DIESTERS OF GLUTAMINIC ACID: SYNTHESIS AND PRIMARY PHARMACOLOGICAL INVESTIGATIONS

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The pharmacological spectrum of glutaminic acid (Glu) and its structural analogs is very broad. After discovery of the mediator properties of Glu on the central nervous system (CNS) of mammals the interest in these compounds has increased considerably [9]. However, derivatives and analogs of Glu, which carry three ionic charges at physiological pH values, are highly polar compounds and penetrate badly through the hematoencephalic barrier. Therefore, the effect of these compounds is extremely weakly expressed in the case of systemic administration. One of the ways to increase the transport properties of medicines is the production of prodrugs, compounds that penetrate in the tissue or target organ and are there decomposed with liberation of the active compound [7]. In the case of carboxylic acids the prodrugs may be their esters.

With this in mind the subject of this article is the synthesis and investigation of the pharmacological properties of a series esters of Glu that have been selected as model compounds for the creation of prodrugs of stimulating amino acids.

The literature contains isolated articles containing contradictory data on the biological properties of some diesters of Glu: diethyl, propyl, and diisobutyl esters [2], and two diesters of higher alcohols: didecyl and dicetyl esters [4, 8, 10]. Investigation of homologous series of chemical compounds show that in a series of homologs not only the physicochemical but also the biological properties of the compounds may change sharply [1].

The synthesis of a number of diesters (VIII-XIII) of Glu (I) was carried out according to the following scheme:

$$\underset{\text{I}}{H_2NCH(COOH)CH_2CH_2COOH} + \underset{\text{II-VII}}{HOR} \xrightarrow{HX} \rightarrow \underset{\text{VIII-XIII}}{H_2NCH(COOR)CH_2CH_2COOR \cdot HX}$$

EXPERIMENTAL (CHEMICAL)

The specific optical rotation was measured on an automatic Polamat A polarimeter. TLC was carried out on glass plates (Merck), spots were visualized with ninhydrin and chlorine-benzidine, eluent n-butanol–pyridine–acetic acid–water, 21:12:2:15. Potentiometric titrations were carried out on a Ionomer I-130M instrument, glass electrode ECL-63.07.

Dicetyl L-Glutaminate, p-Toluenesulfonate (XIII). To 0.090 mole of cetyl alcohol, 0.030 mole of L-glutaminic acid, and 0.033 mole of p-toluenesulfonic acid monohydrate is added 75 ml of water-free benzene and the mixture is refluxed in a round-bottomed flask at a Dean–Stark condenser for 6-8 h till the separation of water stops. Then 200 ml of dry ether is added and the mixture is stored in the refrigerator overnight. The precipitate is filtered off and washed thoroughly on the filter to remove the starting alcohol. The residue is recrystallized from aqueous alcohol. Yields and constants are listed in Table 1.

Diesters of L-Glutaminic Acid, Hydrochlorides (VIII, XI, XII). A suspension of 0.025-0.027 mole of the corresponding p-toluenesulfonate prepared by the method described above (without recrystallization) in 200 ml of ethyl acetate is extracted with a 5% $NaHCO_3$ solution (2 × 200 ml). The ethyl acetate solution of the free base is dried over $MgSO_4$ and to it is added one equivalent of a solution of gaseous hydrochloric acid in dry ethyl acetate. The hydrochloride of the corresponding diester of L-glutaminic acid precipitates; this is filtered off and washed with ethyl acetate.

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TABLE 1. Diesters of L-Glutaminic Acid: VIII-XIII

№	R	X	$[\alpha]_D^{20}$	mp, °C	Yield, %	Empirical formula
VIII	CH_2Ph	Cl	+9	100—102	—	
IX	C_2H_5	Cl	+22	113—114	—	
X	$C_{10}H_{21}$	Cl	+16	79—80	25	$C_{25}H_{50}NO_4Cl$
XI	$C_{12}H_{25}$	Cl	+13	86—87	63	$C_{29}H_{58}NO_4Cl$
XII	$C_{14}H_{29}$	Cl	+10	92—93	53	$C_{33}H_{66}NO_4Cl$
XIII	$C_{16}H_{33}$	TosO	+9	67—69	78	$C_{44}H_{81}NO_7S$

TABLE 2. Spectrum of Pharmacological Activities of Diesters of L-Glutaminic Acid

Name of the test	VIII	IX	X	XI	XII	XIII
Spontaneous locomotive activity	+	0	0	—	—	—
Hexenal-induced sleep	0	0	0	0	0	+
Antagonism to corazole with respect to the convulsive effect	0	0	0	0	0	++
Antihypoxic effect	0	0	+	0	0	0
Preservation of CRPA	+	0	0	0	0	0

Notes. +) Increase of the effect; 0) no visible effect; −) lowering of the effect.

Didecyl L-glutaminate (X) was prepared according to [5].

Diethyl L-glutaminate hydrochloride (IX) was prepared according to [3].

Hydrolysis of Diesters of L-Glutaminic Acid (VIII and IX). In a thermostated potentiometric cell at 37°C are placed 20 ml of a 0.5 M potassium chloride solution, 0.5 ml of phosphate buffer (ionic strength 0.10, pH 7.4) and with stirring is added 2.5 ml of rat blood serum. The mixture is allowed to stand till the pH is constant, then 0.1 N hydrochloric acid is added to pH 7.35, and 0.02 mole of the diester of glutaminic acid is added. The acid produced by hydrolysis is titrated with 0.1 N KOH solution.

