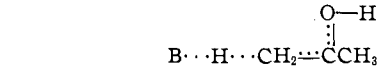
A classic example of a reaction whose rate follows eq. 21 is the enolization of acetone catalyzed by acetic acid and by acetate ion. Swain and co-workers[24-26] have presented convincing arguments that the acetic acid-catalyzed enolization proceeds through a prior equilibrium protonation of the ketonic oxygen followed by a rate-determining abstraction of a proton from carbon by acetate ion, and that the acetate ion-catalyzed enolization involves a similar proton abstraction by acetate ion assisted by hydrogen bonding of water to the oxygen. The hydrogen ion-catalyzed and uncatalyzed rates are attributable to analogous mechanisms with water taking the place of acetate as nucleophile.[24]

The transition states for the enolizations catalyzed by acetic acid or by hydrogen ion thus have the structure III, where B is, respectively, acetate ion or water.



III

The rate data of Bell and Jones[27] may be used to obtain values of $pK_a^{\ddagger}$ for the ionization of the proton from oxygen in these transition states. The values obtained are 4.8 when B is water and 4.0 when it is acetate ion.

According to the rule of Swain and Thornton,[1] changing B from water to acetate should lengthen the B···H bond and shorten the carbon–oxygen bond in the acetone moiety. This latter shortening should decrease the basicity of the oxygen as is observed. This observation provides a verification of the Swain–Thornton rule which is independent of isotope effect arguments and measures the effect of changing the base on the orbital covering the enol proper instead of the effect on the orbital utilized in the B···H bond (which is measured by the α-hydrogen isotope effect).

The 0.8 pK unit shift in the acidity of the proton which accompanies the change in nucleophile is of some interest as a quantitative measure of the deviation from the assumption[24] that the reactivity of the electrophile remains constant when the nucleophile is changed. This shift corresponds to a change of 1.1 kcal./mole in the free energy of the proton–oxygen bond in the transition state. This difference could produce a change in rate of a factor of six, which is small compared to the factor of more than 10^4 calculated[24] for the nucleophilicity of acetate relative to water using the assumption that the reactivity of any electrophile remains constant.

(24) C. G. Swain, *J. Am. Chem. Soc.*, **72**, 4578 (1950).
(25) C. G. Swain, E. C. Stivers, J. F. Reuwer, Jr., and L. J. Schaad, *ibid.*, **80**, 5885 (1958).
(26) C. G. Swain, A. J. DiMilo and J. P. Cordner, *ibid.*, **80**, 5983 (1958).
(27) R. P. Bell and P. Jones, *J. Chem. Soc.*, 88 (1953).

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, NEW BRUNSWICK, N. J.]

Glycine Analogs of Bradykinin[1]

BY MIKLOS BODANSZKY, JOHN T. SHEEHAN, MIGUEL A. ONDETTI AND SAUL LANDE

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The synthesis of three analogs of the nonapeptide bradykinin is described: 6-glycine bradykinin, 7-glycine bradykinin and L-arginyl-heptaglycyl-L-arginine. The preparation of the decapeptide, 7-glycine kallidin, an analog of 1-L-lysyl bradykinin, is also reported. Biological activities of these peptides are tabulated.

For a study of the relationship between structure and activity of the nonapeptide bradykinin[2] some analogs were prepared, in which glycine replaced one or more of the amino acids in the sequence of the naturally occurring peptide.

The initial structure proposed[3] for bradykinin was that of a linear octapeptide in which the proline residue in position 7 of the nonapeptide structure was omitted. When this octapeptide was synthesized[4] it was found to be devoid of activity. The question whether the amino acid in position 7 had to be proline or whether the latter could be replaced by another amino acid, without loss in activity, still remained open. 7-Glycine bradykinin (XII) was prepared in an effort to explore this question. The second analog, 6-glycine bradykinin (VI), wherein serine is replaced by glycine, was synthesized in order to ascertain to what degree the serine side chain contributed to biological activity. This latter analog was also converted into 1-L-lysyl-6-glycine bradykinin (7-glycine kallidin) (XXII), the glycine analog of the biologically active decapeptide kallidin,[5] by adding a lysine residue to the N-terminal arginine. Finally, a glycine analog embodying two characteristic features of bradykinin, namely, the terminal arginine residues separated by a peptide chain of seven amino acid residues, L-arginyl-heptaglycyl-L-arginine (XX) was prepared on the premise that these features might represent the minimum structural requirements for biological activity.

The schemes for the synthesis of 6-glycine- and 7-glycine bradykinin are shown in Fig. 1 and 2, respectively. These schemes differ, of course, in that they require two different protected C-terminal pentapeptide moieties III and IX, respectively, but follow parallel courses from there on. Thus the protected C-terminal pentapeptide ester, methyl benzyloxycarbonyl-L-phenylalanylglycyl-L-propyl-L-phenylalanylnitro-L-argininate (III), required for 6-glycine bradykinin was obtained in crystalline form and in good yield by allowing the *p*-nitrophenyl ester of the protected tripeptide benzyloxycarbonyl-L-phenylalanylglycyl-L-proline (II) to react with the dipeptide ester methyl-L-phenylalanyl-nitro-L-argininate. The *p*-nitrophenyl ester of II was prepared by coupling benzyloxycarbonyl-L-phenylalanine-*p*-nitrophenyl ester with glycyl-L-proline in aqueous pyridine followed by esterification with *p*-nitrophenol.

The protected C-terminal pentapeptide of 7-glycine bradykinin, methyl benzyloxycarbonyl-L-phenylalanyl-L-serylglycyl-L-phenylalanyl-nitro-L-arginine (IX), was secured by coupling the protected dipeptide benzyl-

(1) Some of the compounds described here were first discussed at The New York Academy of Sciences, Conference on Structure and Function of Biologically Active Peptides: Bradykinin, Kallidin and Congeners, March 22–24, 1962.
(2) (a) M. Rocha e Silva, W. T. Beraldo and G. Rosenfeld, *Am. J. Physiol.*, **156**, 261 (1949); (b) E. Werle, *Angew. Chem.*, **73**, 689 (1961).
(3) D. F. Elliott, G. P. Lewis and E. W. Horton, *Biochem. J.*, **76**, 16 (1960).
(4) (a) R. A. Boissonas, St. Guttmann, P. A. Jaquenoud, H. Konzett and E. Stürmer, *Experientia*, **16**, 326 (1960); (b) R. Schwyzer, W. Rittel, P. Sieber, H. Kappeler and H. Zuber, *Helv. Chim. Acta*, **43**, 1130 (1960); (c) E. D. Nicolaides, H. A. DeWald, P. G. Shorley and H. O. J. Collier, *Nature*, **187**, 773 (1960).
(5) J. V. Pierce and M. E. Webster, *Biochem. Biophys. Res. Commun.*, **5**, 353 (1961).

