PEPTIDE ANALOGS OF PYRACETAM AS LIGANDS FOR HYPOTHETICAL NOOTROPIC RECEPTORS

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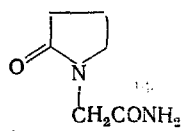
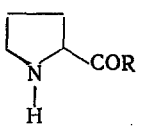
Pyracetam (N-carbamoylmethylbutyrolactam (I)) is the first representative of nootropic agents, a novel class of psychotropic drugs [18]. Nootropic agents are generally accepted as being drugs which have selective effects on the brain, enhancing the control of the cortex over the subcortical systems and stimulating the higher integrative functions of the brain (thought, attention, and memory) [2]. Pyracetam is used in medicine for improving intellectual and memory function in children with retarded intellectual development [25], to reduce the severity of the psycho-organic syndrome in cerebral changes due to aging [15], and to improve the rate of restoration of memory in patients with traumatic [21] or cerebrovascular [1] disturbances. The nootropic activity of pyracetam is however inadequate [19]. Attempts have been made to increase the effectiveness of pyracetam by structural changes, namely by varying the size of the ring [8], the structure of the side chain [9, 10], and the substituents at the exocyclic nitrogen [12]. A search has been made for a compound with nootropic activity amongst hetero-analogs of pyracetam [11].

In a search for new types of compounds with the desired nootropic activity, we adopted the working hypothesis that specific nootropic pyracetam receptors are present in the CNS. On this assumption, the basis of the nootropic activity of pyracetam could be its structural similarity to the endogenous ligands of these hypothetical receptors. An important step in proving the validity of this working hypothesis would be the identification of candidates for the role of such endogenous ligands, and neuropharmacological examination of their effects, in particular comparison of their behavioral effects with that produced by pyracetam,* measurement of selective agonistic and antagonistic effects, and the occurrence of stereospecificity.

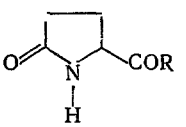
Biogenic compounds with chemical structures related to that of pyracetam include the cyclic aminoacids proline (II) and pyroglutamic acid (IV), derivatives of which could be the first candidates for the endogenous ligands mentioned above.



I



II, III
II R = OH;
III R = $OC_{16}H_{33}$;



IV, X
IV R = OH;
X R = $NH(CH_2)_nCONH_2$

On this basis, and considering the principal property of (I), namely normalization of disturbances of memory, we compared the effects of (I) with those of some derivatives of (II) and (IV), and we also evaluated the interactions of these compounds.

It is known that L-proline [14], when introduced directly into the brain of neonatal chicks, reduces the number of appropriate conditioned reflex responses. We have previously shown [5] that the lipophilic cetyl ester of L-proline (III) can also penetrate the blood-brain barrier when the brain is mature.† In experiments on rats with learned passive

*For the significance of behavioral methods in neurotropic studies, see [20].
†We used this method to obtain a GABA derivative which was capable of penetrating the CNS [3], and the method has been used subsequently by several other workers [16, 17].

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TABLE 1. Properties of Dipeptide Esters (VI)

Compound	Yield, %	mp[a], °C	$[\alpha]_D^b$, deg	R_f^c	Found, %			Molecular formula	Calculated, %			M+
					C	H	N		C	H	N	
L-pGlu-Gly-OCH_3	60	100—1	—17,8	0,65	47,95	6,04	14,05	$C_8H_{12}N_2O_4$	47,99	6,05	13,98	200
L-pGlu-Gly-OC_2H_5		119—20	—20,8	0,65	50,16	6,43	13,21	$C_9H_{14}N_2O_4$	50,41	6,60	13,07	214
D-pGlu-Gly-OC_2H_5	65	118—20	+27,6	0,65	50,21	6,58	12,98	$C_9H_{14}N_2O_4$	50,41	6,60	13,07	214
L-pGlu-D-Ala-OC_2H_5	70	...	+37,5	0,72	52,43	7,21	12,03	$C_{10}H_{16}N_2O_4$	52,61	7,08	12,27	228
L-pGlu-β-Ala-OC_2H_5	66	50—1	—10,0	0,62	52,56	7,27	12,12	$C_{10}H_{16}N_2O_4$	52,61	7,08	12,27	228
L-pGlu-GABA-OC_4H_9	74	77—8	—1,7	0,80	58,08	8,03	10,46	$C_{13}H_{22}N_2O_4$	57,75	8,21	10,35	270
L-pGlu-GABA-$OC_{16}H_{33}$	49	96—7	—36,2[a]	0,25[e]	68,16	10,51	6,36	$C_{25}H_{46}N_2O_4$	68,43	10,59	6,38	438

