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A. M. Yurkevich, I. I. Kolodkina, N. A. Preobrazhenskii

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Abstract

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A. M. Yurkevich, I. I. Kolodkina, N. A. Preobrazhenskii

2′,3′-Phenylboronic Ester of Adenosine in the Synthesis of Adenosine 5′-Monophosphate

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In syntheses of mononucleotides, nucleotide coenzymes, and oligonucleotides, the choice of suitable protecting groups that make it possible to achieve selectivity in carrying out the phosphorylation reaction is of great importance. The most widely used have been the 2′,3′-*O*-isopropylidene and 2′,3′-*O*-benzylidene derivatives of nucleosides ([1]). However, their preparation presents a number of preparative difficulties, and removal of the protecting groups in the final products requires the use of rather harsh conditions.

For protection of the 2′,3′-*cis*-glycol grouping during phosphorylation of the 5′-hydroxyl of adenosine (I), we use phenylboronic acid. It is known that phenylboronic acid readily forms cyclic esters with 1,2-, 1,3-, and 1,4-diols, pyrogallol ([2]), and glycosides ([3]), which are stable under the conditions of acylation reactions but are readily subjected to hydrolysis and alcoholysis. The phenylboronic ester of adenosine (II) obtained is phosphorylated with P^1-diphenyl P^2-morpholinopyrophosphochloridate ([4]), and after acid hydrolysis adenosine 5′-monophosphate (III) is obtained in good yield.

The phenylboronic ester of adenosine (II) was obtained by us from adenosine (I) and phenylboronic acid in pyridine by azeotropic distillation of water. The cyclic ester II is a crystalline substance, readily soluble in pyridine and dioxane, but it is easily hydrolyzed when dissolved in water. The protective group can be readily removed from the nucleoside or nucleotide by simple treatment in aqueous solutions, after which phenylboronic acid is separated by extraction with chloroform. To establish the structure of the adenosine phosphate (III) obtained, paper chromatography in borate solution and thin-layer chromatography on cellulose were carried out in the presence of reference samples of 5′-, 2′-, and 3′-phosphates of adenosine. The obtained R_f values indicate the formation of adenosine 5′-monophosphate (III). Chromatography on Dowex-1 resin and ($HCOO^{\ominus}$) ([5]) of the phosphorylation reaction product showed that, as a result, a mixture is formed consisting of 77.5% adenosine 5′-phosphate and 13% adenosine diphosphate (Fig. 1).

Fig. 1. Chromatography on Dowex-1 resin of the product of phosphorylation of 2′,3′-phenylboronate ester of adenosine: I —adenosine (I); II — adenosine-5′-monophosphate (III); III —adenosine-3′-monophosphate; IV, V — substances not identified; VI —adenosine diphosphates; VII, VIII —adenosine polyphosphates

Figure 1: Fig. 1. Chromatography on Dowex-1 resin of the product of phosphorylation of 2′,3′-phenylboronate ester of adenosine: I —adenosine (I); II — adenosine-5′-monophosphate (III); III —adenosine-3′-monophosphate; IV, V — substances not identified; VI —adenosine diphosphates; VII, VIII —adenosine polyphosphates

Experimental Part

For chromatography we used Whatman-1 paper and solvent systems: 1) isopropyl alcohol—conc. NH_3—water (7:1:2); 2) ethanol—0.2 N solution of ammonium acetate, saturated with boric acid (5:2); thin-layer chromatography was carried out on 9 × 15 cm plates on an unfixed layer of cellulose; 3) saturated solution of ammonium sulfate—sodium acetate—isopropyl alcohol (80:18:2). Paper electrophoresis was carried out in a PEF-1 apparatus at a voltage gradient of 16–20 V/cm for one hour, in 0.05 M triethylammonium bicarbonate solution, pH 7.5. Fraction collection and recording in ion-exchange chromatography were carried out in a "Radirak" apparatus with a "Uvicord" attachment; quantitative determinations were made with an SF-4 spectrophotometer.

Fig. 1. Chromatography on Dowex-1 resin of the product of phosphorylation of 2′,3′-phenylboronate ester of adenosine: *I* —adenosine (I); *II* —adenosine-5′-monophosphate (III); *III* —adenosine-3′-monophosphate; *IV, V* —substances not identified; *VI* —adenosine diphosphates; *VII, VIII* —adenosine polyphosphates.

2′,3′-Phenylboronate ester of adenosine (II). To a suspension of 3.6 g of adenosine (I) in 150 ml of pyridine, with stirring, a solution of 1.66 g of phenylboronic acid in 50 ml of pyridine is added. The reaction mixture is boiled for 2 hours while simultaneously carrying out azeotropic removal of water. As the solvent is removed, 70–100 ml portions of pyridine are periodically added to the flask. The solution is evaporated, the residue is washed with ether, dissolved in dioxane, and the 2′,3′-phenylboronate ester (II) is isolated. Yield 3.8 g (80%). M.p. 223–224° (from dioxane—ether).

Found, %: C 54.26; H 4.68; N 19.95
$C_{16}H_{16}N_5O_4B$. Calculated, %: C 54.42; H 4.57; N 19.83

UV spectrum in dioxane: 218 mμ, lg ϵ 4.41; 260 mμ, lg ϵ 4.31.

Adenosine-5′-monophosphate (III). To a solution of 0.204 g of morpholidophosphorodichloridate and 0.25 g of diphenylphosphoric acid in 2 ml of dioxane, 0.340 ml of 2,6-lutidine ([5]) is added, and after 15 min, 0.176 g of the phenylboronate ester (II). The reaction mixture is left for 48 hours (electrophoretic

control), after which 30 ml of water and 1 N hydrochloric acid to pH 2.0 are added. After two days the resulting clear solution is concentrated to a small volume, diphenylphosphoric and phenylboronic acids are extracted with chloroform, and the solution is evaporated in vacuo. Adenosine-5′-monophosphate is isolated by adding ethyl alcohol. Yield 0.146 g (67%) (spectrophotometric determination), R 0.12 (system 1), 0.02 (system 2), 0.407 (system 3).

Chromatographic separation. The acidic hydrolysate obtained after removal of diphenylphosphoric and phenylboronic acids is diluted to 75 ml, alkalinized with 3% ammonia to pH 8.0, and passed through a column with 1 ml of Dowex-1 × 4 resin, 200-400 mesh ($HCOO^-$). The column is washed with water. The substances are eluted using a linear gra-

formic acid concentration gradient: 1) 100 ml water—100 ml 0.125 M formic acid. 2) 50 ml 0.125 M acid—50 ml 0.5 M acid. 3) 50 ml 0.5 M acid—50 ml 1 M acid, 4) 5 M acid. Fraction volume 5 ml. Fractions 11-17 contain adenosine (I) (2.8%—spectrophotometric determination); 21-35—adenosine 5′-monophosphate (III) (77.5%); 41-44—adenosine 3′-monophosphate (1.5%); 83-88—adenosine diphosphates (13%); 99-100—adenosine polyphosphates (1.0%); fractions 58-63 and 75-78 contain, in total, about 3% of substances that were not identified (Fig. 1).

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Note: Figure translations are in progress. See original paper for figures.