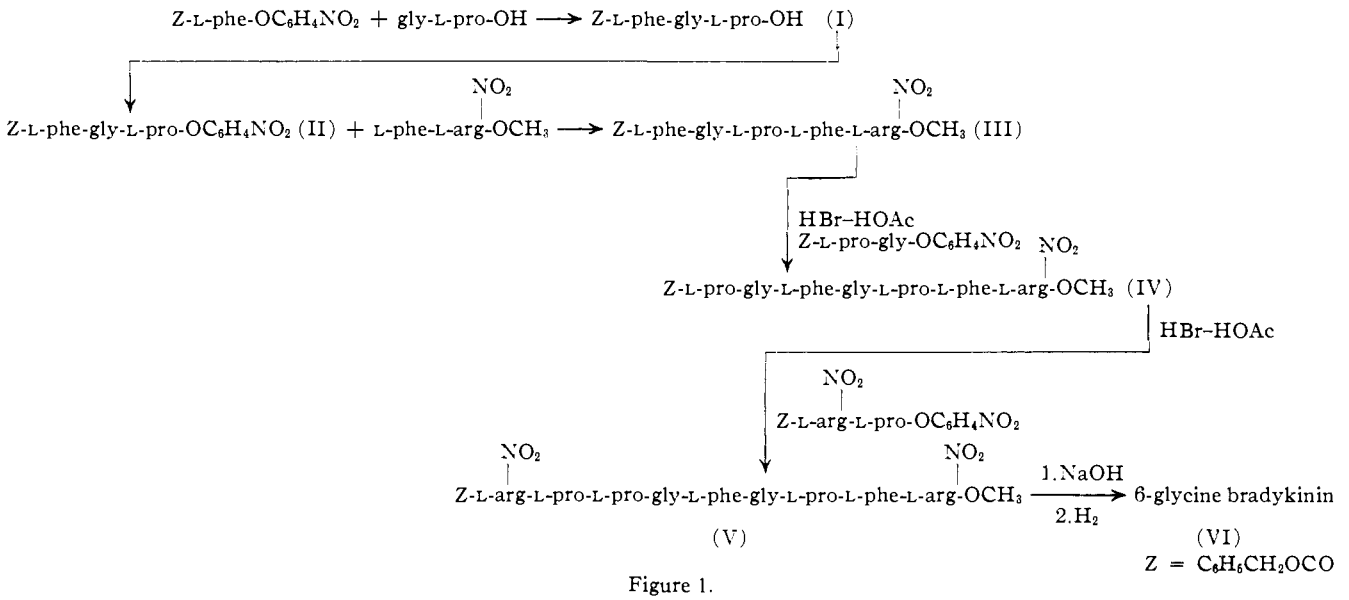


Figure 1.

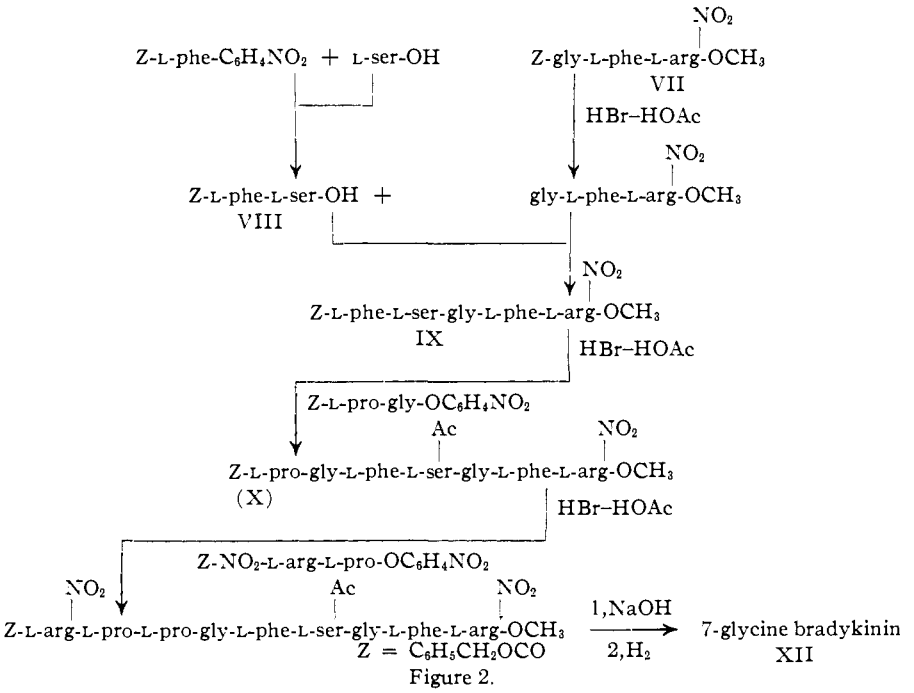


Figure 2.

oxycarbonyl-L-phenylalanyl-L-serine (VIII) with the tripeptide derivative methyl glycyl-L-phenylalanyl-nitro-L-argininate. N-Ethyl 5-phenylisoxazolium-3′-sulfonate was used as the coupling agent[6] in this step because of the presence of the unprotected alcoholic hydroxyl group. In all other cases, the nitrophenyl ester method[7] was used for the formation of peptide bonds. The tripeptide partner for the above coupling reaction was prepared from the dipeptide L-phenylalanyl-nitro-L-arginine methyl ester and the nitrophenyl ester of benzyloxycarbonylglycine to give the amorphous protected tripeptide ester, methyl benzyloxycarbonyl-glycyl-L-phenylalanyl-nitro-L-argininate (VII), which was subsequently converted into the free tripeptide methyl ester by treatment with hydrogen bromide in acetic acid. The dipeptide partner benzyloxy-carbonyl-L-phenylalanyl-L-serine (VIII) was readily obtained from benzyloxycarbonyl-L-phenylalanyl-*p*-nitrophenyl ester and L-serine in aqueous pyridine.

The two foregoing protected C-terminal pentapeptide esters, after the removal of the benzyloxycarbonyl group,[8] were in each case lengthened successively with the *p*-nitrophenyl esters of benzyloxycarbonyl-L-prolyl-glycine and N^{α}-benzyloxycarbonyl-nitro-L-arginyl-L-proline to yield the protected nonapeptide esters methyl N-α-benzyloxycarbonyl-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-nitro-L-argininate (V) and methyl N^{α}-benzyloxycarbonylnitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-serylglycyl-L-phenylalanyl-nitro-L-argininate (XI), respectively.

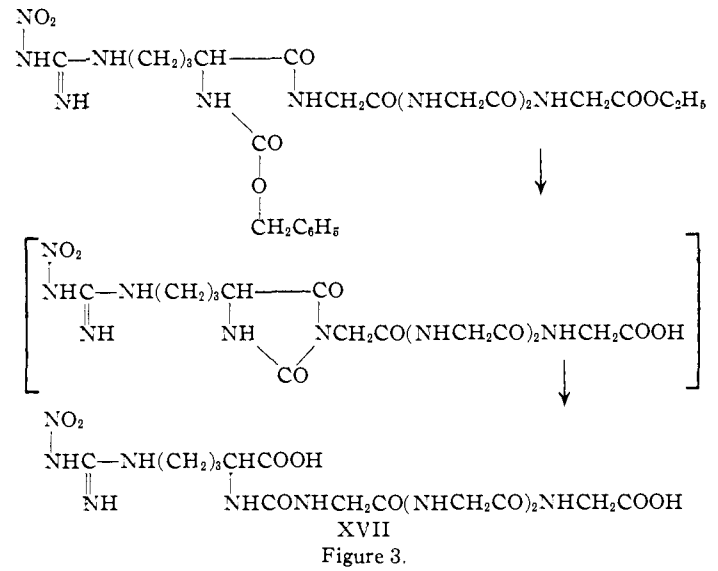
The free nonapeptides were obtained from the above fully protected compounds by an alkaline hydrolysis of the ester groups[9] followed by catalytic hydrogenation

(6) R. B. Woodward, R. A. Olofson and H. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).