Notes. [a] After purification by column chromatography. [b] c = 2.0, methanol. [c] Kieselgel, dioxane—water, 10:1. [d] c = 2, chloroform. [e] Kieselgel, chloroform—methanol, 19:1.

TABLE 2. Properties of Dipeptide Amides (X)

Compound	Yield, %	mp, °C	$[\alpha]_D^a$, deg	R_f^b	Found, %			Molecular formula	Calculated, %			M+
					C	H	N		C	H	N	
L-pGlu-Gly-NH_2	100	195—6[c]	—9,1[c]	0,30	...	...	22,69	$C_7H_{11}N_3O_3$	...	...	22,68	185
D-pGlu-Gly-NH_2	100	195—6	+8,0	0,30	...	...	22,70	$C_7H_{11}N_3O_3$	...	...	22,68	185
L-pGlu-D-Ala-NH_2	98	193—4	+33,6	0,64[d]	44,35	6,99	19,61	$C_8H_{13}N_3O_3 \cdot H_2O$	44,23	6,97	19,33	199
L-pGlu-β-Ala-NH_2	98	210—2	—10,0	0,33	48,32	6,58	20,88	$C_8H_{13}N_3O_3$	48,23	6,59	21,07	199
L-pGlu-GABA-NH_2	70	152—3[e]	—2,6	0,28	50,44	7,21	19,72	$C_9H_{15}N_3O_3$	50,65	7,11	19,70	213

Notes. [a] c = 1.5, methanol. [b] Kieselgel, dioxane—water, 10:1. [c] Literature mp [22], 185-9°C; $[\alpha]_D^{25}$ -44.6° (c 1.0, acetic acid). [d] Kieselgel, dioxane—water, 20:3. [e] After column chromatography; literature mp [6], 159-160°C.

TABLE 3. Activity of Test Compounds and Mixtures in the PACR Test

Compound	Dose mg/kg	Dose μmole/liter	Number of animals	Activity, % A_n	Activity, % A_l	Activity, % A_t	P_t
Pyracetam	200	1400	40	18,2	19,0	20,3	0,025
N-($AcNH_2$)pGlu-NH_2 (VII)	250	1400	19	11,1	12,8	18,2	0,05
L-pGlu-Gly-NH_2	0,5	2,7	30	33,0	46,0	33,0	0,01
	5,0	27	30	14,0	22,4	12,5	0,5
	10,0	54	20	—33,3	—39,7	—45,0	0,025
D-pGlu-Gly-NH_2	0,5	2,7	20	—40,0	—43,0	—39,0	0,05
L-pGlu-D-Ala-NH_2	1,0	5,0	10	52,8	51,8	51,0	0,025
	10,0	50	10	35,7	34,5	31,0	0,1
L-pGlu-β-Ala-NH_2	1,0	5,0	59	25,4	25,8	26,4	0,01
L-pGlu-GABA-NH_2	1,1	5,0	20	16,6	12,2	11,5	0,01
L-pGlu-GABA-OC_4H_9	1,4	5,0	10	14,3	14,4	14,2	0,1
L-pGlu-GABA-$OC_{16}H_{33}$	2,2	5,0	49	17,3	19,2	19,2	0,01
L-pGlu-Gly-NH_2 + L-Pro-$OC_{16}H_{33}$	0,5 30	2,7 8,8	20	—28,0	—27,6	—16,5	0,01
L-Pro-$OC_{16}H_{33}$ + pyracetam	30 200	8,8 1400	34	—71	—63,8	—94,5	0,01
L-Pro-$OC_{16}H_{33}$	30	8,8	24	—16,7	—7,3	—11,3	0,01
L-Glu-GABA-$OC_{16}H_{33}$ + bicuculin	2,2 1,0	5,0 2,7	16	37,5	43,3	36,1	0,01

