SYNTHESIS OF DEPSIPEPTIDE ANALOGS OF BRADYKININ

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In recent years, several research groups have synthesized a number of analogs of bradykinin (I) in which the amino acid residues in various parts of the peptide chain have been varied [1].

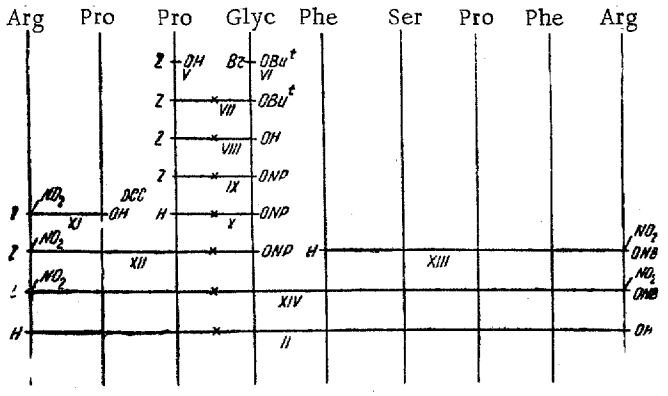
Continuing investigations in the field of depsipeptide analogs of biologically active peptides [2, 3], we have undertaken the synthesis of analogs of bradykinin in which some amino acid residues have been replaced by residues of the hydroxy acids corresponding to them.

As the first subject of synthesis we selected 4-glycolylbradykinin (II) in which the glycine has been replaced by glycolic acid. It was also of interest to carry out the synthesis of 6-glycolylbradykinin (III), since it follows from Bodanszky's [4] and Schröder's [5] results that the replacement of the serine located in the molecule of bradykinin in position 6 by glycine does not appreciably reduce the biological activity of the peptide. In addition, to elucidate the question of the influence of an accumulation of ester bonds in the molecule on the biological activity of bradykinin, we have synthesized 4, 6-diglycolylbradykinin (IV).

Arg	*Pro*	*Pro*	*Gly*	*Phe*	***Ser***	*Pro*	*Phe*	*Arg*,*	(I)
1	2	3	4	5	6	7	8	9	
Arg	*Pro*	*Pro*	*Glyc*	*Phe*	*Ser*	*Pro*	*Phe*	*Arg*,	(II)
Arg	*Pro*	*Pro*	*Gly*	*Phe*	*Glyc*	*Pro*	*Phe*	*Arg*,	(III)
Arg	*Pro*	*Pro*	*Glyc*	*Phe*	*Glyc*	*Pro*	*Phe*	*Arg*.	(IV)

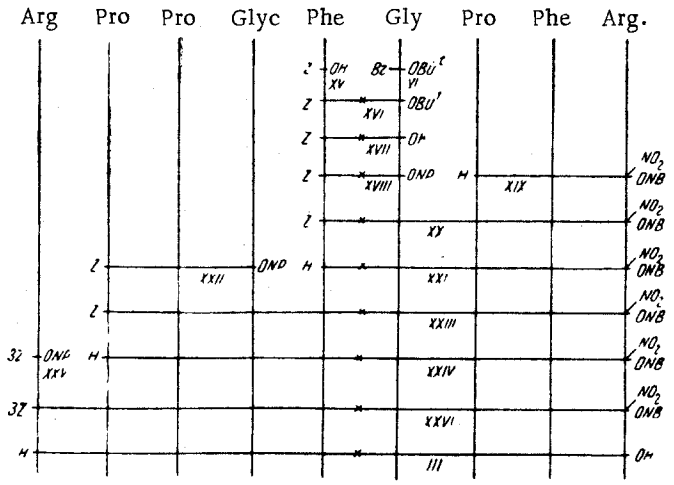
The synthesis of the depsipeptide analogs of bradykinin (II)–(IV) was effected by schemes 1–3 drawn up with a view to the possibility of using the peptide fragments (XIII), (XIX), and (XXII) that we obtained previously in the synthesis of bradykinin [6].

