SYNTHESIS OF THE ANTINEOPLASTIC PREPARATION FOPURIN, LABELED WITH THE PHOSPHORUS ISOTOPE ^{32}P, AND STUDY OF ITS METABOLISM IN THE ANIMAL ORGANISM

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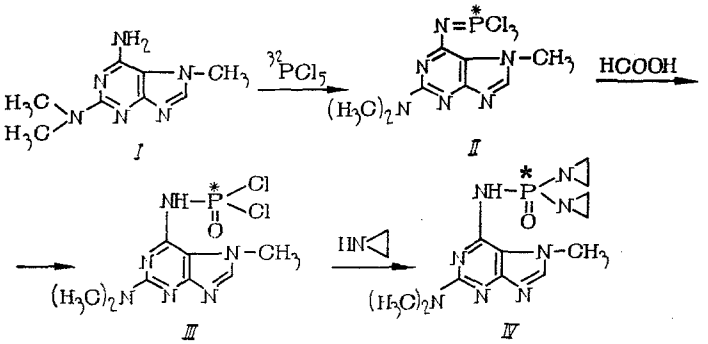
2-Dimethylamino-6-diethylenimidophosphamido-7-methylpurine (fopurin), synthesized at the S. Ordzhonikidze All-Union Chemicopharmaceutical Scientific Research Institute, is now under clinical study. According to the preliminary data, fopurin is effective in the treatment of reticulodermias and leukemias.

Continuing studies of the behavior of antineoplastic preparations from the class of ethylenimino-derivatives in the organism [1, 2], we undertook the synthesis of fopurin labeled with the phosphorus isotope ^{32}P and began an investigation of its metabolism.

Phosphorus pentachloride, containing the phosphorus isotope ^{32}P with specific activity 12.5 μCi/mg, was used as the initial radioactive compound. By boiling 2-dimethylamino-6-amino-7-methylpurine [1] with $^{32}PCl_5$ in chloroform, we obtained 2-dimethylamino-6-trichloro-[^{32}P]phosphazo-7-methylpurine (II), which was converted to the dichloride of 2-dimethylamino-7-methylpurinyl-6-amido[^{32}P]phosphoric acid (III) by the action of formic acid. When the acid chloride was treated with an aqueous alkaline solution of ethylenimine, it was converted to 2-dimethylamino-6-diethylenimido[^{32}P]phosphamido-7-methylpurine (IV; [^{32}P]fopurin).

The yield of [^{32}P]fopurin after two recrystallizations from alcohol (1:5) was 30-35% (on the basis of the initial $^{32}PCl_5$), specific activity 5.0-7.5 μCi/mg.



An analysis of the radiochemical purity of the labeled preparation obtained and its identification were performed by the method of thin-layer chromatography (TLC) on Silufol plates, followed by measurement of the level of radioactivity along the chromatograms on an FH-452 radiochromatograph. The following solvent systems were used as the mobile phases: chloroform—ethanol—methoanl (2:2:1), propanol—ammonia (7:3), and butanol—0.1 N NaOH (1:1). The identity of [^{32}P]fopurin with a known pure sample of the preparation was confirmed by the same R_f values in the indicated solvent systems. In all cases one peak was obtained on the radiochromatograms, corresponding in R_f value to fopurin.

A study of the metabolism of [^{32}P]fopurin in the animal organism was conducted by the method of TLC on urine in the solvent systems used for the identification of the preparation, followed by measurement of the radioactivity level along the chromatograms. The experiments were conducted on mice with lympholeukemia L 1210 and on tumor-free animals. The preparation was introduced intravenously, intraperitoneally, and intragastrically in a dose of 70 mg/kg. The preliminary data obtained are presented in Table 1.

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TABLE 1. Content (in percent) of [^{32}P]Fopurin and Its Radioactive Metabolites (I-III) in the Urine of Experimental Animals After Intravenous Injection of the Preparation

Time after administration	Metabolite I (R_f 0,0)	[^{32}P]Fopurin (R_f 0,20)	Metabolite II (R_f 0,35)	Metabolite III (R_f 0,50)
Mice with lympholeukemia L 1210				
0	—	97,5	—	—
5 min	6,4	93,6	—	—
15 min	13,9	86,1	—	—
30 min	21,0	79,0	—	—
2,5 h	45,4	50,0	4,6	—
4 h	54,0	35,2	8,8	2,0
5 h	58,6	28,0	10,4	3,0
24 h	22,6	15,9	46,5	15,0
Tumor-free mice				
0	—	97,5	—	—
30 min	20,1	79,9	—	—
1 h	37,8	62,2	—	—
2 h	53,0	47,0	—	—
5 h	60,0	37,3	2,7	—
8 h	63,0	33,0	4,0	—
20 h	50,0	14,1	31,9	4,0
24 h	36,4	8,2	49,4	6,0

Note. Chromatography in the system chloroform—ethyl acetate—methanol (2:2:1).

An analysis of the results obtained showed that [^{32}P]fopurin is rapidly transformed in the organism. Thus, the content of the unchanged preparation in the urine of mice with lympholeukemia 5, 15, and 30 min, 2.5, and 5 h after intravenous injection was 93.6, 86.1, 79.0, 50.0, and 28.0% relative to the total radioactivity excreted with the urine. In this case, together with fopurin (R_f 0.20), three radioactive substances (I-III) with R_f 0.0, 0.35, 0.50, respectively, were detected. The first of them is evidently the main product of biotransformation of [^{32}P]fopurin. It already appears in the urine 5 min after the use of the preparation; after 15 min its content almost doubles, while after 4-5 h it is more than 50% of the labeled fopurin introduced into the animal organism.