Notes. A_n is the activity expressed in terms of the mean number of animals which did not enter the darkened chamber (n); A_l the activity in respect of the mean latent period for the first entry into the darkened chamber; A_t the activity in respect of the mean residence time of the animals in the light chamber; P_t is the reliability of the factor t, calculated by the Wilcoxon—Mann—Whiting method; and the sign " — " denotes amnesic activity.

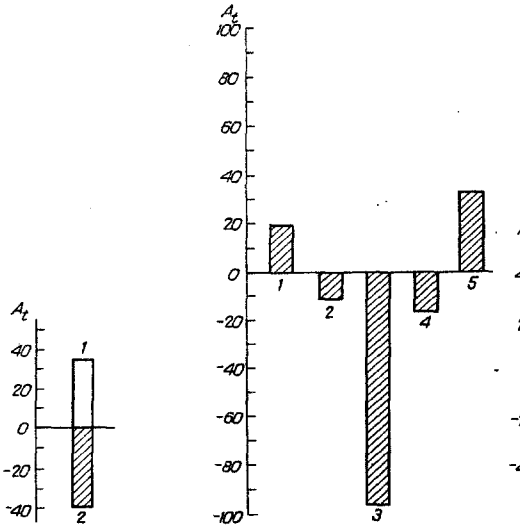


Fig. 1

Fig. 2

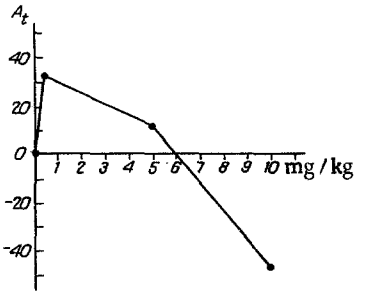


Fig. 3

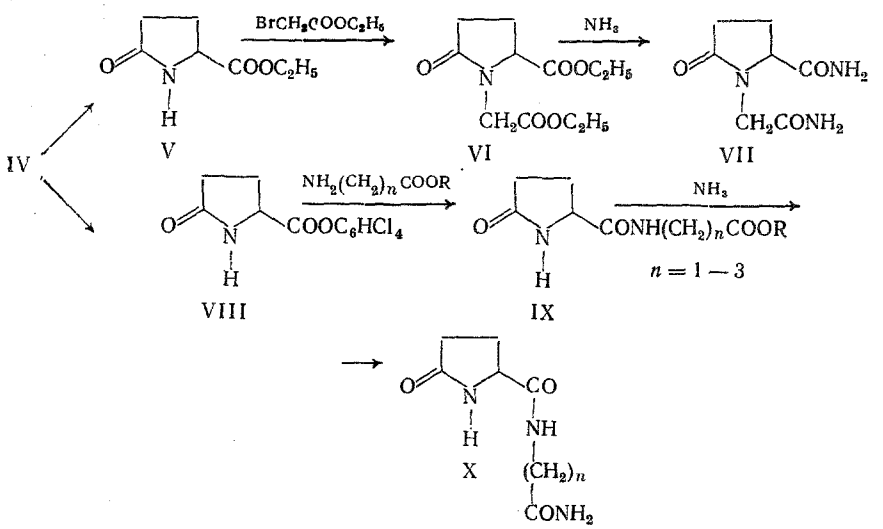
Fig. 1. Stereospecific activity of pyroglutamylglycine amide in the PACR test. Here and in Figs. 2 and 3, A_t is the mnesic activity in respect of the factor t (in %). 1) L-pyroglutamylglycine amide; 2) D-pyroglutamylglycine amide.