As can be seen from schemes 1–3, the p-nitrophenyl ester method was used to add the depsipeptide fragment (XII) to the peptides (XIII) and (XXI), and the depsipeptide (XVIII) to the peptide (XIX). This synthetic route proved to be particularly advantageous for the preparation of the nonadepsipeptide (XIV), since preliminary experiments showed that the addition of the depsipeptide (VIII) to the pentapeptide (III) by the acid chloride method or the mixed anhydride method leads to an unresolvable mixture of substances the formation of which is evidently connected with the presence of the hydroxy group of serine in the peptide (XIII).



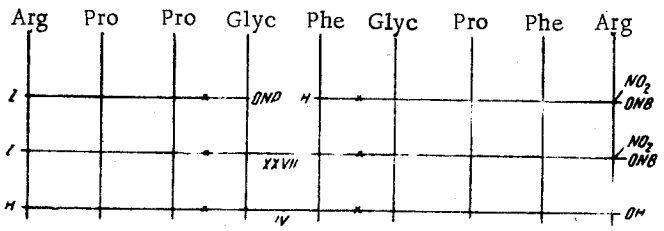
* The symbols for the amino acids and their derivatives are in agreement with the decision of the 5th European Symposium, Oxford, September, 1962. In addition, TEA = triethylamine, DMF = dimethylformamide, DCC = dicyclohexylcarbodiimide, and $-\times-=$ an ester bond.

In the synthesis of the intermediate depsipeptide fragments it was found that the free bases of some depsipeptides are very unstable. Thus, in the isolation of the free base of depsipeptide (X), the complete loss of proline took place almost simultaneously.



The same feature was found with the heptadepsipeptide Z. Pro Glyc Phe Ser Pro Phe Arg (NO_2) ONB (XXVIII) (see the Experimental section). The decarbobenzoxylation of this peptide with hydrogen bromide in glacial acetic acid gave a stable hydrobromide, but in the subsequent isolation of the free base, the N-terminal proline was lost completely. At the same time, the base of the depsipeptide (XXI) formed by treating the corresponding hydrobromide with sodium hydrogen carbonate proved to be a completely stable compound. It was also found that the ester bond between proline and glycolic acid is split only when the fragment Pro-Glyc is present at the N-end of the depsipeptide, while the depsipeptide analogs of bradykinin (II) and (IV), in which this fragment is remote from the N-end of the depsipiptide, are substances completely stable in aqueous solutions.

Biological tests of the depsipeptide analogs of bradykinin (II)–(IV) (table) have shown that all three compounds retain the characteristic action of bradykinin, although their activities are from one to four orders of magnitude lower.



These results permit the conclusion that a continuous peptide chain is not an indispensable condition for the appearance of the specific biological activity of bradykinin.

Biological Activity of Depsipeptide Analogs of Bradykinin

Substance	Threshold doses		
	Contraction of the horn of the rat uterus, g/ml bath	Increase in the permeability of the capillaries of the skin g/ml	reduction of the blood pressure in the rat, g/kg
Bradykinin [6]	$1 \cdot 10^{-9} - 1 \cdot 10^{-11}$	$1 \cdot 10^{-9}$	$4 \cdot 10^{-7}$
4-Glycolybradykinin	$1 \cdot 10^{-6} - 2 \cdot 10^{-7}$	$1.5 \cdot 10^{-6}$	$0.55 \cdot 10^{-3}$
6-Glycolybradykinin	$5 \cdot 10^{-10} - 5 \cdot 10^{-11}$	$2.5 \cdot 10^{-9}$	$3.4 \cdot 10^{-6}$
4, 6-Diglycolybradykinin	$1 \cdot 10^{-5} - 3.2 \cdot 10^{-6}$	$2.5 \cdot 10^{-5}$	$2 \cdot 10^{-3}$

Experimental

The purity of all the substances was checked by chromatography on Leningrad paper, type B in the systems butan-1-ol–water–acetic acid (4: 5: 1) $R_{f(1)}$ and isoamyl alcohol – pyridine – water (35 : 35 : 30) ($R_{f(2)}$). The spots were revealed with ninhydrin, chlorine-potassium iodide, and Sakaguchi's reagent.