(7) M. Bodanszky, *Nature*, **175**, 685 (1955). The yields with the *p*-nitrophenyl ester method in the syntheses reported in the present paper are generally lower than those observed earlier, *e.g.*, in the stepwise synthesis of oxytocin (M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959)), but no attempts were made this time to establish optimal conditions.

(8) In the course of this removal of the benzyloxycarbonyl group, the hydroxyl group of the serine in the C-terminal pentapeptide of 7-glycine bradykinin is acetylated by the reagent.

(9) The protected nonapeptide acid XIII of the 6-glycine bradykinin was obtained in crystalline form.



XVII

Figure 3.

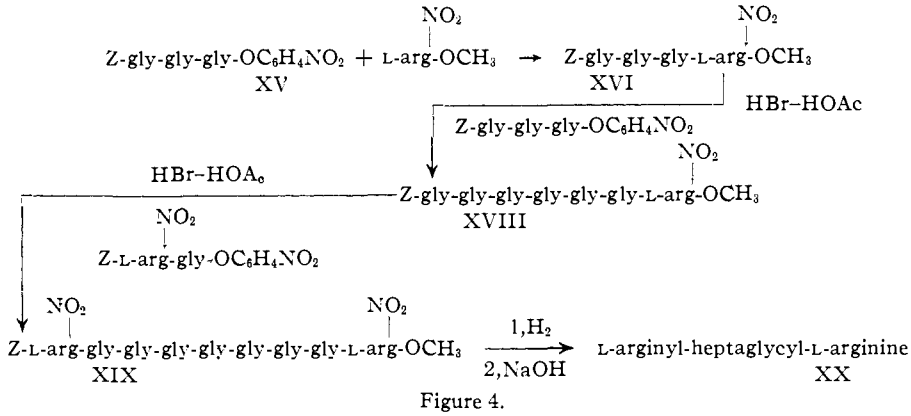


Figure 4.

to remove the benzyloxycarbonyl and nitro protecting groups. The kinin analog 6-glycine bradykinin (VI) was isolated by lyophilization from an aqueous solution. Both paper chromatography and paper electrophoresis indicated that the substance was homogeneous and quantitative amino acid analysis gave the expected ratio of the amino acids present. For the isolation of 7-glycine bradykinin (XII) purification over a carboxymethylcellulose column was required. Elution with ammonium acetate solution furnished a product that was homogeneous by the criteria listed above for 6-glycine bradykinin.

The synthesis of L-arginyl-heptaglycyl-L-arginine (XX) was to be achieved by coupling N^{α}-benzyloxycarbonyl-nitro-L-arginyltetraglycine with the tetrapeptide ester, methyl triglycyl-nitro-L-argininate, using the carbodiimide method.[10] The above protected tetrapeptide ester was readily obtained in excellent yield when benzyloxycarbonyl triglycine *p*-nitrophenyl ester (XV) was allowed to react with methyl nitro-L-argininate and the product in turn converted into the free tetrapeptide ester with hydrogen bromide in acetic acid. The protected N-terminal pentapeptide ester ethyl N^{α}-benzyloxycarbonyl-nitro-L-arginyltriglycyl glycinate (XIV) was prepared by coupling benzyloxycarbonyl-nitro-L-arginylglycine *p*-nitrophenyl ester to ethyl diglycylglycinate. When this protected pentapeptide ester was treated with dilute alkali there was formed, instead of the desired protected pentapeptide acid, a dibasic acid the infrared spectrum of which no longer showed absorption characteristic of the phenyl group and the elemental analysis of which favored the dibasic acid of structure XVII (Fig. 3). Evidently, cyclization with loss of benzyl alcohol, followed by hydrolytic opening of the hydantoin intermediate as shown in Fig. 3, had occurred in addition to the desired hydrolysis reaction. Such rearrangements are not unknown[11] and have been shown to occur during alkaline treatment of peptide esters, in which glycine is adjacent to the benzyloxycarbonyl N-terminal residue.

The original approach having failed, an alternative route to the heptaglycine bradykinin analog was chosen (Fig. 4). The C-terminal tetrapeptide ester XVI prepared above was acylated with benzyloxycarbonyl triglycine *p*-nitrophenyl ester (XV). After the removal of the benzyloxycarbonyl protecting group the heptapeptide ester methyl hexaglycyl-nitro-L-argininate (XVIII) was obtained. This was in turn condensed with benzyloxycarbonyl-nitro-L-arginylglycine *p*-nitrophenyl ester to form the fully protected desired nonapeptide XIX in pure form. To avoid the formation of the undesirable hydantoin intermediate, the hydrogenolytic removal of the benzyloxycarbonyl (and nitro) groups was allowed to precede the ester hydrolysis step. The free nonapeptide XX was purified by chromatography on a carboxymethylcellulose column and was obtained in homogeneous form as indicated by paper chromatograms and paper electrophoresis. Quantitative amino acid analysis gave a ratio of glycine to arginine of 3.4:1.

The bradykinin-like activity of these glycine analogs on the isolated rat uterus[12] (Table I) indicates that

(10) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(11) (a) J. A. Maclaren, *Austral. J. Chem.*, **11**, 360 (1958); (b) F. Wessely and E. Kemm, *Z. physiol. Chem.*, **174**, 306 (1928), and **180**, 64 (1929).

(12) Pharmacology Department Manual, Emory University, Georgia, 1951, p. 39.

TABLE I

		Relative biological activity[12]
Bradykinin	L-arg-L-pro-L-pro - gly-L-phe-L-ser-L-pro-L-phe-L-arg	1
7-Glycine bradykinin	L-arg-L-pro-L-pro - gly-L-phe-L-ser - gly-L-phe-L-arg	1/100
6-Glycine bradykinin	L-arg-L-pro-L-pro - gly-L-phe - gly-L-pro-L-phe-L-arg	1
L-Arginyl-heptaglycyl-L-arginine	L-arg - gly - gly - gly - gly - gly - gly - gly-L-arg	0
7-Glycine kallidin	L-lys-L-arg-L-pro-L-pro - gly-L-phe - gly-L-pro-L-phe-L-arg	1/4–1
	1 2 3 4 5 6 7 8 9 10	

alteration of the structure of the natural kinin can lead to substantial loss in activity. Replacement of the proline residue in the 7-position by glycine reduces the activity a hundredfold. On the other hand, the fact that some activity is found in this analog shows that a length of nine amino acids in the peptide chain *per se* does play a role. This does not mean, however, that length of the peptide chain alone with terminal arginine residues is sufficient to impart activity to a nonapeptide as can be seen by the complete lack of activity of the L-arginyl-heptaglycyl-L-arginine (XX) analog.

Finally, the replacement of serine by glycine provides a bradykinin analog with activity very nearly equal to that of the natural kinin, demonstrating that not all the structural features of the natural peptide are necessary for activity: *e.g.*, the hydroxyl group of serine is not required.

The high order of activity shown by 6-glycine bradykinin (VI) prompted us to prepare the analogous decapeptide derivative 7-glycine kallidin (XXII). For its preparation the protected nonapeptide ester of 6-glycine bradykinin (V) was treated with hydrogen bromide in acetic acid to remove the benzyloxycarbonyl group and the resulting peptide ester was acylated with bis-(benzyloxycarbonyl)-L-lysine-*p*-nitrophenyl ester. The ester group was removed from the protected decapeptide derivative by alkaline hydrolysis to give a crystalline protected decapeptide acid (XXI) which in turn was hydrogenated and the free decapeptide L-lysyl-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-L-arginine (XXII) isolated as a homogeneous material.