Fig. 2. Antagonism of the cetyl ester of L-proline to pyracetamide and L-pyroglutamylglycine amide. 1) pyracetam (200 mg/kg); 2) pyracetam (200 mg/kg + L-proline cetyl ester (30 mg/kg); 3) L-proline cetyl ester (30 mg/kg); 4) L-pyroglutamylglycine amide (0.5 mg/kg) + L-proline cetyl ester (30 mg/kg); 5) L-pyroglutamylglycine amide (0.5 mg/kg).

Fig. 3. Dose plot of L-pyroglutamylglycine. Horizontal axis, dose (mg/kg); vetical axis, A_t (%).

avoidance conditioned reflex (PACR) this compound, when administered systemically, caused amnesia which was alleviated by pyracetam. This leads to the conclusion that L-proline and some of its derivatives are antagonists for the nootropic receptors. In a search for agonists for these receptors (nootropes) which were more effective than (I), we prepared and examined a number of derivatives of pyroglutamic acid, which is structurally related to pyracetam and proline, and which is present as an N-terminal moiety in many peptide regulators.

A number of compounds were synthesized in which the structure of (I) was converted into the structures of oligopeptide derivatives of pyroglutamic acid (X), and their nootropic activity examined.



Reaction of the sodio-derivative of ethyl L-pyroglutamate (V) with ethyl bromoacetate gave ethyl N-(ethoxycarbonylmethyl)-L-pyroglutamate (VI), converted by treatment with ammonia into N-carbamoylmethyl-L-pyroglutamide (VII).

Dipeptides derived from pyroglutamic acid were synthesized by the activated ester method, from 2,3,4,6-tetrachlorophenyl pyroglutamate (VIII) and the hydrochlorides of the alkyl esters of the appropriate aminoacids, in dimethylformamide at room temperature in the presence of triethylamine (Table 1). The dipeptide esters (IX) were isolated and purified by chromatography on ion exchange resins and silica gel (Table 2). In the separation of the ethyl ester of pyroglutamylglycine on an ion exchange resin in methanol, transesterification occured to give a mixture of the methyl and ethyl esters, which were than separated by high-performance liquid chromatography (HPLC). The esters (IX) were converted into the amides (X) by keeping them for 24 h in methanol saturated with ammonia.

Learning and memory, as assessed by the PACR test, were chosen as models of the higher integrative functions of the brain. The nootropic effect is known to be not apparent under intensive learning conditions. In order to detect this activity, it is necessary to use initially untrained animals, or animals subjected to the influence of an amnesic factor. In the present study, conditions were used in the study of nootropic activity which gave on average 50% learning. The antihypoxic activity is not necessarily related to nootropic (antiamnesic) activity [4, 12].

The N-carbamoylmethyl derivative of pyroglutamide (VII) had the same experimental activity as (I), amounting to 18% at a dose of 250 mg/kg. Hence, the introduction of a carbamoyl group into the 5-position of pyracetam has no effect on activity. In the case of L-pyroglutamylglycine amide (XX, n = 1), the activity was 33% even at a dose of 0.5 mg/kg (i.e., 2.7 μmole/kg). Thus, moving the glycine moiety from position 1 to position 5 of the ring (using the numbering of the substituents in pyracetam) considerably enhances activity. Subsequent changes in the structure comprised increasing the distance between the pyrrolidone ring and the amide group by introducing β-alanine amide or γ-aminobutyric acid (GABA) amide. It was found that in this series activity decreased from L-pyroglutamylglycine amide (XX,

n = 1) to L-pyroglutamyl-β-alanine amide (XX, n = 2) (33 and 26% respectively) and to L-pyroglutamyl-GABA amide (XX, n = 3) (12%), in equimolar doses (Table 3).