The solutions, previously dried with sodium sulfate, were evaporated in vacuum. The substances for analysis were dried in vacuum (0.1–0.5 mm) at ~ 60° C. The melting points of the substances were determined on a "Boethius" stage.

p-Nitrophenyl ester of carbobenzoxy-L-prolylglycolic acid (IX). A mixture of 4.98 g of carbobenzoxy-L-proline (V) [7], 4.1 g of tert-butyl bromoacetate (VI) [8], and 2.02 g of TEA in 50 ml of ethyl acetate was boiled for 2 hr. The reaction mixture was washed with water and with 4% sodium hydrogen carbonate solution and was evaporated to give 6 g (82%) of the tert-butyl ester of carbobenzoxy-L-prolylglycolic acid (VII) in the form of a light yellow oil giving a single spot on chromatography [loose Al_2O_3 in the benzene – ethyl acetate (8 : 2) system].

A volume of 20 ml of CF_3COOH was added to 5.7 g of substance (VII), and after 15 min, the solution was evaporated in vacuum at 20°–25° C; the residue was dissolved in ether, the solution was washed with water, and extracted with 8% sodium hydrogen carbonate solution, and the extract was acidified with hydrochloric acid.

The oil which separated out was extracted with ether, and evaporation of the solvent gave 4.16 g (86%) of substance (VIII).

Found, %: mol. wt. 303 (titration). $C_{15}H_{17}O_6N$. Calculated: mol. wt. 307.

At 0° C, 6.0 g of DCC was added to a solution of 7.8 g of (VIII) and 3.6 g of p-nitrophenol in 20 ml of ethyl acetate, and the mixture was left for 18 hr at 20° C. The precipitate was separated off and the solution was evaporated in vacuum, after which the residue was dissolved in ether and left for 48 hr at 5° C. This gave 7.7 g (70%) of crystalline substance (IX) with mp 75° C, $[\alpha]_D^{21}$ – 86.7° (c 1; DMF), $R_{f(1)}$ 0.90, $R_{f(2)}$ 0.95.

Found, %: C 58.90; H 5.57; N 6.54. Calculated for $C_{21}H_{20}N_2O_8$, %: C 58.87; H 4.73; N 6.56.

p-Nitrophenyl ester of L-prolylglycolic acid (X). A solution of 1 g of compound (IX) in 5 ml of glacial acetic acid was treated with 5 ml of a 4 N solution of hydrogen bromide in glacial acetic acid. The mixture was kept for 30 min at 20° C and 100 ml of ether was added. The precipitate which formed was washed with ether. The yield of the hydrobromide of the depsipeptide (X) was 0.8 g (84%), $R_{f(1)}$ 0.95, $R_{f(2)}$ 0.93.

Found, %: Br 22.9. Calculated for $C_{13}H_{14}O_6N_3 \cdot HBr$, %: Br 23.04.

p-Nitrophenyl ester of carbobenzoxynitro-L-arginyl-Lprolyl-L-prolylglycolic acid (XII). At – 5° C, 0.2 ml of TEA and a solution of 0.42 g of DCC in 0.5 ml of ethyl acetate were added to a solution of 0.68 g of the hydrobromide (X) and 0.81 g of substance (XI) [9] in 10 ml of DMF. The reaction mixture was left for 3 hr at – 5° C and for 17 hr at 20° C, the dicyclohexylurea was filtered off, the filtrate was treated with 50 ml of ethyl acetate, and the solution was washed with water and 1 N hydrochloric acid. The residue after evaporation was triturated with ether. This gave 0.6 g of an amorphous substance. It was established chromatographically that it contained neither the initial ester (X) nor p-nitrophenol. The content of the tetrapeptide (XII) in the material obtained was found by a spectrophotometric determination of the p-nitrophenol produced by the alkaline hydrolysis of a sample, and amounted to 91%. Yield of substance (XII) 50%, $R_{f(1)}$ 0.94, $R_{f(2)}$ 0.92.

