SYNTHESIS OF PYRIMIDIN-2-YLAMINOPHOSPHONIC DI(ETHYLENEAMIDE) (FOSFEMID) LABELED AT THE PHOSPHORUS ATOM

Yu. I. Savin, A. S. Singin, N. V. Sazonov, A. A. Kropacheva, and T. S. Safonova

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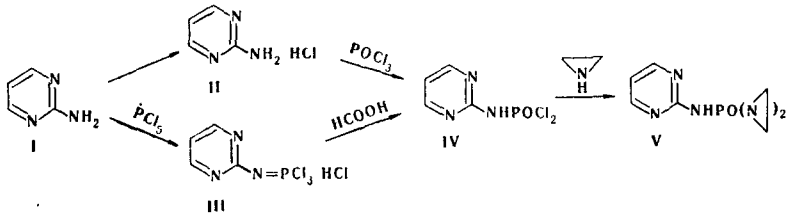
Pyrimidin-2-ylaminophosphonic di(ethyleneamide) labeled at the phosphorus atom (fosfemid-^{32}P) has been synthesized both in an anhydrous medium and also using an aqueous alkaline solution of ethyleneimine.

Pyrimidin-2-ylaminophosphonic di(ethyleneamide) (fosfemid) possesses a high antitumoral activity and is currently used in clinical practice for the treatment of hemodermias [1, 2]. However, at the present time there is no information in the literature on the fate of the compound in the organism. In order to study this question, we have undertaken the synthesis of pyrimidin-2-ylaminophosphonic di(ethyleneamide) labeled at the phosphorus atom.

On the basis of available literature information, the synthesis of pyrimidin-2-ylaminophosphonic di(ethyleneamide) (V) can be performed by two routes differing in the nature of the phosphorylating agent.



According to the first route, pyrimidin-2-ylaminophosphonic dichloride (IV) is obtained by a method described for the preparation of aliphatic and aromatic phosphoramides [3]. The starting material is 2-aminopyrimidine hydrochloride (II), and phosphorus oxychloride is used as phosphorylating reagent. A fundamental defect of this method is that the reaction is performed in an excess (1 : 4) of phosphorus oxychloride, which leads to difficulties in the purification of the di(acid chloride) (IV). The presence of phosphorus oxychloride has an adverse effect on the following stage – the preparation of the di(ethyleneamide) (V). Furthermore, this method is unsuitable for radiochemical synthesis, since the excess of phosphorus oxychloride leads to the incomplete utilization of the radioactive raw material and to low radiochemical yields.

According to the second route, the di(acid chloride) (IV) is obtained by a method described for the preparation of the dichlorides of sulfanilamido- and carbacylamidophosphonic acids [4]. The reaction of equimolecular amounts of 2-aminopyrimidine and phosphorus pentachloride in benzene gives trichloro(pyrimidin-2-ylimino)phosphorane hydrochloride (III), which is converted into the di(acid chloride) (IV) by the action of 98% formic acid [5, 6]. This method has a number of advantages over the first one. It eliminates the stage of the preparation of 2-aminopyrimidine hydrochloride and the necessity for the isolation and purification of the di(acid chloride) (IV). In addition, it permits the radioactive raw material ($^{32}PCl_5$) to be utilized completely.

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On the basis of what has been said above, we selected the second route for the synthesis of the fosfemid-^{32}P. The di(acid chloride) (IV) obtained by this method was brought to reaction with ethyleneimine without isolation and purification.

The following variants of the method were investigated in this stage of the synthesis: the performance of the reaction of the di(acid chloride) (IV) with anhydrous ethyleneimine in benzene in the presence of triethylamine as hydrogen chloride acceptor, and the performance of this reaction with the use of an aqueous alkaline solution of ethyleneimine. The yield of labeled fosfemid by the first route amounted to 70-80%, and by the second route to 50-60%.

The specific activity of the fosfemid-^{32}P amounted to 5-10 mCi/g (depending on the specific activity of the initial $^{32}PCl_5$).