L-pyroglutamylglycine amide (X, n = 1) and the other pyroglutamic acid derivatives (X, n = 2, 3) had the same type of effect on all the parameters measured, namely the latent period for the entry of the rats into a darkened chamber, the numbers of animals which did not enter the darkened chamber, or the mean time of residence of the animals in the lighted chamber. The activities calculated from these observations were similar to each other, and the choice of the factor had no effect on the relative activities of the compounds.

The activities of the compounds which we synthesized and examined in the PACR test were not related to general stimulant activity, since they had no effect on motor activity.

It may also be assumed that the active entity in compounds (X) is glutamic acid formed on their hydrolysis, which activates the phosphorylation of the proteins of the cerebral cortex [23], thereby participating in memory processes. It appears, however, that these compounds cannot be regarded as a means of transporting glutamic acid across the blood-brain barrier, since diethyl glutamate, which is also able to pass this barrier, has no effect on learning in rats as assessed by the PACR test. This leads to the conclusion that these pyroglutamic acid derivatives act on the CNS in the unchanged cyclic form.

The effect of L-pyroglutamyl-GABA amide (XX, n = 3) on learning is not due to GABA-ergic mechanisms, since the GABA antagonist bicuculin did not affect the influence of this compound on learning. In a dose of 1 mg/kg, bicuculin itself had no effect on the development and maintenance of PACR in rats.

These derivatives of L-pyroglutamic acid have nootropic activity, since their activity is 1.5–2 times greater than that of pyracetam, in substantially lower doses, showing that they have high affinity for the hypothetical receptors. By the intraperitoneal route, they are metabolized much more readily than pyracetam, so that their true effective doses must be even lower. These findings are in accordance with the hypothesis that these compounds interact selectively with nootropic receptors.

Important additional proof of the occurence of specific nooreceptors in the CNS is our observation of stereospecifity in the effects of pyroglutamylglycine amides on learning and memory. In a dose of 0.5 mg/kg, L-pyroglutamylglycine amide improves memory, whereas D-pyroglutamylglycine amide in the same dose has amnesic effects (Fig. 1).

Support for the reaction of pyracetam with the same receptors is provided both by its structural similarity to L-pyroglutamylglycine, and by the antagonism of both these compounds to L-proline cetyl ester (III). It has been found that the amnesic activity of L-proline cetyl ester is almost completely negated by 1/3 of an equimolar dose of L-pyroglutamylglycine amide (X, n = 1), in a similar way to pyracetam in the usual dose of 200 mg/kg (Fig. 2). The dose–activity plot of L-pyroglutamylglycine has the dome shape characteristic of neuropeptides, with inversion of the effect at high doses (Fig. 3).

These results lead to some preliminary conclusions as to the structure of the binding site for the ligand to the nootropic receptor. It probably contains regions for binding the pyrrolidine ring and the carbamoyl group, which may be either the carbamoyl group in the 5-position, or the acetamide group in position 1 of the ring (in Dreiding molecular models, these groups can be spatially coincident).

When the above groups are present, the carbonyl group in the 2-position is not essential for binding, but it of considerable significance in determining the physiological end-result. Its removal results in the conversion of an agonist, L-pyroglutamylglycine amide, into an antagonist, an L-proline derivative. The configuration of the carbon atom in the 5-position is similarly important. A change in this configuration results in the conversion of an agonist, L-pyroglutamylglycine amide, into an antagonist, D-pyroglutamylglycine amide. In all likelihood, there is also a binding region for the second aminoacid, the nature of which influences the affinity of the ligand for the receptor, although its configuration is not of great significance, since L-pyroglutamyl-D-alanine amide is also effective in improving learning in the PACR test (Table 3).

