. The solution was washed (water, brine), dried, and evaporated. In certain cases, noted in Table 1, dichloromethane (with or without added butanol) was used as a more powerful solvent in place of ethyl acetate in this procedure. For isolation by the Amberlyst method, the product was normally applied to the resin in ethyl acetate–ether (1:1); non-basic contaminants were washed off the column by the same mixture of solvents. Elution of the product was by pyridine in dimethylformamide (25% by vol.) at 0—5 °C.

N^{α}-*Acetyl*-L-*alanyl*-L-*alanyl*-L-*alanyl*-N^{ε}-*benzyloxycarbonyl*-L-*lysine* 4-*Picolyl Ester* (**12**).—The t-butoxycarbonyl group was removed from the protected tetrapeptide (**16**) (Table 1) (0.40 g) by trifluoroacetic acid as usual; trifluoroacetate anion was replaced by acetate by means of Amberlite IRA-93 (acetate form) and the product was acetylated by acetic anhydride (1.54 ml) (10 min at 0 °C, 50 min at room temperature). After the addition of water, the product was isolated by the Amberlyst procedure (solvent, dimethylformamide–ethyl acetate, 2:1), giving the *acetyl derivative* (**12**) (0.31 g, 85%), m.p. 212—214 °C, R_f 0.07 (E4) and 0.44 (P2), $[\alpha]_D^{20}$ −22.5° (*c* 1.0 in Me_2NCHO) (Found: C, 58.75; H, 6.7; N, 13.4. $C_{31}H_{42}N_6O_8 \cdot 0.5H_2O$ requires C, 58.57; H, 6.82; N, 13.21%).

L-2-*Acetamido*-4-(3-*pyridyl*)*butyryl*-L-*alanyl*-L-*alanyl*-L-*alanyl*-N^{ε}-*benzyloxycarbonyl*-L-*lysine* 4-*Picolyl Ester*. (**9**).—This was prepared from protected pentapeptide (**8**) as described for the above acetyl derivative (**12**); yield 87%. The *acetyl derivative* (**9**) had m.p. 215—217 °C, R_f 0.17 (P2) and 0.89 (G1); $[\alpha]_D^{20}$ −14° (*c* 0.5 in Me_2NCHO) [Found: C, 60.25; H, 6.65; N, 14.1. $C_{40}H_{52}N_8O_9 \cdot 0.5H_2O$ requires C, 60.21; H, 6.70; N, 14.04%. Found after acid hydrolysis: Lys + Ala(3 Pm), 2.03; Ala, 3.0].

N^{α}-*Acetyl*-L-*alanyl*-L-*alanyl*-L-*alanyl*-L-*lysine* (**13**).—N^{α}-Acetyl-L-alanyl-L-alanyl-L-alanyl-N^{ε}-benzyloxycarbonyl-L-lysine 4-picolyl ester (**12**) in 80% aqueous acetic acid was hydrogenolysed over palladium on charcoal (5%) during 20 h. The filtered solution was evaporated and the residue was applied in 5% acetic acid to a Sephadex G10 column, with 5% acetic acid as eluant. The *acetyl tetrapeptide* (**13**) *acetate* was recovered in 89% yield, R_f 0.10 (J) and 0.19 (H), $[\alpha]_D^{20}$ −61° (*c* 0.35 in acetic acid) (Found: C, 47.35; H, 7.85; N, 14.55. $C_{17}H_{31}N_5O_6 \cdot MeCO_2H \cdot H_2O$ requires C, 47.59; H, 7.78; N, 14.6%).

Appendix

The inhibition assays were carried out as follows. The inhibitor solution (100 μl) in dimethyl sulphoxide and the enzyme solution (20 μl) were added to the assay buffer (10 mM phosphate, 500 mM sodium chloride, pH 7.6; 10% Me_2SO, by vol.; 2.78 ml) contained in a 3 ml cuvette and the mixture was brought to 25 °C in the cell compartment of a Cary 210 spectrophotometer. The reaction was initiated by injection of 100 μl of substrate in dimethyl sulphoxide solution. The progress of the reaction was measured by monitoring at 410 nm the release of 4-nitroaniline from succinyl-Ala-Ala-Ala-*p*-nitroanilide (for porcine pancreatic elastase) or from *O*-methyl-succinyl-Ala-Ala-Pro-Val-*p*-nitroanilide (for human leucocytic

elastase). Absorbances were measured continuously and were then digitised and stored in an Apple II mini-computer. Inhibitor concentrations were varied between 5×10^{-6}M and 5×10^{-4}M. Initial velocities were calculated by a fit of the experimental data to a linear dependence on time by linear least squares. Apparent inhibition constants $(K_i)_{app}$ were determined from Dixon plots and the true inhibition constant K_i was obtained from the equation:

$$K_i = (K_i)_{app}/(1 + [S]/Km)$$

where [S] is the substrate concentration and K_m is the Michaelis constant. For porcine pancreatic elastase, [S] = Km/10 = 0.76 mM and for human leucocytic elastase [S] = Km = 0.16 mM.

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