USE OF tert-BUTOXYCARBONYL PROTECTION IN THE SYNTHESIS OF THE PEPTIDE CORRESPONDING TO SEQUENCE 17-19 OF ACTH

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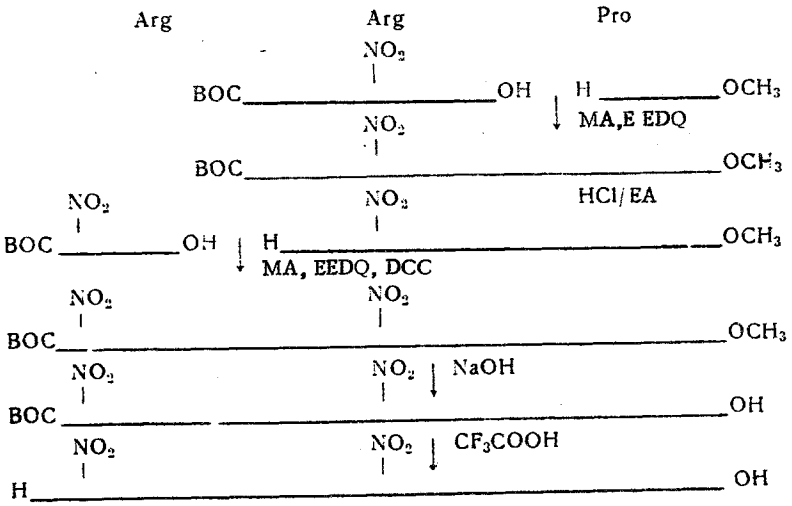
The paper gives the results of the synthesis of the tripeptide arginyl-arginyl-proline performed with the use of various condensing agents. N^{α}-tert-Butoxycarbonyl-N^{ω}-nitroarginine was used as the starting compound. Some physicochemical characteristics of the tripeptide derivatives (melting point, angle of optical rotation, elementary composition) are given. The ^{13}C NMR method was used to identify the structures of the compounds. The chemical shifts of the signals and their assignments are given.

In the scheme of synthesis of the tripeptide arginyl-arginyl-proline – fragment 17-19 of the sequence of adrenocorticotropic hormone (ACTH) – used by Kopelevich et al. [1] the peptide chain was built up from the N-terminal proline ester using the benzyloxycarbonyl group as temporary protection for the N^{α} atom of arginine [1]. At the same time, the tert-butoxycarbonyl group is finding ever wider use for the temporary protection of amino groups. This is also favored by the development in recent years of a convenient method for obtaining tert-butoxycarbonyl derivatives of amino acids with the aid of di-tert-butyl pyrocarbonate [2].

We have synthesized the above-mentioned tripeptide and another of its derivatives from N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginine* by the following scheme:



The first stage of the condensation of N^{ω}-tert-butoxycarbonyl-N^{ω}-nitroarginine with proline methyl ester was performed by the mixed anhydride (MA) method using ethyl chloroformate or 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in methylene chloride as condensing agent.

The high tendency of activated arginine to undergo lactamization and the, apparently, inadequate activity of the dipeptide arginylproline in the condensation reaction did not permit these condensing agents to be used effectively in the following condensation stage. The

*All the amino acids used in the work were the L forms.

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TABLE 1

Functional groups	Carbon atoms	NO_2 | BOC-Arg_1-ProOMe	NO_2 | Arg_1-ProOMe·HCl	NO_2 NO_2 | | BOC-Arg_1 Arg_2-ProO-OMe	NO_2 NO_2 | | BOC-Arg_1 Arg_2-ProO-OH	NO_2 NO_2 | | $HArg_1$ - Arg_2-ProOH·CF_3COOH
Pro	C=O	172,12	171,74	172,15	173,03	172,99
	C_α	53,45	53,85	53,52	58,63	53,65
	C_β	28,36	28,53	28,49	28,58	28,58
	C_γ	24,45	24,63	24,50	24,48	24,48
	C_δ	46,30	46,86	46,55	46,56	46,61
Arg_1	C=O	170,44	167,37	169,80	169,67	169,35
	C_α	51 59	50,48	49,85	49,82	50,50
	C_δ	24,45	23,59	24,27	24,26	24,48(23,48)
	C_γ	28,10	27,12	28,49	28 58	28,44
	C_δ	40,33	40,17	40,32	40,33	40,33
	C_ε	159,42	159,53	159,44	159,50	159,50
Arg_2	C=O			171,90	171,90	168,32
	C_α			54,13	54,17	51,89
	C_β			24,98	24,98	23,98(24,48)
	C_γ			29,17	29,15	28,19
	C_δ			40,32	40,33	40,03
	C_ε			159,44	159,50	159,50
BOC	C=O	155,09		155,20	155,27	
	$-\overset{\vert}{C}-O$	78,13		78,33	78,45	
	CH_3	28,10		28,07	23,12	
OMe	CH_3	51,46	51,84	51,62		

required tripeptide was obtained with low yield (20-30%) and with low purity. A positive result was obtained on using dicyclohexylcarbodiimide (DCC) in the dimethylformamide–acetonitrile system. It must be mentioned that the solvent plays a not unimportant role in this stage, since when coupling was performed in the presence of DCC in methylene chloride the yield and purity of the product fell sharply.