Product II is detected in the urine only after 2.5 h (up to 5%), while by 4-5 h its content reaches 9-10%. Product III appears in the urine in an even smaller amount (2-3%) and at later periods after the administration of fopurin (4-5 h).

Analogous data were obtained in a chromatographic analysis of the substances contained in the urine of tumor-free animals: The unchanged preparation and radioactive products, identical in chromatographic mobility with I-III, were detected. However, in this case II and III appear in the urine at later periods in comparison with tumor-bearing animals. Thus, II is determined only after 5 h (up to 2.7%); then its content in the urine increases, and by the end of 24 h it reaches almost 50%. At this period the amount of unchanged preparation in the urine is only 8%; the content of metabolite I decreases substantially (from 60-63% after 5-8 h to 36% after 24 h), and metabolite III begins to appear in the urine (up to 4-6%).

Considering the presence of labile, extremely reactive groups in the fopurin molecule, we believe that II and III are products of more profound decomposition of the labeled product, probably at the N—P bond.

In the case of intraperitoneal injection of [^{32}P]fopurin into tumor-free animals, by 2 h after the injection of the preparation, product I was already detected in the urine (93%), along with a small amount of unchanged preparation (about 6%) and traces of II.

After intragastric administration of the preparation to tumor-free animals, for 3 h only the radioactive product I was determined in the urine, with traces of [^{32}P]fopurin. At this time products II and III were not detected even by autoradiography.

Thus, on the basis of the data obtained we can conclude that [^{32}P]fopurin in unchanged form is present in the organism in significant amounts (90-50%) only during the first 2 h after intravenous injection of the preparation. Then processes of its biotransformation to

I-III begin to predominate. In the case of intraperitoneal and intragastric methods of administration of the preparation, the rate of biotransformation of [^{32}P]fopurin is even higher. These data are of great practical importance, since they permit the most rational system of use of the preparation to be outlined.

EXPERIMENTAL

Chemical

2-Dimethylamino-6-diethylenimidophosphamido-7-methylpurine (IV, [^{32}P]Fopurin). To a suspension of 2.7 g (0.013 mole) $^{32}PCl_5$ with specific activity 18.5 μCi/g in 25 ml of chloroform, washed free of alcohol and thoroughly dried over calcium chloride, 2.5 g (0.013 mole) I was introduced at room temperature with mixing. The mixture was heated to boiling, boiled for 5 h, and filtered if a precipitate was present. To the filtrate, 0.61 g (0.013 mole) formic acid (d = 1.22) was added at 20°C with mixing. The chloroform was distilled off under vacuum at 30-40°C. To the foamy residue we added 50 ml of technical chloroform, and with vigorous mixing and external cooling with ice (the temperature of the mass should not exceed 20°C), a mixture of 1.163 g (0.027 mole) ethylenimine with a solution of 2.7 g (0.0195 mole) potash in 13.5 ml of water was added. The mixture of the potash solution and ethylenimine was prepared directly before loading. The reaction mixture was mixed for another 30 min at this temperature, then the chloroform layer was removed, dried over anhydrous sodium sulfate, filtered, and the chloroform distilled off at a temperature no higher than 40°C. We obtained 2.5 g (60% on the basis of $^{32}PCl_5$) technical [^{32}P]fopurin, which was purified by crystallization from alcohol (1:5) with activated charcoal. After repeated crystallization (without activated charcoal), 1.25 g [^{32}P]fopurin was obtained. The specific activity of the preparation was 5.0 μCi/g, mp 249-250°C with decomposition. The preparation was chromatographed on Silufol plates in the solvent systems chloroform—ethyl acetate—methanol (2:2:1), propanol—ammonia (7:3), and butanol—0.1 N NaOH (1:1). In a measurement of the level of radioactivity along the chromatogram on an FH-452 radiochromatograph, in all cases one peak was obtained, with R_f 0.20, 0.46, and 0.63, respectively, containing 95-97% radioactivity. Autoradiograms were taken from the chromatograms obtained, and in all cases one spot was obtained on them, corresponding in R_f value to fopurin.

Radiochromatography of Urine. The investigation was conducted on hybrid mice ($C_{57}BL(6 \times DBA)_2F$ with lympholeukemia L 1210 and tumor-free animals weighing 20-25 g. Four groups of animals, five in each, were used in the experiment (the first group consisted of mice with lympholeukemia, intravenous injection of the preparation; groups 2, 3, and 4 were tumor-free animals, with intravenous, intraperitoneal, and intragastric administration, respectively). [^{32}P]Fopurin was administered in a single 2 mg dose (10 μCi) per animal. After definite intervals following the introduction of the preparation (5, 15, 30 min, 1, 2, 5, 8, and 24 h), urine samples were collected from the animals, chromatographed on Silufol plates in the systems chloroform—ethyl acetate—methanol (2:2:1) and propanol—ammonia (7:3), and the level of radioactivity measured along the chromatograms on an FH-452 radiochromatograph. The percent content of [^{32}P]fopurin and its radioactive metabolites was calculated according to the area of the peaks.

LITERATURE CITED

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