Experimental

All melting points are uncorrected.

The amino acid analyses reported herein were performed after hydrolysis of the peptides in constant boiling hydrochloric acid in evacuated sealed ampoules at 110° for 16 hours. The Stein–Moore procedure of quantitative amino acid analysis, as modified by Technicon, Inc., Chauncey, N. Y., was used.

Benzyloxycarbonyl-L-phenylalanylglycyl-L-proline (I).—A solution of 9.3 g. of glycyl-L-proline[13] in 160 ml. of a pyridine–water mixture (1:1) was brought to *p*H 8.9 by the addtion of 5 *N* sodium hydroxide. While stirring at room temperature, alkali (5 *N* sodium hydroxide) and portions of 21 g. of benzyloxycarbonyl phenylalanine nitrophenyl ester[14] were alternately added to maintain *p*H 8.5. The addition was complete in 1 hour after which the mixture was stirred for an additional hour at room temperature. To this solution 25 g. of sodium bicarbonate and 125 ml. of water were added and the whole extracted ten times with 50-ml. portions of ethyl acetate. From the combined ethyl acetate extract an aqueous layer separated which was drawn off and added to the main aqueous layer. This aqueous solution was acidified with 6 *N* hydrochloric acid (congo paper). An oil separated which quickly granulated. Filtered, washed with water and air-dried, it weighed 20.5 g. (90%) and melted at 205–206°. One gram of material crystallized from 75 ml. of boiling ethanol gave 0.7 g., m.p. 212°, $[\alpha]^{20}D$ −62.6° (*c* 1.0, dimethylformamide).

Anal. Calcd. for $C_{24}H_{27}N_3O_6$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.51; H, 6.19; N, 9.52.

Benzyloxycarbonyl-L-phenylalanylglycyl-L-proline Nitrophenyl Ester (II).—A solution of 15.5 g. of benzyloxycarbonyl-L-phenylalanylglycyl-L-proline and 5.3 g. of *p*-nitrophenol in 150 ml. of dimethylformamide was cooled to 0° in an ice–water bath and, while stirring, 7.2 g. of dicyclohexylcarbodiimide was added. The mixture was stirred at 0° for 2 hours and for an additional 3 hours at room temperature. After the addition of 0.5 ml. of glacial acetic acid the dicyclohexylurea was filtered off and washed with a small amount of ethyl acetate. The filtrate and washings were combined and added with stirring to 1.5 liters of cold water. A semi-solid mass separated. The supernatant liquor was decanted and the residue taken up in 200 ml. of ethyl acetate and dried over anhydrous magnesium sulfate. The desiccant was filtered off and the solvent evaporated *in vacuo* leaving a glassy residue. This was repeatedly treated with ether, the solvent evaporated and this repeated until a solid, ether-insoluble material was obtained. This (17 g.) was dissolved in 100 ml. of warm ethanol containing 1% acetic acid and filtered. On standing at room temperature, 5 g. of crystalline product separated. An additional 3.5 g. (total yield 44%) was obtained on long standing in the cold (−5°). This ester softens at 65° and melts at 85–90°, $[\alpha]^{26}D$ −87.5° (*c* 1, dimethylformamide containing 1% acetic acid).

Anal. Calcd. for $C_{30}H_{30}N_4O_8$: C, 62.71; H, 5.26; N, 9.75. Found: C, 62.72; H 5.14; N, 9.78.

Methyl Benzyloxycarbonyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanylnitro-L-argininate (III).—A solution of hydrobromic acid in acetic acid (36%; 36 ml.) was added to a suspension of methyl benzyloxycarbonyl-L-phenylalanylnitro-L-argininate[15] (5.5 g.) in acetic acid (40 ml.) and freeze-dried after 1 hour standing at room temperature. The solid residue was dissolved in dimethylformamide (14 ml.), benzyloxycarbonyl-L-phenylalanylglycyl-L-proline *p*-nitrophenyl ester (II) (5.75 g.) was added and the mixture was made alkaline with tributylamine (4.5 ml.) and triethylamine (1 ml.). After 2.5 days at room temperature the reaction mixture was diluted with ethyl acetate, washed once with *N* hydrochloric acid, once with water, six times with *N* ammonium hydroxide and four times with water. The solution was dried over magnesium sulfate and the solvent removed *in vacuo*. The residue was crystallized from ethyl acetate. The protected pentapeptide ester (5.95 g., 66%) melts at 114–116°, $[\alpha]^{22}D$ −57.7° (*c* 1.1, dimethylformamide). Recrystallization from methanol raises the m.p. to 185–186° without significant changes in the rotation and analysis.

Anal. Calcd. for $C_{40}H_{39}N_9O_{10}$: C, 58.87; H, 6.05; N, 15.45. Found: C, 58.67; H, 6.27; N, 15.01.

Methyl Benzyloxycarbonyl-L-prolylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanylnitro-L-argininate (IV).—The benzyloxycarbonyl group was removed from compound III (4.88 g.) and the resulting ester hydrobromide was allowed to react with benzyloxycarbonyl-L-prolyl glycine *p*-nitrophenyl ester[16] (2.84 g.) in the presence of tributylamine (2.8 ml.) and triethylamine (1 ml.). After 2.5 days the reaction mixture was treated as described for III. The residue after evaporation of the ethyl acetate was dissolved in methanol and precipitated with ether. The protected heptapeptide ester (4.8 g., 82%) melts at about 126–134°, $[\alpha]^{20}D$ −54.5° (*c* 1.0, dimethylformamide).

Anal. Calcd. for $C_{47}H_{59}N_{11}O_{12}$: C, 58.20; H, 6.13; N, 15.88. Found: C, 58.11; H, 6.19; N, 15.64.

Methyl N^{α}-Benzyloxycarbonylnitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-glycyl-L-prolyl-L-phenylalanylnitro-L-argininate (V).—The benzyloxycarbonyl group was removed from compound IV (3.7 g.) as described above. The solid residue obtained after freeze-drying was dissolved in dimethylformamide (7.5 ml.), N-α-benzyloxycarbonylnitro-L-arginyl-L-proline *p*-nitrophenyl ester (2.2 g.),[16] tributylamine (2.5 ml.) and triethylamine (0.4 ml.) were added and the solution kept at room temperature for 4.5 days. The reaction mixture was diluted with ethyl acetate–acetonitrile (2:1), washed once with normal hydro-

(13) Benzyloxycarbonylglycyl-L-proline was obtained by coupling *p*-nitrophenyl benzyloxycarbonylglycinate ("Biochemical Preparations," Vol. 9, John Wiley and Sons, Inc., New York, N. Y., 1962, p. 110) and L-proline in aqueous pyridine (1:1) at *p*H 8.8. The product was hydrogenolyzed to give glycyl-L-proline with the same properties as described by M. Bergmann, L. Zervas, H. Schleich and F. Leinert, *Z. physiol. Chem.*, **212**, 72 (1932), and H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.*, 3642 (1956).

(14) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 6072 (1959).

(15) K. Hofmann, W. D. Peckham and A. J. Rheiner, *ibid.*, **78**, 238 (1956).

(16) M. Ondetti, *J. Med. Chem.*, **6**, 10 (1963).

chloric acid, three times with water and dried over magnesium sulfate. After removing the solvent *in vacuo* the oily residue was triturated with ethyl acetate when it turned into a solid (2.75 g., 56%, m.p. 115–135°). A small sample was dissolved in methanol and precipitated with ethyl acetate (m.p. 135–150°, softening at 120°); $[\alpha]^{20}D$ −57.3 (*c* 1.0, dimethylformamide).

