SUMMARY

1. The tripeptide arginylarginylproline of sequence 17-19 of ACTH has been synthesized from $N^{\alpha}$-tert-butoxycarbonyl-$N^{\omega}$-nitroarginylproline.

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

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# ANALOGS OF D(+)-PANTOTHENIC ACID.

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

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UDC 547.164.14.074

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

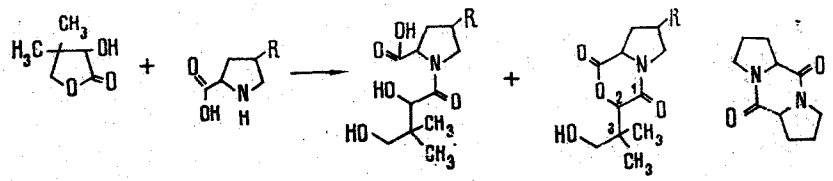
N-D-Pantoyl-L-proline (Ia) was synthesized by a general method for obtaining pantothenic acid and its derivatives [1, 2] involving the interaction of D-pantolactone with the sodium

All-Union Scientific-Research Vitamin Institute, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 378-383, May-June, 1979. Original article submitted January 22, 1979.

 0009-3130/79/1503-0328$07.50

salt of L-proline in boiling methanol followed by treatment of the reaction mixture with KU-2 cation-exchange resin ($H^+$ form) with a yield of ∿50%. Compound (Ia) formed an oily product the structure of which agreed well with its spectral characteristics.

It is known that optical isomers may possess different pharmacological activities, and in view of this we have performed the synthesis of all four possible combinations of isomers of N-pantoylproline.



I II a. R=H III
b. R=OH

Together with substance (Ia), a crystalline product was isolated in low yield (∿10%) from the reaction mixture the IR spectrum of which showed the bands of tertiary amide and ester carbonyls and the mass spectrum of which contained the peak of the molecular ion with m/e 227, i.e., 18 units less than in compound (Ia). These facts gave us grounds for assuming the structure of anhydro-(Ia), (IIa), for the byproduct isolated; this can be formed as the result of an intramolecular reaction between the carboxy group of the proline residue and one of the hydroxy groups of the molecule of (Ia).

Similarly, the reaction of D-pantolactone with the sodium salt of L-hydroxyproline gave N-D-pantoyl-L-hydroxyproline (Ib) and its anhydro derivative.

When D-pantolactone was fused with DL-proline in the absence of a solvent at 140°C, we did not isolate the isomer (Ia). Under these conditions, the reaction led to the formation of D-DL-anhydro-(Ia) and to the anhydride of DL-prolylproline (III) with yields of 16 and 67%, respectively. Compound (III) was identical with the substance that we obtained by fusin DL-proline at 140°C. The preparation of the anhydride L-(III) from the ethyl ester of L-proline has been described previously [5].

A few examples of the formation of 2,5-dioxomorpholine derivatives from dipeptolides containing α-hydroxy acids as the result of intramolecular cyclization are known in the literature [6, 7]; however, such compounds were previously unknown among derivatives of pantoic acid. In this connection, to answer the question of the structure of the compounds obtained and, in particular, the size of the ring formed, we investigated the IR, NMR, and mass spectra of compounds (Ia) and (IIa).

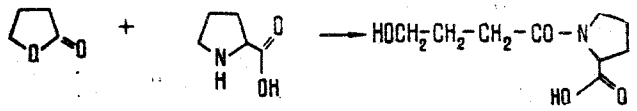
In the IR spectra of N-D-pantoyl-L-proline (Ia) and its cyclic derivative (IIa) (in a thin film), strong bands at 1755 and 1645 $cm^{-1}$ in (Ia) and at 1750 and 1650 $cm^{-1}$ in (IIa) are due to the absorption of an unionized carbonyl group and of the amide group, respectively. The displacement of $\nu_{\substack{C=O \\ | \\ N}}$ by dissolution ($CHCl_3$ + $CCl_4$ (1:2)) to 1675 $cm^{-1}$ in (Ia) and 1662 $cm^{-1}$ in (IIa) shows the considerable noncoplanarity of the amide group. The $\nu_{\substack{C-N \\ | \\ (O)}}$ absorption band reported in the literature [8, 9] in the 1400-1430 $cm^{-1}$ region for cyclodipeptides containing a proline residue and other amino acid residues does not appear in the spectra of (Ia) and (IIa). It is apparently shifted to higher or lower frequencies and is masked by the strong bands of the deformation vibrations of $CH_2$ and $CH_3$ groups at 1460 and 1380 $cm^{-1}$.

