PROLINE-BASED TOPOLOGIC PYRACETAM ANALOGS AND THEIR NOOTROPIC ACTIVITY

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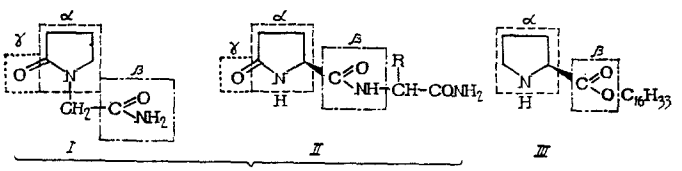
Pyracetam [methylpyrrolidone-2-N-carbamide] (I) is the most characteristic representative of the class of nootropic compounds, which specifically facilitate learning and memory processes. The search for other nootropic agents among pyracetam analogs has so far been conducted mainly by attempts to complicate its molecule while preserving the N-substituted pyrrolidone skeleton [7, 11, 13]. In this paper a fundamentally new direction for such a search is developed.

The writers have postulated [1-3] that pyracetam is an exogenous analog of the endogenous peptide ligand, not yet discovered, of hypothetical nootropic (pyracetam) receptors, with the aid of which learning and memory processes are controlled. This hypothesis has received the following confirmation. Peptide structural analogs of pyracetam with N-terminal pyroglutamic acid (II) exhibit nootropic activity similar to that of pyracetam – they facilitate learning in rats in passive and active avoidance tests, they affect the transcallosal response, and counteract retrograde amnesia induced by electric shock; other forms of psychotropic activity have not been found in them [1, 5, 6, 10]. Compounds II, in small doses (100-10 μg/kg, intraperitoneally) have an action which is stereospecific. Antagonists of the nootropic effect, with only minor structural differences from II, have been found [1, 2]. Compounds II thus represent a new type of substances with nootropic activity.

One of the antagonists of the nootropic effect is the cetyl ester of L-proline (III), described by the writers [4], which induces amnesia in rats when administered systematically. This amnesia is abolished by the action of both I and II. Considering the similarity of the structures of I-III, we postulated that III acts on the same receptors as I and II.



I II III

Agonists Antagonist

It will be clear from the formulas accompanying this paper than compounds I-III have a number of common structural elements, which are essential for manifestation of their activity. For instance, they all contain a pyrrolidine ring (pharmacophore α), and a change in size of the ring leads to a decrease or loss of activity [9]; a carbamido or carbalkoxyl group (pharmacophore β), and lactam carbonyl group (pharmacophore γ), absence of which in III converts the agonist into an antagonist [4]. These pharmacophores are arranged similarly in space, and despite the fact that pharmacophore β in I is bound with pharmacophore α through a different system of covalent bonds than in II and III, it can be clearly seen in Dreiding's molecular models that the amido nitrogen or oxygen of pharmacophore β in I can be spatially coincident with the nitrogen or oxygen of this same pharmacophore in II and III. We have suggested that pharmacophore γ can also be bound with pharmacophore α through a different system of covalent bonds, for example, it can be introduced in III into the N-acyl group. It might also be expected that the antagonist III, differing from I and II in the absence of pharmacophore γ, on N-acylation is converted into an agonist of nootropic receptors. To test this hypothesis we synthesized a number of N-acyl derivatives of L-proline, and also the amide of N-carbamido methyl-L-proline in accordance with the following scheme:

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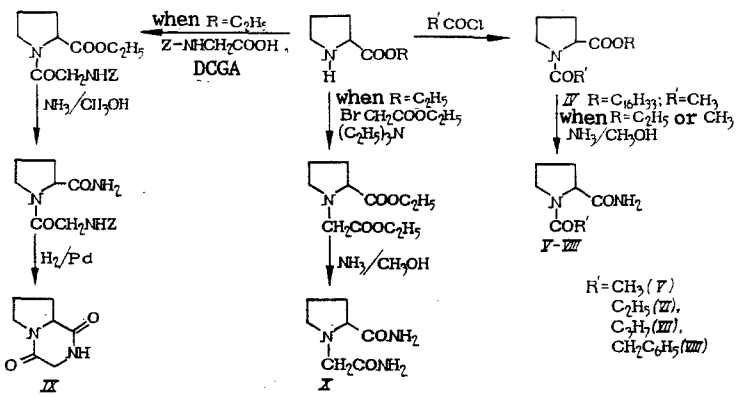
TABLE 1. Activity of Topologic Pyracetam Analogs IV-X in the PALT