Anal. Calcd. for $C_{58}H_{77}N_{17}O_{16}$: C, 54.92; H, 6.12; N, 18.77. Found: C, 54.72; H, 6.31; N, 18.73.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-L-arginine (VI).—To a solution of compound V (130 mg.) in methanol (2 ml.) and water (0.3 ml.) a solution of 2 *N* sodium hydroxide (0.15 ml.) was added. After 2 hours at room temperature, water (1 ml.) was added. The solution was left for 15 minutes at room temperature and then another milliliter of water was added. After a total of 2.5 hours the solution was acidified with *N* hydrochloric acid (congo reaction) and kept overnight in the refrigerator. The supernatant was decanted and the residue treated with hot methanol when it became crystalline and insoluble. The mixture was cooled, filtered and the product, N^{α}-benzyloxycarbonylnitro-L-arginyl-L-prolyl-L-prolyl-glycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanylnitro-L-arginine (XIII), was washed with methanol (83.4 mg., m.p. 155–170°, sintering at 150°). Recrystallization from methanol–water gave 72 mg., m.p. 165–180°, sintering at 160°. This partially protected nonapeptide was dissolved in a mixture of acetic acid–water (2:1, 12 ml.) and hydrogenated at normal pressure for 48 hours using 5% Pd-on-barium sulfate as a catalyst. After removal of the catalyst by filtration the solvents were removed *in vacuo*, the residue dissolved in water and freeze-dried. The free nonapeptide (73 mg., $[\alpha]^{27}D$ −86.0° [*c* 1.0, *N* acetic acid]) is homogeneous on paper chromatograms (butanol–acetic acid–water, 4:1:5, R_f 0.15; butanol–pyridine–water, 5:5:2, R_f 0.44; isoamyl alcohol–pyridine–water, 3.5:3.5:3.0, R_f 0.12) using the ninhydrin and Sakaguchi reagents. It was also seen to be homogeneous in paper electrophoresis (using pyridine acetate buffer *p*H 4 and ammonium citrate buffer *p*H 5.3) after development with the above-mentioned reagents. The quantitative amino acid analysis gave the following ratio of amino acids: proline:glycine:phenylalanine:arginine, 2.9:2.0:2.0:2.0.

Methyl Benzyloxycarbonylglycyl-L-phenylalanylnitro-L-argininate (VII).—Methyl benzyloxycarbonyl-L-phenylalanylnitro-L-argininate (8.24 g.)[15] was treated with hydrobromic acid in acetic acid. The resulting hydrobromide was dissolved in dimethylformamide (24 ml.) together with benzyloxycarbonylglycine *p*-nitrophenyl ester (5.7 g.) and the solution made alkaline with a mixture of tributylamine (8 ml.) and triethylamine (1 ml.). After 6 days at room temperature the mixture was diluted with ethyl acetate, washed twice with *N* hydrochloric acid and finally with water. The organic layer was dried and concentrated to dryness and the oily residue triturated with ether until it became solid. For purification this solid was dissolved in *hot* acetonitrile, and precipitated with ether. The oil thus obtained was dissolved in hot ethyl acetate, and hexane was added until slight turbidity. On cooling, the product precipitated as an amorphous solid (6.1 g., 66%) of m.p. 110–112° (flows at 116°), $[\alpha]^{20}D$ −9.0° (*c* 2.09, dimethylformamide).

Anal. Calcd. for $C_{26}H_{33}N_7O_8$: C, 54.63; H, 5.82; N, 17.15. Found: C, 54.95; H, 5.94; N, 17.38.

Benzyloxycarbonyl-L-phenylalanyl-L-serine (VIII).—To a solution of L-serine (1.57 g.) in a mixture of water (25 ml.) and pyridine (25 ml.) benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester[14] (6.3 g.) was added in small portions while the *p*H was kept constant at 8.7 by addition of 4 *N* sodium hydroxide (in total 5.5 ml.). After 16 hours the clear yellow solution obtained was diluted with water (to 150 ml.), saturated with sodium bicarbonate and washed several times with ethyl acetate. The aqueous layer was acidified and extracted four times with ethyl acetate. The organic phase was washed once with water, dried over magnesium sulfate and concentrated to dryness. The crystalline residue was suspended in a mixture of ethyl acetate and ether (1:4), filtered and washed with ether; 3.8 g. (65%), m.p. 155–156° (sint. 154°), $[\alpha]^{21}D$ −2.6° (*c* 1.0, dimethylformamide).

Anal. Calcd. for $C_{20}H_{22}N_2O_6$: C, 62.16; H, 5.74; N, 7.25. Found: C, 62.04; H, 5.58; N, 7.25.

Methyl Benzyloxycarbonyl-L-phenylalanyl-L-serylglycyl-L-phenylalanylnitro-L-argininate (IX).—To a suspension of benzyloxycarbonyl-L-phenylalanyl-L-serine (VIII) (386 mg.) and N-ethyl-5-phenylisoxazolium-3′-sulfonate (253 mg.) in acetonitrile (4 ml.), triethylamine (0.18 ml.) was added and the mixture stirred in an ice–water-bath until a clear solution was obtained (2 hours). The solution of the activated dipeptide acid was added to that of methyl glycyl-L-phenylalanyl-nitro-L-argininate hydrobromide (obtained from 571 mg. of VII by hydrobromic acid–acetic acid treatment) in a mixture of dimethylformamide (1 ml.) and triethylamine (0.2 ml.). The reaction mixture was stirred overnight at room temperature, when a considerable amount of precipitate formed. The suspension was diluted with ethyl acetate and the product filtered, washed with ethyl acetate and ether and finally suspended in water, filtered again and washed with water. For further purification it was dissolved in hot acetonitrile–water (9:1) where on cooling it separated as an amorphous solid; 410 mg. (50%), m.p. 146–148°, sintering at 130°, $[\alpha]^{21}D$ −12.6° (*c* 2.0, dimethylformamide).

Anal. Calcd. for $C_{38}H_{47}N_9O_{11}$: C, 56.65; H, 5.88; N, 15.63; O-acetyl, 0.0. Found: C, 56.51; H, 5.97; N, 15.30; O-acetyl, 0.0.

Methyl Benzyloxycarbonyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-serylglycyl-L-phenylalanylnitro-L-argininate (X).—To a solution of compound IX (405 mg.) in acetic acid (3 ml.) a solution of hydrobromic acid in acetic acid (3 ml.) was added. After 2.5 hours at room temperature ether was added and the solid pale yellow precipitate which formed was washed several times with fresh ether, and finally dried *in vacuo* over sodium hydroxide. The solid residue was dissolved in dimethylformamide (1.5 ml.) together with benzyloxycarbonyl-L-prolylglycine *p*-nitrophenyl ester[16] (260 mg.) and the solution made alkaline with a mixture of tributylamine (0.24 ml.) and triethylamine (0.05 ml.). After 6 days at room temperature the mixture was diluted with an ethyl acetate–acetonitrile mixture (2:1) and the organic phase washed once with 0.2 *N* hydrochloric acid and twice with water. On evaporation to dryness an oily residue was obtained which upon treatment with fresh ethyl acetate turned into a solid; 210 mg. (42%), m.p. 168–170° sintering from 160°, $[\alpha]^{21}D$ −27.8° (*c* 1.0, dimethylformamide).