p-Nitrobenzyl ester of L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine (XIII). 0.45 g of the hydrobromide of the pentapeptide (XIII) [6] was dissolved at – 5° C in a mixture of 1.5 ml of methanol, 10 ml of ethyl acetate, and 10 ml of sodium hydrogen carbonate solution; the organic layer was separated off and the aqueous fraction was extracted with 20 ml of ethyl acetate containing 1 ml of methanol. The combined ethyl acetate solutions were dried and evaporated in vacuum. The yield of amorphous pentapeptide (XIII) was 0.41 g (97%), $R_{f(1)}$ 0.90, $R_{f(2)}$ 0.85.

p-Nitrobenzyl ester of carbobenzoxynitro-L-arginyl-L-prolyl-L-prolyl-glycolyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine (XIV). A solution of 0.3 g of (XII) and 0.4 g of (XIII) in 5 ml of DMF was left for 5 days at 22°–25° C. Then 100 ml of ethyl acetate was added and the solution was washed with water and 1 N hydrochloric acid and was evaporated in vacuum. After recrystallization [from a mixture of ethyl acetate and ether (1 : 1)] the yield of protected nonadepsipeptide (XIV) was 0.54 g (79%); mp 132°–134° C, $[\alpha]_D^{21}$ – 41.7° (c 1; DMF), $R_{f(1)}$ 0.95, $R_{f(2)}$ 0.93.

Found, %: C 54.74; H 5.81; N 17.10. Calculated for $C_{65}H_{81}O_{20}N_{17}$, %: C 54.96; H 5.74; N 16.76.

L-Arginyl-L-prolyl-L-prolylglycolyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine (II). In solution in 15 ml of glacial acetic acid and 7 ml of methanol, 0.27 g of substance (XIV) was hydrogenated over palladium black for 48 hr at 25°–28° C, and the solution was filtered. The solvent was evaporated off and the residue was

triturated with ether. The resulting amorphous substance was dissolved in 10 ml of water and the solution was freeze-dried. The yield of the tetrahydrate of the nonadepsipeptide (II) was 0.20 g (90%), mp 181°–184° C, $[\alpha]_D^{21}$ −63.3° (c 1; H_2O), $R_{f(1)}$ 0.4; $R_{f(2)}$ 0.25.

Found, %: C 52.78; H 6.81; N 17.36; H_2O 5.9. Calculated for $C_{50}H_{22}N_{14}O_{12} \cdot 4H_2O$, %: C 53.00; H 6.94; N 17.32; H_2O 6.35.

p-Nitrophenyl ester of carbobenzoxy-L-phenylalanylglycolic acid (XVIII). This was obtained from carbobenzoxy-L-phenylalanine [7] and tert-butyl bromoacetate [8] in a similar manner to substance (IX). The over-all yield was 48%, mp 112°–113° C, $[\alpha]_D^{21}$ −45.8° (c 1; DMF), $R_{f(1)}$ 0.93; $R_{f(2)}$ 0.94.

Found, %: C 62.29; H 4.56; N 5.91. Calculated for $C_{25}H_{22}O_8N_2$, %: C 62.76; H 4.83; N 5.86.

p-Nitrobenzyl ester of carbobenzoxy-L-phenylalanylglycolyl-L-prolyl-L-phenylalanylnitro-L-arginine (XX). A solution of 1.24 g of (XVIII) and 1.6 g of (XIX [6] in 9 ml of DMF was kept for 4 days at 25° C and was then treated with 100 ml of ethyl acetate. The resulting solution was washed with 1 N hydrochloric acid and with water, and was dried and evaporated in vacuum. The residue was triturated with hot ether (5 × 10 ml) and was precipitated from 5 ml of ethyl acetate with 50 ml of ether. This gave 1.8 g (71%) of the pentadepsipeptide (XX) in the form of an amorphous powder with $[\alpha]_D^{25}$ −54.3° (c 0.75; DMF), $R_{f(1)}$ 0.92, $R_{f(2)}$ 0.94.