Comp.	Dose		Activity A_t, %
	mmole/kg	mg/kg	
IV	0,078	30,0	+35 ($P_t < 0,05$)
V	0,089	14,0	+50 ($P_t < 0,01$)
	0,006	1,0	Insufficient
VI	0,088	15,0	»
VII	0,086	16,0	»
VIII	0,082	19,0	−32 ($P_t < 0,01$)
IX	0,006	1,0	+23 ($P_t = 0,05$)
X	0,070	12,0	Insufficient

Legend. A_t) Activity based on mean length of stay of animals in lit compartment, calculated by the formula in [1]; P_t) significance of the parameter t, calculated by Wilcoxon–Mann–Whitney method; −) amnestic activity.



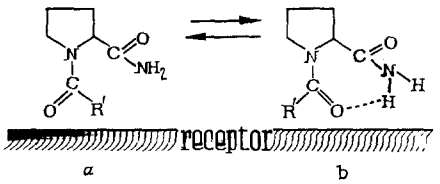
The nootropic activity of the compounds synthesized was taken to be equal to the relative improvement of initially untrained animals to learn in the passive avoidance learning test (PALT) and was expressed as a percentage of the maximal possible effect [1].

The tests showed that introduction of a carbonyl group into III in the form of an N-acetyl residue converts the antagonist III into an agonist, namely the cetyl ester of N-acetyl-L-proline (IV), with nootropic activity of 35%. It will be recalled that the pharmacophore β in the structure of the agonists I and II is represented by a carbamido group which, unlike the carbalkoxyl group, may be not only an acceptor, but also a donor of protons during hydrogen bond formation with the receptors. Replacement of the carbalkoxyl group in IV by an amido group in fact led to an increase of nootropic activity to 55% (amide of N-acetyl-L-proline, V, Table 1). Antiamnestic activity, which is usually cited in the literature for pyracetam, also was found for V (a more complete comparison of the spectra of nootropic activity of these compounds with that of pyracetam will be given in a special publication).

Compounds I (N-carbamidomethyl-pyrrolidone-2), II (L-pyroglutamyldipeptide), and V (amide of N-acetyl-L-proline) belong to different chemical series. However, since the mutual arrangement of the basic pharmacophores in them is on the whole preserved, they can be regarded as topologic analogs, and their biological activity can be explained by the complementary nature of their topology with that of the active center of the nootropic receptor.

Comparison of the general formula of molecules I, II, and V shows that a hollow in the binding zone of the receptor must correspond to the acetyl residue in V and to the acetamide residue in I. The depth of this hollow does not exceed the size of the acetyl group, for its lengthening by only one CH_2 group (N-propionyl-L-proline amide, VI) leads to loss of its activity (Table 1). However, lengthening of the N-acyl radical does not lead to loss of the ability of the topologic pyracetam analog to bind with the nootropic receptor, for N-phenylacetyl-L-

proline amide (VIII) exhibits amnestic activity in the PALT. Within the framework of our ideas, this can be explained as follows. Topologic pyracetam analogs can bind with the active center of the receptor in two conformations: *a* and b.



a b

Conformational equilibrium *a* ⇄ b for N-acyl derivatives of L-proline amide V-VIII in solutions was investigated by PMR- and IR-spectroscopic methods, and this will also be dealt with in a special communication. Here it will simply be stated that the position of conformational equilibrium in solution is independent of the volume of the N-acyl radical. However, in a complex with the receptor, steric hindrances from the base of the hollow in the binding zone evidently cause equilibrium in the case of bulky N-acyls (VI-VIII) to be shifted toward the b conformation, which is inactive because of the incorrect orientation of the pharmacophore γ. Stabilization of the hypothetically active *a* conformation in cyclo(propylglycine) (IX) actually led to a tenfold decrease in the threshold dose of IX compared with that of V (Table 1). This also is evidence that the amido nitrogen of pharmacophore β is in a cis-oid conformation with the nitrogen of the pyrrolidine ring. Shifting pharmacophore γ by one CH_2 group away from pharmacophore *a* (N-carbamidemethyl-L-proline amide, X) leads to disappearance of activity. This may be connected with difficulty of hydrogen bond formation with the receptor, and also with transition of the nitrogen of pharmacophore α into another, sp^3-hybridized state, and the appearance of a positive charge on it under physiological conditions.