To remove the BOC group from the tripeptide we used trifluoroacetic acid. In order to exclude trifluoroacetylation as a side reaction in the synthesis of the tripeptide, the BOC group was eliminated from the dipeptide with a solution of hydrogen chloride in ethyl acetate (EA). The ester group was eliminated by alkaline hydrolysis in methanol. The tripeptide derivatives and intermediate products were obtained in high yields and were distinguished by chromatographic homogeneity.

To identify the structures of the compounds obtained we used the ^{13}C NMR method. The values of the chemical shifts of the signals in ppm and their assignments are given in Table 1. The assignment of the signals in the 45-80 ppm and 155-175 ppm region was made on the basis of literature data [4, 5]; the multiplicities in the spectra obtained under "off-resonance" conditions (the O–Me signals and those of the quaternary carbon atom of the tert-butoxycarbonyl group and of the C^δ atom of the proline residue); the nature of the splitting of the lines by long-range spin–spin coupling constants J_{C-H} in the spectra obtained under the "gated decoupling" regime (the C=O signals of the Pro, Arg_1, and Arg_2 residues); and the magnitudes of the change in the chemical shifts on passing from one compound to another. Facts given in the literature [4, 5], and also the values of the changes in the chemical shifts in the series of compounds considered are in favor of the suggested assignment of the signals in the 20-41 ppm region.

EXPERIMENTAL

The work was carried out with dry freshly-distilled solvents. Organic solvents were evaporated in a rotary evaporator at a residual pressure of 10-15 mm Hg and a bath temperature of 40-50°C. The products were dried in a vacuum drying chamber at a residual pressure of 1-5 mm Hg and a temperature of 50-60°C. Melting points were determined in open capillaries without correction, and angles of rotation in a polarimeter. The polarographic purities and mobilities of the substances were determined by thin-layer chromatography (TLC) on "Silufol-254" plates (Czechoslovakia) in the chloroform–methanol (4:1 by volume) system. The electrophoretic homogeneities and mobilities of the substances were determined by paper electrophoresis on a laboratory apparatus in the pyridine–acetic acid–water (1.2:1.0:100) system.

The samples for elementary analysis were dried in the vacuum drying chest over phosphorus pentoxide and potassium hydroxide for 10-12 h.

The ^{13}C NMR spectra of solutions of the peptides in deuterated dimethyl sulfoxide, c = 100 mg/ml, were recorded on a Brüker WP-80DS spectrometer (GFR) with a working frequency of 20.115 MHz. The conditions for recording the spectra with broad-band proton decoupling were: volume of the memory for accumulating the spectra 8 K and for reproduction 8 K; machine resolution 0.45 Hz; duration of a pulse 2-3 μsec (25-36°); time for one scan 1.092 sec; number of accumulations 3000-6000. The chemical shifts are given in the δ scale relative to tetramethylsilane. The chemical shifts were determined relative to the signal of the solvent and were recalculated in the δ scale relative to tetramethylsilane by means of the formula: $\delta_{TMS} = \delta_{DMSO} + 39.6$ ppm.

1. Preparation of **BOC-Arg-Pro-OMe**. A. To a solution prepared from 8.6 g (52 mmole) of the hydrochloride of proline methyl ester in 40 ml of methylene chloride cooled to –10°C was added 5.35 g (53 mmole) of triethylamine (TEA), and the reaction mixture was stirred at the temperature given for 30 min, after which the precipitate that had formed was filtered off on a glass filter and was washed with methylene chloride. In parallel, 4.2 ml (44 mmole) of ethyl chloroformate was added to a solution prepared from 12.8 g (40 mmole) of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginine and 4.1 g (40 mmole) of TEA in 30 ml of methylene chloride cooled to –15°C, and the reaction mixture was stirred at the given temperature for 15 min. To the resulting mixed anhydride, at –10°C, a solution of proline methyl ester obtained by the method described above was added, and the reaction mixture was stirred at 0 to –5°C for 2 h and at room temperature for 2 h. Then the reaction products were washed successively with aqueous citric acid, water, aqueous sodium bicarbonate, and water again. The organic layer was dried by filtration through a layer of absorbent cotton and sodium sulfate on a chemical funnel, and the solvent was driven off in vacuum. This gave 12.1 g of chromatographically homogeneous product; yield 70% of theory, R_f = 0.64, mp 69-70°C; $[\alpha]_D^{22}$ –59.5° (c 1.0; MeOH).

