SYNTHESIS OF p-BIS(β-CHLOROETHYL)AMINOANILIDES OF PYRIDINECARBOXYLIC ACIDS AND A STUDY OF THEIR ANTITUMORAL ACTIVITY

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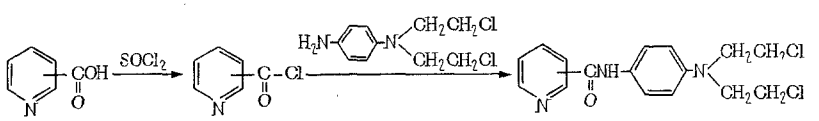
Some antimetabolites of nicotinamide such as 6-aminonicotinamide possess a considerable antitumoral activity [1-3]. The antitumoral activity of the antimetabolites of nicotinamide, which is a precursor of the pyridine nucleotides [4], is evidently connected with a deficiency in the cancer cell of the pyridine nucleotides which participate in its biosynthetic and oxidative reactions [5].

Some derivatives of pyridine containing cytotoxic groups—for example, 1-bis(β-chloroethyl)aminopyridine, 3-bis(β-chloroethyl)-aminomethylpyridine, etc.,—possess a considerable antitumoral effect in animal experiments, and 3-(β-chloroethylaminomethyl)-5-hydroxy-4-methoxy-6-methylpyridine has been used in clinical medicine and has proved effective [6]. The N,N-bis(β-chloroethyl)hydrazides of β- and γ-pyridinecarboxylic acids have also been synthesized [7], but their activity was found to be no higher than that of N,N-bis(β-chloroethyl)hydrazine [8].

In view of this, it appeared of interest to synthesize and study the antitumoral activity of analogs of nicotinamide containing cytotoxic groupings as compounds with a potential antitumoral effect. In view of the antitumoral activity of lymphochin [6, 9] and what has been said above, it appeared of interest to synthesize and study the antitumoral activity of the p-bis(β-chloroethyl)aminoanilide of nicotinic acid. A comparison of the action on malignant neoplasms of cytotoxic-group-containing amides of α-, β-, and γ-pyridinecarboxylic acids is also of definite interest for the elucidation of the connection between structure and biological activity.

We have synthesized the p-bis(β-chloroethyl)aminoanilides of α-, β-, and γ-pyridinecarboxylic acids (I-III) in the following way:



α—(I) β—(II) γ—(III)

The biological tests of the compounds mentioned showed that the β isomer II (which can be regarded as an antimetabolite of nicotinamide) is the least toxic and is highly active on the Walker carcinosarcoma, which is sensitive to chloroethylamines and has a differentiated action on transplanted leukoses. This compound II extends the life of mice with acute L_a leukosis by 82%, but is ineffective on other leukoses. The γ isomer III retards the development of all four leukoses in almost equal degree (Table 1), and is highly active on the Walker carcinosarcoma. The α isomer did not exhibit activity when it was studied on two different leukoses.

According to the literature [10, 11], a definite connection exists between the relative rate of hydrolysis of the chlorine in the bis(β-chloroethyl)amines and their biological activity. Consequently, it was of interest to compare the rate of hydrolysis of the chlorine in compounds I-III with their antitumoral effect. The rates of hydrolysis of the chlorine in compounds I-III, determined as described in the literature [12], proved to be similar (the percentage hydrolysis of the chlorine was 35-40 after 1 h, 50-55 after 2 h, and

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TABLE 1. Antitumor Activity of p-Bis(β-chloroethyl)aminoanilides of the Isomeric Pyridinecarboxylic Acids*

Compound, isomer	Dose (in mg/kg)		Effect (percentage extension of the lives of animals with leukosis and retardation of the growth of solid tumors				
	LD_{100}	therapeutic	Hemocytoblastosis L_a	lympholeukosis N_k/L_y †	lympholeukosis L_{1210}	reticulosis-erythroblastosis	Walker's carcinosarcoma
I, α	1 000	15	No effect	No effect	–	–	–
II, β	No losses from 1000	150 (for mice), 80 (for rats	82	No effect	–	–	92
III, γ	500	50 (for mice), 45 (for rats)	40	29	57	34	96

*The biological tests were carried out in the Laboratory of Experimental Chemotherapy (Director: Corresponding Member of the Academy of Medical Sciences of the USSR Prof. L. F. Larinov) of the Institute for Experimental and Clinical Oncology of the Academy of Medical Sciences of the USSR.
†Abbrev. for Nemeth-Kellner Ascites Lymphoma.