p-Nitrobenzyl ester of L-phenylalanylglycolyl-L-prolyl-L-phenylalanylnitro-L-arginine (XXI). A solution of 0.5 g of (XX) in 1 ml of glacial acetic acid was treated with 1 ml of a 4 N solution of hydrobromic acid in glacial acetic acid and was left for 40 min at 20° C; the solvent was eliminated in vacuum and the residue was repeatedly triturated with absolute ether until it had been converted into a powder. The hydrobromide of the pentadepsipeptide (XXI) so obtained was dissolved in a mixture of 1 ml of methanol, 10 ml of ethyl acetate, and 10 ml of 4% sodium hydrogen carbonate solution cooled to −5° C, and the amine (XXI) was isolated in a similar manner to compound (XIII). This yielded 0.39 g (90%) of the pentadepsipeptide (XXI), $R_{f(1)}$ 0.93, $R_{f(2)}$ 0.91.

p-Nitrobenzyl ester of carbobenzoxy-L-prolyl-L-prolylglycyl-L-phenylalanyl-glycolyl-L-prolyl-phenylalanyl-nitro-L-arginine (XXIII). A solution of 0.39 g of the amine (XXI) and 0.26 g of the p-nitrophenyl ester of carbobenzoxy-L-prolyl-L-prolylglycine (XXII) [6] in 3 ml of DMF was left for 5 days at 25° C. Then 50 ml of ethyl acetate was added to the solution and it was washed with water and with 1 N hydrochloric acid, dried, and evaporated in vacuum. The residue was triturated five times with 10 ml of boiling ether and was twice reprecipitated from 20 ml of ethyl acetate by the addition of 100 ml of ether. The yield of octadepsipeptide (XXIII) was 0.41 g (75%), mp 116°–123° C, $[\alpha]_D^{25}$ −54.4° (c 1; DMF), $R_{f(1)}$ 0.96, $R_{f(2)}$ 0.92.

Found, %: C 58.00; H 5.78; N 14.12. Calculated for $C_{58}H_{67}O_{16}N_{12}$, %: C 58.07; H 5.76; N 14.15.

p-Nitrobenzyl ester of L-prolyl-L-prolylglycyl-L-phenylalanyglycolyl-L-prolylnitro-L-arginine (XXIV). A solution of 0.5 g of (XXIII) in 1 ml of glacial acetic acid was treated with 1 ml of a 34% solution of hydrogen bromide in glacial acetic acid and was kept for 40 min at room temperature. The solvent was distilled off in vacuum and the residue was repeatedly triturated with ether. The resulting solid substance was dissolved in 1 ml of methanol, and 6 ml of ethyl acetate and 6 ml of 4% sodium hydrogen carbonate solution were added to 0° to 4°C. The ethyl acetate solution was washed, dried for 40 min at 5° C, and evaporated. This gave 0.39 g (88%) of the amino ester (XXIV) in the form of an amorphous powder, $R_{f(1)}$ 0.92, $R_{f(2)}$ 0.89.

p-Nitrobenzyl ester of tricarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycolyl-L-prolyl-L-phenyalanylnitro-L-arginine (XXVI). A solution of 0.39 g of (XXIV) and 0.25 g of the p-nitrophenyl ester of tricarbobenzoxy-L-arginine (XXV) [10] in 3 ml of DMF was left for 5 days at room temperature. Then 50 ml of ethyl acetate was added to the solution and it was washed with 1 N hydrochloric acid and with water, dried, and evaporated, and the residue was twice reprecipitated from ethyl acetate with ether. This gave 0.43 g (80%) of the protected nonadepsipeptide (XXVI) with mp 110°–113° C, $[\alpha]_D^{20}$ −47.3° (c 2, DMF), $R_{f(1)}$ 0.95, $R_{f(2)}$ 0.93.

Found, %: C 57.58; H 6.00; N 13.43. Calculated for $C_{82}H_{97}O_{21}N_{17} \cdot 3.5\ H_2O$, %: C 57.25; H 6.09; N 13.25.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanylglycolyl-L-prolyl-L-phenylalanyl-L-arginine (III). In solution in 6 ml of glacial acetic acid and 3 ml of methanol, 0.17 g of (XXIV) was hydrogenated over palladium black at 25°–28° C for 24 hr, and the solution was filtered. Then it was evaporated and the residue was triturated with ether until it formed a powder. The substance was dissolved in 5 ml of water. The solution was filtered and freeze-dried. The yield of the nonadepsipeptide (III) was 0.1 g (87%), mp 176°–180° C; $[\alpha]_D^{21}$ −72.5° (c 1; H_2O).