It was important to confirm the purity of the labeled material and its identity with the pharmacopoeia sample of unlabeled fosfemid. For this purpose we used the method of thin-layer chromatography with the subsequent scanning of the radioactivity along the chromatographic plate. The fosfemid-^{32}P was chromatographed on Silufol UV_{254} plates in methanol in the presence of authentic pure unlabeled material as marker. The spots on the chromatograms were revealed with iodine vapor. The R_f values of the marker and of the fosfemid-^{32}P were identical at 0.50. When the radioactivity was scanned along the chromatographic plate on an FH-452 radiochromatograph, only one peak was found, with R_f 0.50, corresponding to the fosfemid-^{32}P.

EXPERIMENTAL

[^{32}P]Pyrimidin-2-ylaminophosphonic Di(ethyleneamide) (V). A. A mixture of 3.698 g (17.8 mmoles) of $^{32}PCl_5$ with a specific activity of 10 mCi/g and 1.688 g (17.8 mmoles) of 2-aminopyrimidine in 30 ml of benzene was boiled with vigorous stirring until the evolution of HCl ceased (2 h 30 min). To eliminate the hydrogen chloride more completely, a current of dry nitrogen was passed into the mixture. The suspension of trichloro(pyrimidin-2-ylimino)phosphorane hydrochloride in benzene was cooled to room temperature and, with cooling, 0.816 g (17.8 mmoles) of 98% formic acid in 15 ml of ether was slowly added. The mixture was stirred for another 1 h and was left to stand for 12-14 h. The benzene and the ether were distilled off in vacuum, 10 ml of benzene was added, the mixture was cooled to 7-10°C, and with stirring a solution of 1.83 g (17.8 mmoles) of ethyleneimine and 4.3 g (35.6 mmoles) of triethylamine in 10 ml of benzene was slowly added. The reaction mixture was stirred for 30 min at 7-10°C and 1 h at room temperature, and was left to stand for 12-14 h. The precipitate of triethylamine hydrochloride was filtered off and washed with benzene, and the filtrate was evaporated to dryness in vacuum to give 3.4 g of the di(ethyleneamide) (V). After recrystallization from ethyl acetate with the addition of activated carbon, 3.25 g (81%, calculated on the 2-aminopyrimidine) of fosfemid-^{32}P was obtained with mp 138-139°C and a specific activity of 7.9 mCi/g.

B. To a solution of 1.14 g (12 mmoles) of 2-aminopyrimidine in 30 ml of chloroform was added 2.5 g (12 mmoles) of $^{32}PCl_5$ with a specific activity of 10 mCi/g. The reaction mixture was heated at 45-50°C for 1 h and was then boiled for 4 h, after which it was cooled to room temperature, 0.55 g (12 mmoles) of 98% formic acid was slowly added, and the mixture was stirred for 3 h and was left to stand for 12-14 h. Then it was evaporated to dryness in vacuum, and the residue was treated with 20 ml of chloroform, with which it was carefully stirred. With constant stirring at a temperature of −8 to −10°C, a solution of 1.24 g (28.8 mmoles) of ethyleneimine and 1.8 g (12.8 mmoles) of K_2CO_3 in 5 ml of water was added to the resulting suspension. The reaction mixture was kept at the same temperature for 30 min and at room temperature for 2 h, the chloroform layer was separated off, the aqueous layer was extracted three times with chloroform, and the chloroform solutions were combined and dried with sodium sulfate. The sodium sulfate was filtered off and the filtrate was evaporated in vacuum to dryness, giving 1.8 g (66%) of the diethyleneamide) (V). After recrystallization from ethyl acetate with the addition of activated carbon, 1.5 g of fosfemid-^{32}P (55% calculated on the 2-aminopyrimidine) was obtained with mp 138-139°C and with a specific activity of 7.9 mCi/g.

When the labeled sample obtained was chromatographed on Silufol UV_{254} plates in methanol, in both cases a single spot with R_f 0.50 was found which coincides with the R_f value of a pharmacopoeia sample of fosfemid. On subsequent scanning of the radioactivity along the chromatograms on an FH-452 radiochromatograph, only one peak was obtained, with R_f 0.50.

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