The above remarks clarify some of the structural characteristics of the complex of the ligand with the binding center of the hypothetical nootropic (pyracetam) receptor. The dipeptide ligand II is located in the binding site so that a hollow the depth of an acetyl group remains between the nitrogen atom of the pyrrolidine ring and the receptor, as a result of which the nitrogen atom of the pyrrolidine ring is unable to form a hydrogen bond with the receptor. The proton donor for hydrogen bond formation with the pharmacophore γ is evidently so positioned that it can form a hydrogen bond both with lactam carbonyl I and II with the carbonyl of the N-acetyl group of V. The hydrogen bond with carbamido nitrogen of pharmacophore β is evidently located on an axis passing through the pyrrolidine and amido atoms, which are in the cis-oid conformation. Hence it follows that the peptide bond of the endogenous ligand formed by N-terminal pyroglutamic acid, in a complex with the receptor, has a cis-conformation; functional groups of this second amino acid fill the hollow described above in the binding center and probably form an intramolecular hydrogen bond with the NH group of the pyrrolidine ring, which takes part in stabilization of the cis-conformation of the peptide bond. These data on topology of the binding center of the hypothetical nootropic receptor may provide the basis for a search for new and effective nootropic agents.

EXPERIMENTAL CHEMICAL

IR-spectra were recorded on a Perkin-Elmer 580B instrument (Sweden) in KBr or in a film between NaCl laminae, and mass spectra were recorded on a Varian MAT-112 chromato-mass-spectrometer (Sweden) with ionizing electron energy of 70 eV, temperature of the ionic source and molecular separator 220°C. PMR-spectra were recorded on a Brucker-250 spectrometer (West Germany). Specific optical rotation was measured on an A-1-EPO automatic polarimeter. Thin-layer chromatography was carried out on DC-Alufolien Kieselgel $60F_{254}$ plates (Merck).

N-Acyl-L-proline Esters (typical method). To a suspension of 0.1 mole of hydrochloride of the methyl of ether ester of L-proline in 20 ml of absolute ethyl acetate was added 0.2 mole of N-methylmorpholine. To the cold reaction mass (water with ice) 0.1 mole of acyl chloride was slowly added and the mixture stirred at room temperature for 15 min. The residue was filtered off, the filtrate evaporated to dryness, and the residue chromatographed on a column with silica-gel (chloroform as eluent), and esters of N-acyl-L-proline were obtained with a yield of 80-90%.

Ethyl Ester of N-Acetyl-L-proline. R_f 0.96 (chloroform–methanol, 9:1), $[\alpha]_D^{25}$ −89.0° (c 1.0; methanol). According to data in the literature [15], $[\alpha]_D^{25}$ −90.6° (c 1.4; methanol).

Methyl Ester of N-Propionyl-L-proline. R_f 0.80 (chloroform–methanol, 9:1) $[\alpha]_D^{23}$ −104.0° (c 1.0; methanol). According to data in the literature [14], $[\alpha]_D^{23}$ −103.8° (c 1.3; methanol).

TABLE 2. Amides of N-Acyl-L-Proline (V-VIII)