Found, %: C 51.83; H 7.28; N 17.05; H_2O 5.4; CH_3COOH 4.65. Calculated for $C_{49}H_{70}O_{11}N_{14} \cdot 3H_2O \cdot CH_3COOH$, %: C 51.34; H 7.04; N 17.12; H_2O 5.24; CH_3COOH 4.80.

p-Nitrobenzyl ester of carbobenzoxynitro-L-arginyl-L-prolyl-L-prolylglycolyl-L-phenylalanylglycolyl-L-prolyl-L-phenylalanylnitro-L-arginine (XXVII). A solution of 0.37 g of (XII) and 0.42 g of (XXI) in 4 ml of DMF was kept for 5 days at 22°–25° C; then 100 ml of ethyl acetate was added and the resulting solution was washed with water, 1 N hydrochloric acid, and water again, and was dried and evaporated in vacuum. The crystalline residue was triturated twice with 50 ml of boiling ether. From a mixture of ethyl acetate and ether, 0.5 g (76%) of the nonadepsipeptide (XXVII) was obtained with mp 128°–131° C, $[\alpha]_D^{20}$ – 45.0° (c 1; DMF), $R_{f(1)}$ 0.96, $R_{f(2)}$ 0.92.

Found, %: C 54.74; H 5.62; N 16.05. Calculated for $C_{64}H_{79}O_{21}N_{16}$, %: C 55.2; H 5.69; N 16.11.

L-Arginyl-L-prolyl-L-prolylglycolyl-L-phenylalanylglycolyl-L-prolyl-L-phenylalanyl-L-arginine (IV). In solution in 10 ml of glacial acetic acid and 5 ml of methanol, 0.16 g (XXVII) was hydrogenated over palladium black for 30 hr. Then the solution was filtered and evaporated in vacuum, and the residue was triturated with ether, dissolved in water, and freeze-dried. This gave 110 mg (84%) of the nonadepsipeptide (IV) in the form of the monoacetate, mp 182°–186° C, $[\alpha]_D^{20}$ – 44.7° (c 0.8; water), $R_{f(1)}$ 0.4, $R_{f(2)}$ 0.25.

Found, %: C 51.17; H 7.00; N 15.92; H_2O 4.95; CH_3COOH 5.32. Calculated for $C_{49}H_{69}O_{12}N_{13} \cdot 3H_2O \cdot CH_3COOH$, %: C 51.34; H 6.94; N 15.89; H_2O 4.72; CH_3COOH 5.23.

p-Nitrobenzyl ester of carbobenzoxy-L-prolylglycolyl-L phenylalanyl-L-seryl-L prolyl-L-phenylalanylnitro-L-arginine (XXVIII). A solution of 0.77 g of (XI) and 1.6 g of (XIII) [6] in 10 ml of DMF was left for 3 days at 25° C, after which 100 ml of ethyl acetate was added and the solution was washed with water, 1 N hydrochloric acid, a 4% solution of sodium hydrogen carbonate until the yellow coloration had disappeared, and with water again, and was dried and evaporated in vacuum. From ethyl acetate 1.7 g (79%) of (XXVIII) was obtained. Mp 126°–132° C, $R_{f(1)}$ 0.92, $R_{f(2)}$ 0.94.

Found, %: C 56.90; H 5.96; N 13.35; H_2O 1.55. Calculated for $C_{54}H_{63}O_{16}N_{11} \cdot H_2O$, %: C 56.84; H 5.74; N 13.55; H_2O 1.57.

The biological tests were carried out by T. S. Pashkina, M. S. Surovikina, and T. K. Egorova (Laboratory of Biologically Active Peptides of the Institute of Biological and Medicinal Chemistry, AMS USSR).

Summary

The syntheses of three depsipeptide analogs of bradykinin have been effected. The replacement of one or even two peptide linkages in the molecule of bradykinin by ester linkages does not affect the nature of its biological activity but only reduces it. These results show that a continuous peptide chain is not unconditionally necessary for the appearance of the specific biological activity of bradykinin.

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