The presence of hydroxy groups is confirmed by a strong broad band with its center at 3400 $cm^{-1}$ overlapping with the absorption of the carboxy group (2400-3200 $cm^{-1}$) in the case of (Ia) and 3380 $cm^{-1}$ in the case of (IIa). Also connected with C—OH vibrations are strong bands at 1050 $cm^{-1}$ (Ia) and 1068 $cm^{-1}$ (IIa), and with C—N and $(CH_3)_2C$ group bands in the 1200 $cm^{-1}$ region.

N-D-Pantoyl-DL-proline has a spectrum similar to that of (Ia) but in solution the amide band shifts to 1690 $cm^{-1}$.

In order to elucidate the nature of the hydrogen bonds and to answer the question of the size of the ring formed, the IR spectra of these compounds, and also of the model compound γ-hydroxybutyryl-L-proline (IV) in which the α-hydroxy group is absent, were studied at high dilution ($10^{-2}$-$2.5 \cdot 10^{-3}$ M). Compound (IV) was obtained by condensing γ-butyrolactone with

the sodium salt of L-proline in boiling methanol. Under these conditions we observed no cyclization of compound (IV).



IV

In the IR spectra of this compound in a thin film and in solution, the $\nu_{C=O}$ of vibrations of the carboxy and amide groups are present at lower frequencies than in D-pantoyl-L-proline - 1735 and 1620 $cm^{-1}$ (in solution, 1740 and 1630 $cm^{-1}$), which is apparently connected with the greater accessibility of these groups for the formation of unstrained hydrogen-bond rings without particular disturbance of the planarity of the amide group. A broad absorption band in the 2400-3500 $cm^{-1}$ region is due to the vibrations of the OH bonds of the hydroxy and carboxy groups. In solution at concentrations of $2.4 \cdot 10^{-2}$ and $6 \cdot 10^{-3}$ M the absorption of the hydroxy group remains unchanged — a broad unresolved band with center at 3400 $cm^{-1}$; there is weak absorption of a free hydroxy group at 3610 $cm^{-1}$.

A consideration of Dreiding models shows the possibility of the formation of several intramolecular hydrogen bonds (intra-HBs) of the hydroxy group, primarily with the amide and carboxy C=O groups. Their lowered frequencies ($\nu_{C=O}$) are also evidence in favor of this hypothesis.

In the spectrum of D-pantoyl-L-proline at concentrations of $10^{-2}$ and $2.5 \cdot 10^{-3}$ M, the intra-HBs likewise remain practically unchanged. Absorption of a free hydroxy group appeared only in the form of a weak shoulder on the strong $3570^{-1}$ band, corresponding to a weak but sterically suitable intra-HB possibly through secondary (and primary) hydroxyls with the OH of the carboxy group. At both concentrations a broad band of lower intensity (threefold at the maximum) at 3450 $cm^{-1}$ is retained which corresponds to a stronger, but sterically less suitable, hydrogen bond possibly through the primary hydroxyl with the amide group but in these circumstances the nonplanarity of the latter may be increased ( $\nu_{C=O}$ 1675 $cm^{-1}$).