TABLE2. p-Bis(β-chloroethyl)aminoanilides of Pyridinecarboxylic Acids (I-III)

Compound, isomer	Yield (in	Melting point (in degrees), solvent for crystallization	External form	Found (in %)				Empirical Formula	Calculated (in %)*			
				C	H	Cl	N		C	H	Cl	H
I, α	54	98-101, benzene+petroleum ether	Yellow needles	56.25	4.92	21.08	12.32	$C_{16}H_{17}Cl_2N_3O$	56.81	5.06	20.96	12.42
II, β	44	156 (decomp., 2-propanol)	Ditto	56.76	5.16	21.04	12.21	$C_{16}H_{17}Cl_2N_3O$	56.81	5.06	20.96	12.42
III, γ	35	136-138, benzene	Lustrous plates	56.40	5.55	20.92	12.11	$C_{16}H_{17}Cl_2N_3O$	56.81	5.06	20.96	12.42

*Calculated on the acid.

65-70 after 3 h), although the biological activities of the compounds were different, i.e. in this case there is no appreciable relationship between the antitumoral activity on the strains studied and the chemical reactivity of the chlorine atoms in the bis(β-chloroethyl)amino groups.

EXPERIMENTAL

p-Bis(β-chloroethyl)aminoanilide of picolinic acid (I). A mixture of 3 g of picolinic acid and 50 ml of freshly distilled thionyl chloride was heated in the water bath for 1 h. The excess of thionyl chloride was distilled off in vacuum, the residue was treated with 100 ml of anhydrous benzene, 6.5 g of aminolymphochin hydrochloride, and 6.7 ml of anhydrous triethylamine and the mixture was heated in the water bath for 2 h. The triethylamine hydrochloride that formed was filtered off, the filtrate was treated with Al_2O_3, and the benzene was distilled off in vacuum to small bulk. The reaction product was then precipitated by the addition of petroleum ether.

p-Bis(β-chloroethyl)aminoanilide of nicotinic acid (II). A mixture of 1.5 g of nicotinic acid and 10 ml of thionyl chloride was boiled for 0.5 h and poured into 60 ml of anhydrous benzene. After cooling in a closed vessel, the nicotinoyl chloride hydrochloride was filtered off, washed with anhydrous benzene, and

rapidly transferred into 100 ml of anhydrous benzene containing 3.3 g of aminolymphochin hydrochloride and 5.3 ml of anhydrous triethylamine. The mixture was boiled for 1 h, and was then filtered hot, and the triethylamine hydrochloride was treated at the boil (10-15 min) with 100 ml of benzene and the mixture was filtered. The filtrates were combined and evaporated to small bulk in vacuum at a temperature not exceeding 40°C. The residue was cooled and the II was filtered off.

p-Bis(β-chloroethyl)aminoanilide of Isonicotinic Acid (III). A mixture of 5.5 g of isonicotinic acid and 10 ml of thionyl chloride was boiled for 1.5 h and poured into 100 ml of anhydrous benzene, and the isonicotinoyl chloride hydrochloride was filtered off, washed with anydrous benzene, and rapidly transferred into a reaction flask containing 200 ml of anhydrous benzene, 11.9 g of aminolymphochin hydrochloride, and 18.6 g of anhydrous triethylamine. The mixture was heated at 80-90°C for 2 h and was filtered hot, the filtrate was cooled, and the product III that had precipitated was filtered off (Table 2).

The relative rates of hydrolysis of the chlorine in the bis(β-chloroethyl)aminoanilides of the isomeric pyridinecarboxylic acids were determined in 50% aqueous ethanol at 50°C with a concentration of the substance under investigation of $0.558 \cdot 10^{-3}$ M.

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