We have therefore accomplished the directed synthesis of pyroglutamic acid derivatives which are peptide analogs of pyracetam, and carried out tests of these compounds in learning models. The results provide neuropharmacological proof of the occurrence in the CNS of specific nootropic receptors. Support for this view is provided by the stereospecificity of

the effects of the pyroglutamic acid oligopeptides examined, their activity at low doses, the occurrence of agonism and antagonism with slight structural changes, and the dome-shaped dose–effect plots. These derivatives of pyroglutamic acid and proline appear to be compounds which are structurally similar to the endogenous ligands of the nootropic receptors.

EXPERIMENTAL (CHEMICAL)

Mass spectra were obtained an a MAT-112 mass spectrometer (Varian, USA), source temperature 100–120°C for the dipeptide esters and 250°C for amides, ionization energy 70 eV, and PMR spectra on a Varian T-60 spectrometer. The specific optical rotation was measured on an A-1-EPO polarimeter.

Ethyl N-Ethoxycarbonylmethyl-L-pyroglutamate (VI). To a suspension of 0.6 g (0.024 mole) of 80% sodium hydride in 50 ml of dry benzene was added a solution of 4 g (0.024 mole) of ethyl L-pyroglutamate (V) in 100 ml of dry benzene. The mixture was stirred for 30 min at room temperature and 10 min at 50-60°C, then cooled to 0°C and 2.65 ml (0.024 mole) of freshly-distilled ethyl bromoacetate added slowly. The mixture was stirred for a further 2 h at room temperature, the solid filtered off, the filtrate evaporated, and the residue chromatographed on a silica gel column (eluent benzene–ethyl acetate–methanol, 4:1:1) to give 2.6 g (50%) of a clear oil, R_f 0.64 (kieselgel, ethyl acetate–benzene–methanol, 4:4:1). M^+ 243, M_{calc} 243 $[\alpha]_D$ -15.05° (c 2, chloroform). PMR spectrum in $CDCl_3$ (δ, ppm): 2.4 (m, $-CH_2-CH_2-$ ring, 4H); 1.2 (t, $O-CH_2-CH_3$, 6H). Found, %: C 53.87; H 7.12; N 5.57. $C_{11}H_{17}O_5N$. Calculated, %: C 54.30; H 7.06; N 5.75.

N-Carbamoylmethyl-L-pyroglutamide (VII). A solution of 0.6 g of ethyl N-ethoxycarbonyl-L-pyroglutamate in 30 ml of methanol saturated with ammonia was kept overnight at room temperature. The solvent was then distilled off, and the residue recrystallized from absolute ethanol to give 0.5 g (VII) (100%) as colorless crystals, mp 178-180°C, R_f 0.58 (kieselgel, dioxane–water, 10:1). M^+ 185, M_{calc} 185. $[\alpha]_D$ 0° (c 2, methanol). PMR spectrum in D_2O: 2.5 (m, $-CH_2-CH_2-$ ring), 3.66 (d, $N-CH_2$), 4.27 (d, $N-CH_2$), J 16 Hz. Found, %: C 45.42; H 6.04; N 22.52. $C_7H_{11}N_3O_3$. Calculated, %: C 45.40; H 5.99; N 22.70. Literature mp 180°C [7].

2,3,4,6-Tetrachlorophenyl L-Pyroglutamate (VIII). To a suspension of 12.9 g (0.1 mole) of L-pyroglutamic acid in 200 ml of dry THF was added 27.8 g (0.12 mole) of 2,3,4,6-tetrachlorophenol followed at 0°C by 21.8 g (0.106 mole) of cyclohexylcarbodiimide in 50 ml of dry THF. The mixture was stirred for 1 h at 0°C, then kept overnight at room temperature. The solid was filtered off, the solvent evaporated under reduced pressure, and the residue recrystallized from propan-2-ol to give 17.4 g (50%) of (VIII), mp 158°C, $[\alpha]_D$ + 17.0° (c 1, DMF.) Found, %: C 38.22; H 2.03; Cl 41.60. $C_{11}H_7NO_3Cl_4$. Calculated; %: C 38.50; H 2.06; Cl 41.39.