Compound	Yield, %	mp, °C	R_f	$[\alpha]_D^{20}$, deg	IR spec., in KBr, cm^{-1}: ν CO of primary amide	IR spec., in KBr, cm^{-1}: ν CO of tertiary amide	PMR-spectrum ($CDCl_3$)	Empirical formula
V	90	145—7	0,55[a]	—109 (*c* 2,0; water)	1695	1627	2,12 (s CH_3, 3H); 2,35—2,49 (m, 3, 4-CH_2, 4H); 3,47—3,70 (m, 5—CH_2, 2H); 4,25—4,33; 4,505—4,515 (m, 2-H, 1H), 5,63; 6,09; 6,42; 7,10 (s, NH_2, 2H)	$C_7H_{12}N_2O_2$
VI	85	141—3	0,61[a]	—79 (*c* 0,43; methanol)	1680	1630	1,1—1,3 (t, CH_3, 3H), 1,9—2,2 (m, CH_2CH_3, 2H); 2,2—2,55 (m, 3,4-CH_2, 4H); 3,35—3,70 (m, 5-CH_2), 4,25—4,33; 4,55—4,65 (m, 2-H, 1H); 5,33; 5,60; 6,05; 7,15 (s, NH_2, 2H)	$C_8H_{14}N_2O_2$
VII	87	113—4	0,68[a]	—67 (*c* 0,12; methanol)	1680	1626	0,8—1,1 (t, CH_3, 3H); 1,55—2,55 (m, 3,4-CH_2, $CH_2CH_2CH_3$, 8H); 4,27—4,35; 4,56—4,70 (m, 2-H, 1H); 5,60; 6,07; 6,32; 7,15 (s, NH_2, 2H)	$C_9H_{16}N_2O_2$
VIII	72	hygroscopic	0.18[b]	—159	1682	1636	1,60—2,75 (m, 3,4-CH_2, 4H); 3,2—4,1 (m, 5-CH_2, $CH_2C_6H_5$, 4H); 4,15—4,95(m, 2-H, 1H); 6,00; 6,16; 6,83; 7,09 (s, NH_2, 2H); 6,70—7,85 (m, C_6H_5, 5H)]	$C_{13}H_{16}N_2O_2$

[a]Dioxan—water 9:1. [b]Chloroform—methanol 9:1.

Methyl Ester of N-Butyryl-L-proline. R_f 0.87 (chloroform—methanol, 9:1). $C_{10}H_{17}NO_3$. IR-spectrum (in film), ν_{max}, cm^{-1}: 1740 (CO ester), 1645 (CO acyl).

Cetyl Ester of N-Acetyl-L-proline (IV). To a suspension of 1.1 g (2.9 mmoles) hydrochloride of the cetyl ester of L-proline [1] in 15 ml of absolute toluene was added 0.74 ml (5.8 mmoles) of N-methylmorpholine, followed drop by drop by 0.28 ml (2.9 mmoles) of acetic anhydride. The reaction mixture was stirred for 4 h at room temperature. The residue was filtered off, the filtrate evaporated, and the residue chromatographed on a column with silica-gel (chloroform as eluent). Yield 0.68 g (62%) of IV in the form of a congealing oil, mp 44-45°C, R_f 0.77 (chloroform—methanol, 19:1), $[\alpha]_D^{23}$ −50° (c 2, chloroform). $C_{23}H_{43}NO_3$. M^+ 381. M_{calc} 381. IR-spectrum (in film), ν_{max}, cm^{-1}: 1740 (CO ester), 1650 (CO acetyl), PMR-spectrum, δ, ppm ($CDCl_3$): 1.2 (c, $C_{16}H_{23}$, 33H), 2.0 (c, $COCH_3$, 3H), 3.2-3.7 (m, 3,4-CH_2CH_2, 4H), 3.9-4.2 (m, 5-CH_2, 2H), 4.25-4.55 (m, 2-CH, 1H).

N-Phenylacetyl-L-proline. To a solution of 5.75 g (0.05 mole) of L-proline in 25 ml of 2N NaOH, during cooling (water with ice) and stirring, 12.5 ml of 4 N NaOH and 6.6 ml (0.05 mole) of phenylacetic acid chloroanhydride were slowly added. After 15 min the reaction mixture was acidified with 1N hydrochloric acid. The separating oil was extracted with chloroform, dried with sodium sulfate, and the solvent was removed by evaporation. Absolute ether was added to the residue, which was allowed to stand for 3 days in a refrigerator. Yield 6.2 g (53%) of N-phenylacetyl-L-proline in the form of white crystals. mp 131-132°C. $C_{13}H_{15}NO_3$.

Methyl Ester of N-Phenylacetyl-L-proline. To a solution of 0.7 g (3 mmoles) of N-phenylacetyl-L-proline in 12 ml of absolute methanol 0.47 g of cation-exchange resin Q-150 in the H^+ form was added. The reaction mixture was boiled for 5 h. The resin was removed by filtration, the filtrate evaporated, and the residue chromatographed on a column with silica-gel (chloroform as eluent). Yield 0.6 g (77%) of the methyl ester of N-phenylacetyl-L-proline in the form of a transparent oil. R_f 0.83 (chloroform—methanol, 10:1; $[\alpha]_D^{23}$ −71.0° (c 1.0; chloroform). According to data in the literature [8], $[\alpha]_D^{29}$ −73.1° (c 1.32; chloroform).