B. To a solution of proline methyl ester prepared from 3.3 g (19.9 mmole) of the hydrochloride, TEA, and 20 ml of methylene chloride by the method described above and cooled to –10°C were added 3.2 g (9.9 mmole) of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginine and 2.7 g (10.9 mmole) of EEDC [3]. The reaction mixture was kept in the refrigerator at 0 to –2° for 10-12 h and then the reaction products were diluted to 50 ml with methylene chloride and were worked up in the manner described above.* This gave 3 g of product; yield 82% of theory. It was completely identical with the product obtained in experiment 1A.

Preparation of **H-Arg-Pro-OMe·HCl**. A solution of 11 g (25.6 mmole) of the methyl ester of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginyl proline in 10 ml of ethyl acetate was treated with 45 ml of ethyl acetate saturated with hydrogen chloride (c = 0.8 g/ml). The reaction mixture was stirred at room temperature for 30 min and the product that deposited was filtered on a glass filter and was washed with ethyl acetate and dried in vacuum. This gave 9 g of product in the form of white hygroscopic crystals. Yield 96% of theory. The product was electrophoretically homogeneous with E = 124 mm, V = 500 V, I = 0.5 A, τ = 2.5 h, G = 15 V/cm, mp 161-163°C, $[\alpha]_D^{22}$ –38.0°C (c 1.0; MeOH).

*For the better washing of the quinoline derivatives, a 1 N solution of HCl was used in place of the citric acid.

NO_2 NO_2
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3. Preparation of **BOC-Arg-Arg-Pro-OMe.** A. A solution of 7.7 g (24 mmole) of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginine in 15 ml of DMFA was added to a solution prepared from 8 g (21.8 mmole) of the hydrochloride of the methyl ester of N^{ω}-nitroarginylproline, 3.3 ml of TEA, and 15 ml of dimethylformamide (DMFA) by the method of experiment 1. The reaction mixture was cooled to −20°C, and 10 ml of acetonitrile and 5 g (24.2 mmole) of ground DCC were added. The reaction mixture was stirred at −15 to −20°C for 5.6 h and at from −2 to 0°C for 12 h. Then it was treated with 1 ml of glacial acetic acid and was cooled to −30 to −40,C, and the precipitate of dicyclohexylurea that had deposited was filtered off on a glass filter and was washed with cold ethyl acetate. The solvent was driven off in vacuum, and the residue was dissolved in the butanol–chloroform (1:1) system. The further working up of the reaction products was performed by the method of experiment 1A, giving 10.3 g of product in the form of a yellowish-white powder; yield 75% of theory. To free it from traces of dicyclohexylurea, it was recrystallized from butanol. The product was chromatographically homogeneous with R_f = 0.52, mp 123-125°C, $[\alpha]_D^{22}$ −6.0 (c 1.0; MeOH).

B. A solution prepared from 1.6 g (4.98 mmole) of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginine, 0.7 ml of TEA, and 5 ml of methylene chloride was cooled to −15°C, and 0.52 ml (5.48 mmole) of ethyl chloroformate was added. The reaction mixture was kept at −10 to −15°C, and to the solution of mixed anhydride prepared in this way was added, at −20°C, a solution of the methyl ester of N^{ω}-nitroarginylproline prepared from 2 g (5.44 mmole) of its hydrochloride, 1.4 ml of TEA, and 5 ml of DMFA by the method of experiment 1A. The reaction mixture was kept at −5 to 0°C for 1 h and at room temperature for 2 h. The reaction products were worked up in the manner described in experiment 1A. This gave 0.75 g of tripeptide; yield 24% of theory. The product was chromatographically inhomogeneous.

C. To a solution prepared from 1.0 g (2.72 mmole) of the hydrochloride of the methyl ester of N^{ω}-nitroarginylproline, 0.7 ml of TEA, and 10 ml of methylene chloride by the method of experiment 1A was added 0.8 g (2.49 mmole) of N^{α}-tert-botoxycarbonyl-N^{ω}-nitroarginine, and then the reaction mixture was cooled to −5°C and 0.9 g (3.6 mmole) of EEDC was added. The reaction mixture was stirred until it was homogeneous and was kept in a refrigerator at −5 to −7°C for 48 h. The reaction products were worked up by the method described in experiment 1A. This gave 0.47 g of tripeptide; 37% of theory. The product was chromatographically inhomogeneous.