Anal. Calcd. for $C_{47}H_{59}N_{11}O_{14}$: C, 56.33; H, 5.93; N, 15.38; O-acetyl, 4.30. Found: C, 56.10; H, 6.21; N, 15.13; O-acetyl, 3.74.

Methyl N^{α}-Benzyloxycarbonylnitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-serylglycyl-L-phenylalanylnitro-L-argininate (XI).—The benzyloxycarbonyl group of the protected heptapeptide ester X (1 g.) was removed with hydrobromic acid in acetic acid and the resulting peptide hydrobromide was allowed to react with benzyloxycarbonylnitro-L-arginyl-L-proline *p*-nitrophenyl ester[16] (0.63 g.) in the usual way. After 5 days at room temperature the partially gelled mixture was diluted with acetonitrile–ethyl acetate (1:1) and the fine precipitate thus obtained was filtered, washed with ethyl acetate, resuspended in ethanol, filtered again, washed with ethanol and dried (710 mg., 54%, m.p. 140–160°; dried *in vacuo* at 110°, m.p. 155–170° with softening from 140°). A small portion (50 mg.) was dissolved in hot acetonitrile, filtered from the small insoluble residue and the filtrate concentrated until it started to form a precipitate and finally cooled, filtered and dried: 25 mg., m.p. 155–170° sintering at 140°, $[\alpha]^{21}D$ −27.9° (*c* 1.0, dimethylformamide).

Anal. Calcd. for $C_{58}H_{77}N_{17}O_{18}$: C, 53.58; H, 5.97; N, 18.31. Calcd. for $C_{58}H_{77}N_{17}O_{18}\cdot 3H_2O$: C, 51.44; H, 6.17; N, 17.58. Found: C, 51.49; H, 6.17; N, 16.91.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-serylglycyl-L-phenylalanyl-L-arginine (XII).—The protected nonapeptide methyl ester XI (131 mg.) was dissolved in boiling methanol (1.5 ml.), cooled down quickly to room temperature and *N* sodium hydroxide (0.3 ml.) was added. After 45 minutes at room temperature the solution was diluted with water (1.5 ml.) and acidified (congo paper) with *N* hydrochloric acid. The oily suspension was kept overnight in the refrigerator, centrifuged, the supernate decanted and the residue dried *in vacuo* over sodium hydroxide. This protected nonapeptide acid (79 mg.) was dissolved in a mixture of acetic acid–water (1:1) and hydrogenated at ordinary pressure in the presence of 5% palladium-on-barium sulfate (80 mg.). The catalyst was removed by filtration and the filtrate freeze-dried. This residue was applied to a column of carboxymethyl cellulose (4 g.) and eluted with a linear gradient of ammonium acetate (from 0.035 *M* to 0.075 *M*). Following the elution by measuring the absorption at 230 mμ the tubes from the main peak were pooled and freeze-dried three times to remove the ammonium acetate. A white powder (40 mg., $[\alpha]^{21}D$ −55.8° [*c* 1.0, 1 *N* acetic acid]) was finally obtained that was homogeneous in paper chromatography (butanol–acetic acid–water, 4:1:5, R_f 0.49; isoamyl alcohol–pyridine–water, 3.5:3.5:3, R_f 0.11) and paper electrophoresis (pyridine acetate buffer *p*H 4, ammonium citrate buffer *p*H 5.3) with the Sakaguchi and ninhydrin developing reagents. The quantitative amino acid analysis gave the following ratio of amino acids: serine:proline:glycine:phenylalanine:arginine, 0.8:2.0:2.0:1.9:2.1.

Benzyloxycarbonylglycylglycine *p*-Nitrophenyl Ester.—A solution of 26.6 g. (0.1 mole) of benzyloxycarbonylglycylglycine[17] in 200 ml. of dimethylformamide was esterified with *p*-nitrophenol and dicyclohexylcarbodiimide reagent as described by Bodanszky and du Vigneaud.[18] After the removal of the dicyclohexylurea the filtrate was diluted with 1.5 l. of cold water and the solid material which separates was filtered off and washed with water. On standing in the air it shrinks to a hard solid which was crystallized from 1.5 l. of ethanol containing 1% of acetic acid. The

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pure product, 28 g. (75%), melted at 164–165° (lit. m.p.[19] 163–165°).

Anal. Calcd. for $C_{18}H_{17}N_3O_7$: C, 55.81; H, 4.42; N, 10.85. Found: C, 55.95; H, 4.49; N, 10.65.

Ethyl Benzyloxycarbonyldiglycylglycinate.—Ethyl glycinate hydrochloride (0.7 g.), benzyloxycarbonyl glycylglycine *p*-nitrophenyl ester (1.94 g.) and tri-*n*-butylamine (1.2 ml.) were dissolved in pyridine (5 ml.) and left at room temperature for 48 hours. The mixture was triturated with methanol and after filtration an equal volume of water was added to the filtrate. The combined precipitates were crystallized from ethyl acetate; yield 1.25 g. (75%), m.p. 167–169° (lit.[20] m.p. 166–167°).

Ethyl Diglycylglycinate Hydrobromide.—Ethyl benzyloxycarbonyl diglycylglycinate (1.25 g.) was dissolved in a saturated solution of hydrobromic acid in acetic acid (10 ml.), and after 1 hour at room temperature ether was added. The precipitate was washed with ether and acetonitrile, desiccated over potassium hydroxide pellets and crystallized from ethanol; yield 0.8 g. (75%), m.p. 191–194°, (lit.[21] m.p. 191°).

***p*-Nitrophenyl N-Benzyloxycarbonylnitro-L-arginylglycinate.**—N^{α}-Benzyloxycarbonylnitro-L-arginylglycine[15] (3.0 g.) and *p*-nitrophenol (1.05 g.) were dissolved in tetrahydrofuran (20 ml.). The solution was cooled to 0° and dicyclohexylcarbodiimide (1.55 g.) was added. The suspension was stored at room temperature for 6 hr., cooled and filtered, and the filtrate was evaporated *in vacuo*. The oily residue was triturated with ether and recrystallized twice from methanol; yield 2.5 g. (63%), m.p. 98–102°, $[\alpha]^{26}D$ −9.5° (*c* 2, dimethylformamide containing 1% acetic acid) (lit.[22] m.p. 90–95°; $[\alpha]^{25}D$ −4.8° [*c* 1.68, dimethylformamide]).

Anal. Calcd. for $C_{22}H_{25}N_7O_9$: C, 49.7; H, 4.7; N, 18.5. Found: C, 50.0; H, 4.9; N, 18.2.

Ethyl N^{α}-Benzyloxycarbonylnitro-L-arginyltriglycylglycinate (XIV).—Ethyl diglycylglycinate hydrobromide (1.6 g.) was dissolved in pyridine (5 ml.) containing tributylamine (0.25 ml.), and *p*-nitrophenyl N^{α}-benzyloxycarbonylnitro-L-arginylglycinate (2.8 g.) was added. After 72 hours at room temperature, the mixture was triturated with ethyl acetate and the precipitate was crystallized three times from methanol–acetonitrile (1:1); yield 2.4 g. (72%), m.p. 174–178°, $[\alpha]^{22}D$ −3.4° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{24}H_{35}N_9O_{10}$: C, 47.3; H, 5.8; N, 20.7. Found: C, 47.5; H, 6.0; N, 20.4.