Obtained similarly was pentachlorophenyl D-pyroglutamate, mp 198°C, $[\alpha]_D$ −18.0° (c 2, DMF.) Found, %: C 35.10; H 1, 75; N 3.44; Cl 46.83. $C_{11}H_6NO_3Cl_5$. Calculated, %: C 34.98; N 1.60; N 3.71; Cl 47.00.

L-Pyroglutamylglycine Ethyl Ester (IX, n = 1). A mixture of 3.43 g (10 mole) of the tetrachlorophenyl L-pyroglutamate, 1.6 g (12 mmole) of glycine ethyl ester hydrochloride, and 1.66 ml (12 mmole) of triethylamine in 40 ml of DMF was stirred for 4 h at room temperature. The precipitated triethylamine hydrochloride was filtered off, the filtrate evaporated under reduced pressure, and the residual oil dissolved in 80% methanol and passed through the ion exchange resin AGI-x8 (OH^- form) and cation exchange resin Q-150 (H^+ form) to give 1.2 g of a chromatographically homogeneous compound with R_f 0.65 (kieselgel, dioxane–water, 10:1). HPLC on a 250 × 35 mm column with Likhrosorb S-8.5 μm, gradient elution with water–methanol 0–100%, 10 ml/min, 60 min (Analyst 7900 apparatus, LDTs, Ireland–USA) separated the compound into two peaks with retention times of 26.5 and 35 min. Evaporation of the fractions corresponding to the first peak gave 0.6 g of L-pyroglutamylglycine methyl ester, mp 100-101°C (hygroscopic). M^+ 200, M_{calc} 200, $[\alpha]_D$ −17.8° (c 2, methanol). UV spectrum: λ_{max} 217.7 nm. Found, %: C 47.95; H 6.04; N 14.05. $C_8H_{12}N_2O_4$. Calculated, %: C 47.99, H 6.05; N 13.98.

Evaporation of the fractions corresponding to the second peak gave 0.6 g of L-pyroglutamylglycine ethyl ester, mp 119.5-120.5°C (literature mp 118-120°C [24]). UV spectrum: λ_{max} 207.7 nm, M^+ 214, M_{calc} 214. $[\alpha]_D$ −20.8° (c 2, methanol). Found, %: C 50.16; H 6.43; N 13.21. $C_9H_{14}N_2O_4$. Calculated, %: C 50.41; H 6.60; N 13.07.

Alkyl Pyroglutamates (IX) (General Method). To a solution of 10 mmole of the tetrachlorophenyl or pentachlorophenyl pyroglutamate in 40 ml of DMF was added 12 mmole of the hydrochloride of the alkyl ester of the appropriate aminoacid and 12 mmole of triethylamine. The mixture was stirred for 5 h at room temperature, and kept overnight. The solvent was removed under reduced pressure, and the residue chromatographed on a column of silica gel (eluent dioxane–water, 10:1). The yields and constants of the resulting esters are given in Table 1.

Pyroglutamylaminoamides (X) (General Method). A solution of 0.02 mole of the pyroglutamate ester in 50 ml of methanol saturated with gaseous ammonia was kept for 1–2 days at room temperature, the solvent removed under reduced pressure, and the residue washed with cold absolute ethanol and dry ether. If necessary, the product was chromatographed on a column of silica gel. The yields and properties of the pyroglutamylaminoamides obtained are given in Table 2.