Thus, a comparison of the model compound with D-pantoyl-L-proline shows that the predominant formation of intra-HBs of the OH group takes place in the first place through the carbonyls of the amide and carboxy groups and in the second place through the hydroxyl of the latter while in the case of the D-L configuration with the nonplanar structure of the proline ring (inversion of the nitrogen) [10, 11] the most suitable form will be a structure with an intra-HB of the secondary hydroxyl with the OH of the carboxyl (nonplanar eight-membered ring) with a simultaneous intra-HB of the primary hydroxyl with the secondary (planar six-membered ring).

In cyclo-D-pantoyl-L-proline, in addition to a medium-intensity band of a free hydroxy group at 3633 $cm^{-1}$ at both concentrations ($10^{-2}$ and $2.5 \cdot 10^{-3}$ M) a strong broad band at 3490 $cm^{-1}$ and a weak one at 3310 $cm^{-1}$ of a hydroxyl bound by an intra-HB are retained. It must be mentioned that in the cyclic compound the intra-HB is stronger than in the open compound (shift to lower frequencies by 80 $cm^{-1}$) and it is apparently formed mainly through the "ester" oxygen; the frequencies of the carbonyl and amide groups are fairly high (1760 and 1662 $cm^{-1}$).

On the basis of a consideration of Dreiding models it may be concluded that in the cyclization of the molecule of (Ia) through the secondary hydroxyl, the primary hydroxyl may be either free or bound by an intra-HB, as the spectra show. In the case of cyclization through the primary hydroxyl when the configuration is D-L, with both equatorial and axial hydrogen atoms of the CH group with the secondary hydroxyl, the latter must be free.

A consideration of the PMR spectra of compounds (Ia) and (IIa) also confirms this conclusion. On the cyclization of the hydroxy acid (Ia), the signal of the $CH_2$ at the primary hydroxyl remains unchanged and the signal of the CH at the secondary hydroxyl shifts downfield by 0.64 ppm. The considerable shift of the signal shows that the methine proton is axial in relation to the six-membered dioxomorpholine ring [12].

In the high- and low-resolution mass spectra of compound (IIa) characteristic peaks of ions are observed which confirm the presence of a six-membered dioxomorpholine ring in the (IIa) molecule. According to the results of high-resolution mass spectroscopy, the characteristic peaks of high intensity with m/e have the elementary composition $C_7H_8NO_3$ $[M - C_4H_9O]^+$

and those of medium intensity with m/e 127 and 114 have the compositions $C_6H_9NO_2$ $[M - C_5H_8O_2]^+$ and $C_5H_8NO_2$ $[M - C_6H_9O_2]^+$, respectively. The ion with m/e 154 is formed by the cleavage of the $C_2$—$C_3$ bond, and the ion with m/e 127 with the additional elimination of a CO group.

Thus, it may be concluded that the preferred conformation of compound (IIa) corresponds to a six-membered ring with axial hydrogen at the "ester" oxygen and with an intra-HB formed by it with the primary hydroxyl and the cis arrangement of the axial hydrogen, of the carbonyl groups, and of the pyrrolidine ring. A slight contribution of a structure with the trans arrangement of the carbonyl groups with respect to the axial hydrogen and with an intra-HB of the primary hydroxyl of the carbonyl of the amide group is possible (weak bands at 3310 and 1614 $cm^{-1}$).

## EXPERIMENTAL

The IR spectra were recorded on a UR-10 spectrometer in a thin film, in paraffin oil, and in solution ($CHCl_3$ + $CCl_4$ (1:2 by volume), c = $10^{-2}$-$2.5 \cdot 10^{-3}$ M, d = 2.54, 5, and 20 mm). The PMR spectra were recorded on a Hitachi R-20A instrument with a working frequency of 60 MHz in $D_2O$ (with sodium 2,2-dimethyl-2silapentane-5-sulfonate as internal standard) and in $CDCl_3$ (with tetramethylsilane as internal standard). The mass spectra were recorded on a JEOL JMSOIS G-2 instrument with a system for the direct introduction of the sample into the ion source, at an energy of the ionizing electrons of 75 eV and a temperature of heating the sample of ∿160°C. Thin-layer chromatography was performed on standard Silufol UV-254 plates (Kavalier, Czechoslovakia) in the following systems: 1) butan-1-ol—acetic acid—water (5:2:3); 2) propanol—25% $NH_4OH$—water (6:3:1); 3) ethanol—ether (1:1); 4) methanol—ether—water (8:1:1). Specific rotations were measured on a A-1-EPL instrument.