Amides of N-Acyl-L-proline (typical method). A solution of 0.02 mole of N-acyl-L-proline ester in 50 ml of methanol, saturated with gaseous ammonia at 0°C, was allowed to stand for 7-10 days at room temperature, the solvent was removed by evaporation in vacuo, and the residue washed with dry ether. Yields and properties are given in Table 2.

Carbobenzoxyglycyl-L-proline Amide. To a solution of 3.8 g (15.8 mmole) of the hydrochloride of the benzyl ester of L-proline in 30 ml of DMPA a small excess of triethylamine (16 mmoles) and 3.3 g (15.8 mmoles) of carbobenzoxyglycine were added. To the cold reaction mass was added 3.8 g (17 mmoles) of dicyclohexylcarbodiimide in 10 ml of DMPA, and the contents were stirred for 5 h. The reaction mixture was diluted with chloroform and allowed to stand overnight in a refrigerator. The residue was filtered off, the solvent evaporated in vacuo, and the residue dissolved in a mixture of ethyl acetate and water; the organic layer was washed with a 10% solution of citric acid, saturated with a solution of sodium bicarbonate and sodium chloride, dried over sodium sulfate, and evaporated to dryness, giving a yield of 4.5 g of a translucent oil, R_f 0.87 (butanol–acetic acid–water, 10:1:3.) Next, 1.8 g of the resulting oil was treated with 50 ml methanol, saturated with ammonia, and allowed to stand for 5 days. The solvent was removed by evaporation and the residue recrystallized from a mixture of ethyl acetate and isopropanol. Yield 1.1 g of amide in the form of white crystals, mp 145-146°C. R_f 0.44 (butanol–acetic acid–water, 10:1:3), R_f 0.55 (chloroform–methanol 1:5). $C_{15}H_{19}N_3O_4$. According to data in the literature, mp 150-151°C (methanol–ether).

Cyclo(glycyl-L-proline) (IX) was obtained by the method in [16]. mp 210-212°C $[\alpha]_D^{20}$ −195°C (c 5, water).

N-Carbamidomethyl-L-proline Amide (X). To a solution of 3.6 g (0.02 mole) of the hydrochloride of the ethyl ester of proline in 10 ml absolute benzene and 5 ml chloroform, purified on alumina, were added 5.1 ml (0.046 mole) of N-methylmorpholine and 2.2 ml (0.02 mole) of bromoacetic ester, and the mixture was stirred for 2 h at room temperature. The reaction mixture was washed with water, dried with anhydrous sodium sulfate, the solvent was removed by evaporation in vacuo, and the residue chromatographed on a column with silica gel (chloroform as eluent). Yield 3.6 g (78%) of the ethyl ester of N-carbethoxymethyl-L-proline. R_f 0.85 (chloroform–methanol, 10:1). The amide was obtained by ammonolysis by the typical method, with a yield of 89%, mp 165-167°C (ethanol), R_f 0.48 (dioxane–water, 10:1), $[\alpha]_D^{20}$ −78° (c 2, water). $C_7H_{13}N_3O_2$.

EXPERIMENTAL PHARMACOLOGICAL

The effect of the preparations on learning and memory was studied by a modified PALT model [12]. The investigations were carried out on noninbred male rats weighing 180-200 g. The test preparation was injected intraperitoneally in a volume of 2 ml/100 g body weight 15 min before learning. Animals of the control group were given an injection of 0.9% sodium chloride solution. The rat was then placed in the lit compartment of a two-compartment box (39 × 39 × 39 cm). After 180 sec, it was placed in the dark compartment (15 × 15 × 39 cm) and with the partition closed, unavoidable foot shock was applied with five consecutive ac shocks (50 V, each 1 sec in duration, intervals between shocks 2 sec). Preservation of PALT was determined after 24 h. For this purpose the animal was placed in the lit compartment and the total length of stay T in the dark compartment was measured. Changes in the ability of the rats to learn under the influence of the substances were determined by the formula developed previously [1]:

$$A_t = \frac{T_c - T_e}{T_c - T_0} \times 100\%,$$

where A_t denotes the test activity; T_c the mean length of stay of rats of the control group in the dark compartment; T_e the same for rats of the experimental group; T_0 the same for untrained animals, for the case $T_c < T_e$ and $T_e = 0$, if $T_c > T_e$.

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