NO_2 NO_2
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4. Preparation of **BOC-Arg-Arg-Pro-OH**. A solution of 1.2 g of sodium hydroxide in 6 ml* and 24 ml of methanol was added to 5.6 g (8.8 mmole) of the methyl ester of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginyl-N^{ω}-nitroarginylproline. The reaction mixture was stirred at room temperature for 2-3 h (monitored by TLC from the disappearance of the spot of the initial compound). Then it was diluted with 100 ml of water and was washed with ethyl acetate–butanol (9:1 by volume). With cooling to 0°C, the aqueous layer was brought to pH 2-3 with 2 N HCl, and the oil that separated out was extracted with isopropanol–chloroform (1:1 by weight). After the extract had been dried and the solvent had been driven off in vacuum, 4.35 g of product (yield 80% of theory) was obtained in the form of a white amorphous mass. The product was chromatographically homogeneous, with R_f = 0.43, mp 121-123°C; $[\alpha]_D^{22}$ −21.9° (c 1.1; dimethylformamide).

NO_2 NO_2
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5. Preparation of **H-Arg-Arg-Pro-OH·CF_3COOH** . A solution of 3.1 g (4.9 mmole) of N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginyl-N^{ω}-nitroarginylproline in 7 ml of trifluoroacetic acid was kept at room temperature for 2-2.5 h (monitoring by TLC from the disappearance of the spot of the initial compound). The reaction mixture was treated with diethyl ether, and the resulting precipitate was separated off on a glass filter, washed with ether, and dried in the air. This gave 2.9 g of product in the form of a white powder. Yield 94% of theory, mp 245-248°C (decomp.), $[\alpha]_D$ −14.0° (c 1.0; 95% CH_3COOH).

*As in Russian Original – Publisher.

SUMMARY

1. The tripeptide arginylarginylproline of sequence 17-19 of ACTH has been synthesized from N^{α}-tert-butoxycarbonyl-N^{ω}-nitroarginylproline.

2. Some physicochemical indices of individual derivatives of the tripeptide and of the intermediate products have been determined.

LITERATURE CITED

1. R. Schwyzer and H. Kappeler, Helv. Chem. Acta, 1550 (1963).
2. V. F. Pozdnev, Khim. Prir. Soedin., 764 (1974).
3. L. Fieser and M. Fieser, Reagents for Organic Synthesis, Wiley, New York, Vol. 2 (1969), p. 191.
4. S. H. Rosenthal and T. H. Fendler, Adv. Phys. Org. Chem., 13, 279 (1976).
5. E. Breitmeier and W. Voelter, ^{13}C NMR Spectroscopy. Methods and Applications. Verlag Chemie Gmbh, Weinheim/Bergstr. (1974), pp. 251-267.

ANALOGS OF D(+)-PANTOTHENIC ACID.

V. SYNTHESIS AND INVESTIGATION OF THE STRUCTURE OF N-PANTOYL DERIVATIVES OF PROLINE

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The synthesis of pantothenic acid analogs is described. Boiling the Na salt of L-proline (L-I) with D-(−)-pantolactone (D-II) in MeONa Yielded 53.91% of N-D-pantoyl-L-proline (III), $[\alpha]_D^{20}$ −52° (c 2; MeOH), and 19.18% of cyclo(N-D-pantoyl-L-proline) (IV), mp 119-121°C (ethanol), $[\alpha]_D^{20}$ −68.9° (c 2; MeOH). The following were obtained similarly: N-L-pantoyl-L-proline, cyclo(N-L-pantoyl-L-proline), N-D-pantoyl-D-proline, cyclo(N-D-pantoyl-D-proline), N-L-pantoyl-D-proline, cyclo(N-D-pantoyl-hydroxyproline), and N-γ-hydroxybutyryl-L-proline. By fusing D-II and DL-I at 140°C cyclo(N-D-pantoyl-DL-proline) and prolylproline anhydride (V) were obtained. Compound (V) with mp 136-138°C was synthesized from DL-1 by heating at 140°C. The PMR spectra of compounds (III-V) are given. The IR spectra of compounds (III and IV) are discussed.

We have previously described the synthesis and properties of some analogs of D-pantothenic acid modified in the amino-acid moiety of its molecule [1, 2]. Some of them have proved to be effective drugs which can be explained by an improvement in the capacities of the pantoyl derivatives of amino acids for passing through biological membranes as compared with the initial amino acids. In particular, the introduction of a pantoyl radical into the γ-aminobutyric acid molecule led to a substance readily passing through the blood-brain barrier and possessing a pronounced neuropharmacological activity [1]. In order to obtain new biologically active derivatives of D-pantothenic acid and to investigate the mechanism of their action, it appeared of interest to study the efficacy of the approach described above for other amino acids possessing neuromediator properties, as well. The information recently obtained according to which proline can play the part of a neuromediator in some synapses of the spinal cord [3, 4] led to the choice of this amino acid as the next object of investigatio

N-D-Pantoyl-L-proline (Ia) was synthesized by a general method for obtaining pantothenic acid and its derivatives [1, 2] involving the interaction of D-pantolactone with the sodium

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