N-D-Pantoyl-L-proline (Ia) and Cyclo(N-D-pantoyl-L-proline) (IIa). To a solution of sodium methanolate (from 0.75 g of sodium in 40 ml of methanol) were added 3.8 g of L-proline and, after 1 h, 4.48 g of D(—)-pantolactone. The reaction mixture was stirred at the boil for 10 h, the methanol was evaporated off, and the residue was dissolved in 150 ml of water and was passed through a column containing KU-2 resin ($H^+$ form). The substance was eluted with water until the reaction of the eluates was neutral, and these were concentrated to 120 ml and the D-pantolactone was extracted with ether (3×70 ml). The aqueous solution was treated with chloroform (3×100 ml) and, after the evaporation, the residue was dried in vacuum over phosphorus pentoxide. The yield of compound (Ia) was 4.55 g (53.19%), $[\alpha]_D^{20}$ —52° (c 2; methanol), $R_f$ 0.45 (system 1), 0.64 (system 2), 0.48 (system 3). PMR spectrum ($CDCl_3$, ppm): 1.05 (6H, doublet, J = 7.9 Hz; $C(CH_3)_2$; 2.0 (4 H, multiplet; β,γ protons of the pyrrolidine ring): 3.45 [2 H; doublet, J = 12.7 Hz; $CH_2(OH)$]; 3.5 (2 H, multiplet, δ-protons of the pyrrolidine ring); 3.98 [H, singlet; CH(OH)]; and 4.2 (H, unresolved quartet; α-proton of the pyrrolidine ring). Found, %: C 53.45; H 7.90; N 6.02. $C_{11}H_{19}NO_5$. Calculated, %: C 53.86; H 7.81; N 5.71.

The chloroform extract was evaporated and the residue was dried in vacuum. This gave 1.5 g (19.18%) of compound (IIa). After three recrystallizations from ethanol, mp 119-121°C, $[\alpha]_D^{20}$ —68.9° (c 2; methanol), $R_f$ 0.55 (system 1), 0.75 (system 2), 0.61 (system 3). PMR spectrum ($CDCl_3$, ppm): 1.05 [6H, doublet, J = 7.5 Hz; $C(CH_3)_2$]; 2.0 (4 H, multiplet; β,γ-protons of the pyrrolidine ring); 3.45 (2 H, doublet, J = 1.5 Hz; $CH_2(OH)$], 3.5 (2 H, multiplet; δ-protons of the pyrrolidine ring) 4.2 (H, unresolved quartet; α-proton of the pyrrolidine ring), and 4.62 [H, singlet; CH(OH)]. Found, %: C 58.30; H 7.51; N 6.52. $C_{11}H_{17}NO_4$. Calculated, %: C 58.13; H 7.54; N 6.16.

The following were obtained similarly.

N-L-Pantoyl-L-proline, yield 51.5%, $[\alpha]_D^{20}$ —63° (c 2; methanol); Cyclo(N-L-pantoyl-L-proline), yield 18.5%, $[\alpha]_D^{20}$ —58° (c 2; methanol), mp 118-120°C; N-D-Pantoyl-D-proline, yield 50.5% $[\alpha]_D^{20}$ +64° (c 2; methanol); Cyclo(N-D-pantoyl-D-proline) yield 17.8%, $[\alpha]_D^{20}$ +60° (c 2; methanol), mp 118-119°C; N-L-Pantoyl-D-proline yield 52.3% $[\alpha]_D^{20}$ +62° (c 2; methanol); Cyclo-(N-L-pantoyl-D-proline), yield 18.2%, $[\alpha]_D^{20}$ +78° (c 2; methanol), mp 114-116°.