EXPERIMENTAL (PHARMACOLOGICAL)

Pharmacological tests of compounds (VIII-XIII) were carried out with mice and male rates weighing 18-20 and 180-200 g, respectively. We have determined the acute toxicity (LD_{50}) of the compounds, their effect on the spontaneous locomotive activity, the duration of hexenal-induced (70 mg/kg) sleep, corazole-induced convulsions (anticorazole test), the conditional reaction of passive avoidance (CRPA), electrodermal irritation, and their antihypoxic properties.

In the case of intraperitoneal administration the LD_{50} for all the compounds was more than 2000 mg/kg. At a dose of 100 mg/kg (intraperitoneally) the compounds under investigation differently affected the spontaneous locomotive activity of mice recorded for 30 min after administration on a DAÉR-20 ÉPM instrument of the Academy of Medical Sciences of the USSR. Compounds XI-XIII lowered, and compound VIII, for example, increased the locomotive activities of the animals (Table 2).

Compound XIII increases and compound XI lowers the narcotic activity of hexenal.

The anticorazole test comprised determination of the latent period of the beginning of convulsions caused by intraperitoneal administration of 110 mg/kg of corazole (in the control about 90 sec) and their duration (in the control not more than 8 min) with subsequent death of the animals in 100% of the cases. Compound XIII (100 mg/kg) did not only delay the onset of convulsions but also enabled the survival of 40% of the animals. On the other hand, compound IX promoted the occurrence of corazole convulsions.

With the model of hypoxia with hypercapnia the presence of moderate antihypoxic activity was found in compounds X and XI. When determining the effect of compounds VIII-XIII on the preservation of CRPA in one experiment [6] it was demonstrated with rats that only compound XIII evoked preservation of avoidance experience provided that it was administered immediately after the situation of teaching CRPA. The other comounds were inactive in this model.

By means of potentiometric titration the hydrolytic stability of diesters VIII and IX was studied in blood serum of rats. It was found that the diethyl ester of Glu (IX) is hydrolyzed rather rapidly to free glutaminic acid (the half lifetime is about 12 min). Under the same conditions and in the same time (10-20 min) dibenzyl ester (VIII) is hydrolyzed only to the monobenzyl ester, which was confirmed by TLC.

The data that we have obtained makes it possible to assume that some of the investigated compounds can penetrate through the hematoencephalic barrier and evoke the exciting effects. To this points the capacity of diester (XI) to lower the duration of hexenal-induced sleep,

to speed up the onset of corazole convulsions under the influence of compound (IX), and the capacity of diester (VIII) to considerably increase the locomotive activity and to improve the preservation of CRPA in rats. Further examination of the distinct antagonism of diester (XIII) to corazole is required, which makes it possible to assume that this compound has anticonvulsive properties, possibly as a result of antagonism to Glu. We may assume that the differences in the pharmacological properties of diesters (VIII-XIII) are connected with the different rate of generating Glu from them under influence of blood enzymes.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF AMIDES AND NITRILES OF 2-ARYLAMINO-5-CARBOXY(CARBETHOXY)-6-METHYLNICOTINIC ACIDS AND 1-ARYL-6-CARBETHOXY-7-METHYL-4-OXO-1,4-DIHYDROPYRIDO[2,3-d]PYRIMIDINES

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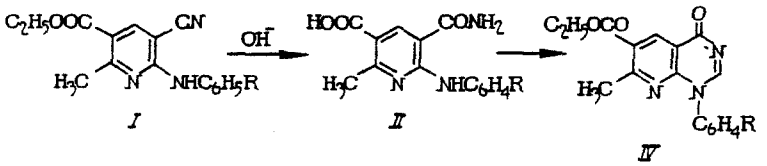
Amides and nitriles of 2-arylaminonicotinic acids have anticonvulsive properties [8] and are also used as starting compounds for the preparation of pyrido[2,3-d]pyrimidines [7]. With the aim of further search for biologically active compounds of this series we have carried out the synthesis of 2-arylamino-5-carbethoxy-6-methylnicotinonitriles (Ia-f, Table 1) and 2-arylamino-5-carboxy-6-methylnicotinamides (IIa-d).



R=2′-MeO (Ia, IIa, IIIa, IVa), 2′-Me (Ib, IIb, IIIb, IVb), 3′-Me (Ic, IIc, IIIc, IVc), 4′-Br (Id IId.IIId.IVd), 4′-Me (Ie), 3′-Br (If).

Nitriles Ia-d were prepared in high yields by heating 5-carbethoxy-6-methyl-2-chloronicotinonitrile with aryl amines in ethanol.

Investigation showed that both the ester group and the nitrile group in compounds Ia-d are easily saponified with an alcoholic potassium hydroxide solution under mild conditions and that in yields of 76-88% 2-arylamino-5-carboxy-6-methylnicotinamides IIa-d are formed. Reaction of amides IIa-d with sodium hydroxide yields the sodium salts of 2-arylamino-5-carboxy-6-methylnicotinamides (IIIa-d).

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