Treatment of Ethyl N^{α}-Benzyloxycarbonylnitro-L-arginyltriglycylglycinate with Alkali.—Ethyl N^{α}-benzyloxycarbonylnitro-L-arginyltriglycylglycinate (2.0 g.) was suspended in *N* sodium hydroxide (5.0 ml.) (solution was complete within minutes) and left at room temperature for 1 hour. After acidification to congo red with 4 *N* hydrochloric acid, the solution was stored at 4° to induce crystallization. The product, 5-[1-carboxy-4-(3-nitroguanidino)-butyl]-hydantoyltriglycine (XVII), was recrystallized from water; yield 0.9 g., m.p. 125–127° (milky soln., decomposition starts at 150°), $[\alpha]^{23}D$ −5.5° (*c* 1.1, dimethylformamide). Infrared absorption spectroscopy showed no absorbance in the region of 700 $cm.^{-1}$, indicating the absence of phenyl groups.

Anal. Calcd. for $C_{22}H_{31}N_9O_{10}$: C, 45.4; H, 5.4; N, 21.7; neut. equiv., 581. Found: C, 37.2; H, 6.4; N, 25.0; neut. equiv., 280. Calcd. for $C_{15}H_{25}N_9O_{10}$: C, 36.7; H, 5.1; N, 25.7; neut. equiv., 246. Found: C, 37.2; H, 6.4; N, 25.0; neut. equiv., 280.

Benzyloxycarbonyltriglycine *p*-Nitrophenyl Ester (XV).—Benzyloxycarbonyltriglycine was esterified with *p*-nitrophenol in the usual way.[18] The product crystallized from boiling ethanol melted at 218–220°.

Anal. Calcd. for $C_{20}H_{20}N_4O_8$: C, 54.05; H, 4.54; N, 12.61. Found: C, 54.19; H, 4.57; N, 12.51.

Methyl Benzyloxycarbonyltriglycylnitro-L-argininate (XVI).—A suspension of benzyloxycarbonyltriglycine *p*-nitrophenyl ester (XV) (0.88 g.) and nitro-L-arginine methyl ester hydrochloride (0.67 g.) in a mixture of dimethylformamide (6 ml.) and tributylamine (0.77 ml.) was stirred until a clear yellow solution was obtained and then kept overnight at room temperature. This reaction mixture was diluted with a mixture of ethyl acetate–acetonitrile (1:1) and washed once with *N* hydrochloric acid and three times with water. The organic phase was dried and concentrated to dryness leaving an oily residue that became solid on treatment with ether. This amorphous solid was crystallized from 95% ethanol to yield 330 mg., m.p. 147–149° (softening at 145°). On standing at room temperature the aqueous phase from the washings mentioned before deposited 480 mg. of a crystalline product with the same melting point, 147–149° (soft. at 145°). Both crops were pooled and recrystallized twice, once from acetonitrile–water (9:1) and once from methanol–water (9:1); 630 mg. (59%), m.p. 148–150° (soft. at 146°), $[\alpha]^{21}D$ −5.1° (*c* 2.0, dimethylformamide).

Anal. Calcd. for $C_{21}H_{30}N_8O_9$: C, 46.84; H, 5.61; N, 20.81. Found: C, 46.55; H, 5.77; N, 20.83.

Methyl Benzyloxycarbonylhexaglycylnitro-L-argininate (XVIII).—Methyl benzyloxycarbonyltriglycylnitro-L-argininate (XVI) (3.0 g.) was dissolved in a saturated solution of hydrobromic acid in acetic acid (15 ml.). After 1 hour at room temperature, ether was added and the precipitate that formed was washed thoroughly with ether and dried over potassium hydroxide pellets. The methyl triglycylnitro-L-argininate hydrobromide (1 g.), prepared as just described, was dissolved in dimethylformamide (20 ml.) containing triethylamine (0.95 ml.). *p*-Nitrophenyl benzyloxycarbonyl-diglycylglycinate (XV) (0.84 g.) was added and the mixture was warmed to 70–80° to redissolve the gel that formed almost immediately. After a few hours at room temperature, heat was again applied to dissolve the gel. After 3 days at room temperature, the mixture was triturated with methanol and the precipitate was crystallized from water; yield 0.85 g. (61%), m.p. 231–234°, $[\alpha]^{23}D$ −4.2° (*c* 1.1, dimethyl sulfoxide).

Anal. Calcd. for $C_{27}H_{39}N_{11}O_{12}$: C, 45.7; H, 5.54; N, 21.7. Found: C, 46.1; H, 6.45; N, 20.8.

Methyl N^{α}-Benzyloxycarbonylnitro-L-arginylheptaglycylnitro-L-argininate (XIX).—Methyl benzyloxycarbonylhexaglycylnitro-L-argininate (XVIII) (0.35 g.) was suspended in a saturated solution of hydrobromic acid in acetic acid (2 ml.), warmed to effect solution, and stored at room temperature. After a total of 1.5 hours exposure to hydrobromic acid, ether was added and the precipitate was washed with ether and desiccated over potassium hydroxide pellets. The methyl hexaglycylnitro-L-argininate hydrobromide formed was dissolved in dimethylformamide (4 ml.). Addition of triethylamine (0.25 ml.) caused the formation of a gelatinous precipitate. *p*-Nitrophenyl benzyloxycarbonylnitro-L-arginylglycinate (0.21 g.) was added and the mixture was warmed to 70–80° until a clear solution was formed. The solution, which slowly jelled, was stored at room temperature for 5 days, triturated with methanol and the precipitate that formed was extracted with boiling methanol; yield of insoluble residue, 0.34 g. (89%), m.p. 195–200° dec., $[\alpha]^{22}D$ −3.1° (*c* 1.1, dimethyl sulfoxide).

Anal. Calcd. for $C_{35}H_{53}N_{17}O_{16}$: C, 43.4; H, 5.52; N, 24.6; OCH_3, 3.20. Found: C, 43.2; H, 6.46; N, 24.9; OCH_3, 3.19.

L-Arginylheptaglycyl-L-arginine (XX).—Methyl benzyloxycarbonylnitro-L-arginylheptaglycylnitro-L-argininate (150 mg.) was suspended in 50% aqueous acetic acid (5 ml.) and hydrogenated over a palladium catalyst for 48 hours. Fresh catalyst was added after 8 and 24 hours. After filtration and lyophilization, the residue was dissolved in water (1.5 ml.) and *N* sodium hydroxide (0.4 ml.) was added (sufficient to maintain *p*H at approximately 10). After 1 hour at room temperature the solution was neutralized with acetic acid and fractionated by ion exchange chromatography on carboxymethylcellulose (3 g.) using a gradient elution technique (0.01 to 0.10 *M* ammonium acetate, *p*H 6.5); the eluate was collected in 10-ml. fractions. Sakaguchi positive material was observed to be present in tubes 5–15 and 40–60. The contents of the latter fraction were pooled, evaporated *in vacuo* to remove the bulk of the solvent and lyophilized three times; yield 50 mg., $[\alpha]^{22}D$ −11.8° (*c* 1.1, *N* acetic acid), R_f −0.06 (butanol–acetic acid–water, 4:1:5), single spot, ninhydrin and Sakaguchi positive; paper electrophoresis (pyridine acetate buffer *p*H 4.5) showed a single component, ninhydrin and Sakaguchi positive, which moved toward the cathode. Quantitative amino acid analysis gave a ratio of glycine:arginine of 3.4:1.0.