N-D-Pantoyl-L-hydroxyproline (Ib). This was obtained from D-pantolactone and L-hydroxyproline by the method used for compound (Ia), yield 52%, $[\alpha]_D^{20}$ —50° (c 1; ethanol), $R_f$ 0.52 (system 1), 0.63 (system 4). Found, %: C 50.25; H 7.02; N 5.20. $C_{11}H_{19}NO_6$. Calculated, %: C 50.56; H 7.33; N 5.36.

Cyclo(N-D-pantoyl-L-hydroxypyroline) (IIb). Yield 12.5%, $R_f$ 0.61 (system 1). Found, %: C 54.05; H 6.85; N 5.86. $C_{11}H_{17}NO_5$. Found, %: C 54.31; H 7.04; N 5.76.

N-γ-Hydroxybutyryl-L-proline (IV). This was obtained from γ-butyrolactone and L-proline in a similar manner to substance (Ia), yield 50.3%, $[\alpha]_D^{20}$ −56° (c 2; methanol), $R_f$ 0.6 (system 1). Found, %: C 53.45; H 7.47; N 7.05. $C_9H_{15}NO_4$. Calculated, %: C 53.72; H 7.51; N 6.96.

Cyclo(N-D-pantoyl-DL-proline). A mixture of 4.48 g of D-pantolactone and 3.8 g of DL-proline was heated at 140°C for 10 h. Then it was cooled and dissolved in 20 ml of water. The aqueous solution was treated with chloroform (5×20 ml). The extract was dried and evaporated. The residue was dissolved in petroleum ether–diethyl ether (1:1) and was chromatographed on a column (500×200) with silica gel M (Woelm).

Petroleum ether–diethyl ether (1:1) (300 ml) eluted 3.82 g (85.3%) of D-(−)-pantolactone and then the column was treated with 500 ml of chloroform. The eluates were collected and evaporated, the residue was triturated with dry ether, and the resulting solid was filtered off and recrystallized from ether–ethanol (5:1). This gave 1.20 g (16.02%) of cyclo(N-D-pantoyl-DL-proline). Mp 70-72°C (from ethanol). $R_f$ 0.55 (system 1), 0.75 (system 2), 0.62 (system 3). Found, %: C 57.86; H 7.38; N 5.99. $C_{11}H_{17}NO_4$. Calculated, %: C 58.13; H 7.54; N 6.16.

Then the column was treated with 350 ml of ethanol, the eluates were evaporated, and the residue was distilled in vacuum. This gave 2.15 g (67.19%) of DL-prolylproline anhydride (III), $R_f$ 0.51 (system 1), 0.31 (system 3); mp 134-136°C. Found, %: C 61.40; H 7.10; N 14.1( $C_{10}H_{14}N_2O_2$. Calculated, %: C 61.85; H 7.20; N 14.40. PMR spectrum ($D_2O$, ppm): 2.1 (8 H, multiplet, β,γ-protons of the pyrrolidine ring); 3.5 (4 H, triplet, J = 5 Hz, δ-protons of the pyrrolidine ring); 4.4 (2 H, multiplet, α-protons of the pyrrolidine ring).

DL-Prolylproline Anhydride (III). DL-Proline (2 g) was heated at 140°C for 12 h and then the melt was cooled, dissolved in 5 ml of water, and treated with chloroform (3×5 ml). The extract was evaporated and the residue was dried in vacuum. Yield 1.2 g (71.0%). Mp 136-138°C, $R_f$ 0.51 (system), 0.31 (system 3).

## SUMMARY

The synthesis of isomers of N-pantoylproline and of cyclo (N-pantoylproline) has been performed by the reaction of D- and L-pantolactones with D- and L-prolines. The structures of the compounds obtained have been studied by IR spectroscopy.

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