Diethyl L-Glutamate Hydrochloride. A mixture of 1.47 g (0.01 mole) of L-glutamic acid and 9.68 g (0.04 mole) of cetyl alcohol in 100 ml of dry dioxane was treated with gaseous hydrogen chloride at 0°C for 30 min, then 15 min at 60°C. The hot solution was decanted, cooled, and the solid which separated was filtered off and washed thoroughly with ether to give 5 g (80%) of a colorless crystalline solid, mp 97.5-98°C (ethanol–ether), R_f 0.84 (kieselgel, butanol–3% aqueous ammonia, 7:3); R_f 0.88 (kieselgel, benzene–acetone, 1:1). M^+ 597, M_{calc} 597; $[\alpha]_D$ + 15.8° (c 1 $CHCl_3$). Found, %: C 69.84; H 11.76; N 2.85; Cl 5.42. $C_{37}H_{74}NO_4Cl$. Calculated, %: C 69.91; H 11.74; N 2.21; Cl 5.64.

Obtained similarly was the cetyl ester of L-proline, mp 80-81°C (ethanol–ether), R_f 0.76 (kieselgel, chloroform–methanol, 4:1); R_f 0.50 (kieselgel, butanol–3% aqueous ammonia, 7:3): $[\alpha]_D$ -19.2° (c 1.5 chloroform). Found, %: C 66.91; H 11.12; Cl 9.56; N 4.01. $C_{21}H_{42}ClNO_2$. Calculated, %: C 67.08; H 11.26; Cl 9.45; N 3.74.

EXPERIMENTAL (PHARMACOLOGICAL)

The effects of the compounds on learning and memory were examined in a modified PACR method in rats [13]. The experimental animals were mongrel male rats weighing 180-200 g. The test drug was administered intraperitoneally in a volume of 0.2 ml per 100 g body weight, 15 min before training. The animals in the control group received 0.9% sodium chloride solution. The rats were then placed in the lighted section of a two-section chamber. After 180 sec, they were subjected in the darkened section to an unavoidable electrical pain stimulus through the floor, consisting of five successive stimuli of alternating current (50 V, one second duration each, interval between shocks 2 sec). Retention of the PACR was estimated after 24 h. For this purpose, the animals were placed in the lighted section, and the latent period before entry into the darkened section (l), the total time of residence in the lightened section (t), and the number of animals which did not enter the darkened section for 180 sec (n) were measured.

Changes in the trainability of the rats under the influence of the drugs were determined by the following expression (which is a modification of the formula given in [12]):

$$A_f = \frac{\bar{f}_{ex} - \bar{f}_{con}}{\bar{f}_{ext} - \bar{f}_{con}} \times 100\%,$$

where A_f is the mnesic activity in terms of f (f = t, l, or n), and is numerically equal to the increase or decrease in trainability of the animals as compare with the controls, as a percentage of the maximum possible effect; $\bar{f}_{ex}$ is the mean value of the parameter for the experimental animals, $\bar{f}_{con}$ the same for the control animals, and $\bar{f}_{ext}$ is the extreme value of the parameter. When $\bar{f}_{ex} > \bar{f}_{con}$ (improved trainability, antiamnesic effect), $\bar{f}_{ext}$ is equal to the value of the parameter for fully trained animals, and when $\bar{f}_{exp} < \bar{f}_{cpm}$ (deterioration in trainability, amnesic effect), $\bar{f}_{ext}$ is equal to the value of the parameter for untrained animals.

The antagonism of the cetyl ester of L-proline to L-pyroglutamylglycine amide was assessed by administering the cetyl ester of L-proline intraperitoneally in a dose of 30 mg/kg (the dose at which this compound antagonizes the effects of pyracetam [15]) 45 min before training, followed by administration of 0.5 mg/kg of L-pyroglutamylglycine amide 15 min before training, followed by training and measurement of trainability as described above.

The effects of the compounds on motor activity were assessed in mice using a Varian Opto-Varimex (Kalamazoo) multichannel apparatus for the recording of motor activity.

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