Methyl N^{α},N^{ω}-Bis-(benzyloxycarbonyl)-L-lysylnitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanylnitro-L-argininate (XXI).—The benzyloxycarbonyl group was removed from the protected nonapeptide ester V (635 mg.) and the resulting hydrobromide was allowed to react (5 days) with N^{α},N^{ω}-bis-(benzyloxycarbonyl)-L-lysine *p*-nitrophenyl ester in the way described for IV. The reaction mixture was diluted with ethyl acetate–acetonitrile (3:1), washed with 0.5 *N* hydrochloric acid and water and after drying over magnesium sulfate it was concentrated to dryness *in vacuo*. The oily residue was triturated with ethyl acetate where it readily solidified. The product filtered off, washed and dried gave 450 mg. (59%) of XXI, m.p. 135–150° (sintering at 120°), $[\alpha]^{21}D$ −64.8° (*c* 1.1, dimethylformamide).

Anal. Calcd. for $C_{72}H_{95}N_{19}O_9$: C, 56.49; H, 6.26; N, 17.39. Found: C, 56.68; H, 6.48; N, 17.33.

L-Lysyl-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-L-arginine (XXII).—To a solution of the above-protected decapeptide methyl ester (147 mg.) in methanol (3 ml.), *N* sodium hydroxide (0.3 ml.) was added. After 1.5

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hours at room temperature, water (5.5 ml.) was added and the clear solution thus obtained was acidified with N hydrochloric acid (congo red). The cloudy suspension was kept overnight in the refrigerator, and then it was centrifuged, the supernatant was decanted and the residue dried *in vacuo*. The amorphous powder (157 mg.) was dissolved in hot methanol from which it crystallized after standing in the refrigerator for 2 days. The crystalline protected decapeptide acid thus obtained (90 mg., m.p. 165–170°, sintering at 160°) was hydrogenated for 48 hours at normal pressure in a 1:1 mixture of acetic acid and water (20 ml.) in the presence of 10% palladium-on-charcoal (three portions of 100 mg. of catalyst were added at equal intervals during the reduction). After removal of the catalyst by filtration the solution was freeze-dried. The free decapeptide (50 mg.), $[\alpha]^{22}D$ −70.7° (c 0.99, N acetic acid), was homogeneous by paper chromatography (butanol–acetic acid–water, 4:1:5, R_f 0.50) and by paper electrophoresis (pyridine acetate buffer pH 4.6 and triethanolammonium acetate buffer pH 6.3) when developed with ninhydrin and Sakaguchi reagents. The quantitative amino acid analysis gave the ratios: lysine:arginine:proline:glycine:phenylalanine, 1.0:2.0:3.4:2.0:1.8.

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[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND NEUROLOGY, COLUMBIA UNIVERSITY, NEW YORK 32, N. Y.]

Sulfonyl Fluorides as Inhibitors of Esterases. I. Rates of Reaction with Acetylcholinesterase, α-Chymotrypsin, and Trypsin[1a]

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The second-order rate constants for inactivation of acetylcholinesterase, α-chymotrypsin, and trypsin by a series of sulfonyl fluorides have been determined. These compounds are most reactive toward chymotrypsin, but the same order of relative reactivities holds for both chymotrypsin and trypsin: phenylmethane- > 2-phenylethane-1- > benzene- > 2-methylpropane-1- > methanesulfonyl fluoride. This order, except for methanesulfonyl fluoride, is rationalized on the basis of steric and polar factors: the data can be fitted to the general Taft relationship. Methanesulfonyl fluoride, however, is about one-ten thousandth as reactive toward chymotrypsin as would be predicted. With acetylcholinesterase only the methane and benzene derivatives react at measurable rates, and limits are set upon the values of the rate constants for the other compounds. A hypothesis compatible with the data is that a reversible complex between the sulfonyl fluoride and enzyme forms before sulfonylation. At present, the evidence that binding markedly increases reactivity is suggestive, but not conclusive.

Introduction

Twenty four years ago Schrader[2] reported that methanesulfonyl fluoride is a potent insecticide. Recently this finding was extended by Myers and Kemp,[3] who found that methanesulfonyl fluoride has a lethal dose for rats comparable to that of diisopropyl phosphorofluoridate (DFP). In these studies it was observed that the symptoms of acute poisoning are similar to those produced by toxic alkyl phosphates. Moreover, they demonstrated that methanesulfonyl fluoride is an inhibitor of rat-brain cholinesterase *in vitro* and suggested that inhibition occurs by sulfonylation of a group in the active site of the enzyme. A year later Hartley and co-workers[4] were able to show that 1-dimethylaminonaphthalene-5-sulfonyl chloride can sulfonylate the active site of chymotrypsin.

Although ever since these investigations the potentiality of sulfonyl fluorides as reagents for characterization of functional groups within the active site was recognized and this information passed on through reviews,[5] no new studies have come to light concerning inhibition of hydrolytic enzymes by these reagents. The work presented here consists primarily of comparative rate studies of a series of sulfonyl fluorides with acetylcholinesterase, α-chymotrypsin, and trypsin. These studies were undertaken in order to lay the groundwork for a chemical investigation of the mechanism of inhibition.

Experimental

Diisopropyl phosphorofluoridate was purchased from Aldrich Chemical Co. Methane-, 2-methylpropane-1-, benzene-, phenylmethane-, and 2-phenylethane-1-sulfonyl fluorides were prepared from the corresponding chlorides by the general method of Tullock and Coffman.[6] Two sulfonyl fluorides are reported here for the first time.

2-Methylpropane-1-sulfonyl Fluoride.—2-Methylpropane-1-sulfonyl chloride[7] (8.4 g., 0.05 mole) was added to a stirred suspension of powdered sodium fluoride (10.2 g., 0.25 mole) in 6 ml. of tetramethylenesulfone. The mixture was heated at 110° for 3 hours, then cooled. After filtration the product was distilled; b.p. 47° (12 mm.), 5.06 g., 72%.

Anal.[8] Calcd. for $C_4H_9FO_2S$: C, 34.27; H, 6.47; S, 22.88. Found: C, 34.46; H, 6.72; S, 23.19.

2-Phenylethane-1-sulfonyl Fluoride.—2-Phenylethane-1-sulfonyl chloride[7] (10.25 g., 0.05 mole), NaF (10.2 g., 0.25 mole), and 10 ml. of dimethylformamide were stirred for 4 hours at 110°. Forty ml. of water and 25 ml. of methylene chloride were added; the organic layer was washed with water, dried, and the solvent evaporated. The residue was dissolved in 5 ml. of chloroform and 10 ml. of petroleum ether (30–60°), treated with charcoal, and filtered. The product crystallized on cooling and was recrystallized twice from chloroform–petroleum ether; 5.83 g., 62%, m.p. 26.5–27°.

Anal. Calcd. for $C_8H_9FO_2S$: C, 51.05; H, 4.82; S, 17.04. Found: C, 51.05; H, 5.23; S, 17.10.

Phenylmethanesulfonyl fluoride, m.p. 91–92°, reported[9] 90–91°; benzenesulfonyl fluoride, b.p. 80.0° (9 mm.), saponification equivalent 80.2 (theor. 80.1); and methanesulfonyl fluoride, b.p. 123°, reported[9] 124°, sapon. equiv. 49.4 (theor. 49.0). The latter two compounds were homogeneous by vapor phase chromatography.

Enzymes.—A purified preparation of acetylcholinesterase from the electric organ of *Electrophorus electricus* was used.[10] It had an activity of 1.4 mmoles of substrate hydrolyzed per min. per mg. of protein when assayed as described below. Twice recrystallized α-chymotrypsin and three times recrystallized trypsin were purchased from Worthington Biochemical Corporation.

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