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ANALOGS OF PURINE NUCLEOSIDES AND PURINE MONO- AND POLYNUCLEOTIDES. VI*. PHOSPHORYLATION OF 9-(1,5-DIHYDROXY-3-PENTYL)PURINES AND THEIR POLYCONDENSATION WITH 1',5'-DIPHOSPHATES OF 6-SUBSTITUTED 9-(1,5-DIHYDROXY-3-PENTYL)PURINES

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Phosphorylation of 6-substituted 9-(1,5-dihydroxy-3-pentyl)purines with 2-cyanoethyl phosphate in the presence of dicyclohexylcarbodiimide in anhydrous pyridine gave their 1',5'-diphosphates. Oligomers containing pyrophosphate and ester bonds were obtained by polycondensation of 1',5'-diphosphates of 6-dimethylamino- and 6-oxo-9-(1,5-dihydroxy-3-pentyl)purines with the appropriate 9-(1,5-dihydroxy-3-pentyl)purines.

In the present research we studied the phosphorylation of our previously synthesized 6-amino-, 6-dimethylamino-, and 6-oxo-9-(1,5-dihydroxy-3-pentyl)purines (I-III) with 2-cyanoethyl phosphate. We obtained 9-(1,5-dihydroxy-3-pentyl)purine 1',5'-diphosphates (IV-VI) when the reaction was carried out by the method in [2] in anhydrous pyridine in the presence of dicyclohexylcarbodiimide. As in the case of phosphorylation of 1-(1,4-dihydroxy-2-butyl) thymine [3], the reagent molar ratio has a considerable effect on the yields of final products IV-VI. Thus the maximum yields of 1',5'-diphosphates IV-VI were obtained when a six-fold excess of 2-cyanoethylphosphate and, respectively, a 12-fold excess of dicyclohexylcarbodiimide were used. 1',5'-Diphosphates IV-VI were isolated by means of preparative chromatography on Dowex 50W × 4 ion-exchange resin (H^+ form). The considerable adsorption of purines on the ion-exchange resin makes it possible to efficiently separate the phosphoric acid, formed in the decomposition of 2-cyanoethyl phosphate, from the phosphorylation products and also makes it possible to separate diphosphorylated purines IV-VI from monophosphorylation products VII-IX and starting purines I-III, which are retained more strongly by the resin. However, the IV-VI isolated in this manner are not sufficiently homogeneous and require additional purification. Preparative rechromatography on A-25 QAE-Sephadex anion-exchange resin proved to be the most effective method to achieve this. In addition, we were able to purify V and VI by conversion to the corresponding barium salts, and VI was also purified by washing with ethanol. Monophosphorylation products VII and VIII were purified with columns by means of A-25 QAE-Sephadex resin. The chromatographically pure mono- and diphosphorylated

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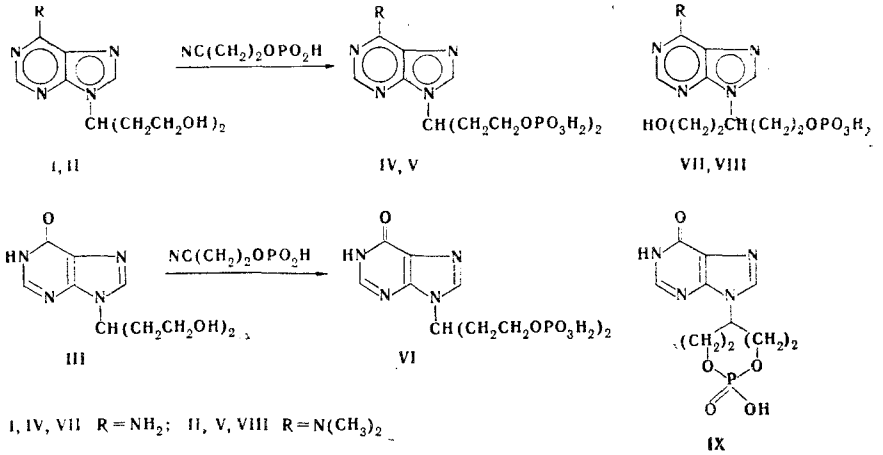
TABLE 1. Electrophoretic Mobilities and R_f Values of the Phosphorylation Products

Compound	Electrophoretic mobility as compared with adenosine 5'-monophosphate		R_f	
	phosphate buffer, pH 7.5	0.01 N HCl	A	B
IV	1,22	1,21	0,31	0,45
V	1,20	1,23	0,56	0,45
VI	1,24	1,26	0,30	0,30
VII	1,04	1,01	0,56	0,66
VIII	1,00	1,00	0,78	0,68
IX	0,76	1,02	0,50	0,60
Adenosine 3',5'-cyclophosphate	0,79	1,05	—	—

products IV-IX obtained in this way were identified on the basis of their electrophoretic mobilities (Table 1).

Thus the lower electrophoretic mobilities of VII and VIII as compared with the mobilities of IV-VI make it possible to assign the 9-(1,5-dihydroxy-3-pentyl)purine monophosphate structure to them and the 9-(1,5-dihydroxy-3-pentyl)purine cyclophosphate structure to IX. Evidence in favor of this assumption is provided by a comparison of the data on the electrophoretic mobilities of VII-IX with the mobilities of adenosine 5'-monophosphate and adenosine 3',5'-cyclophosphate in acidic and neutral media. In neutral media, adenosine 3',5'cyclophosphate and cyclophosphate IX acquire only one negative charge, in contrast to adenosine 5'-monophosphate and monophosphates VII and VIII, which have two negative charges. They are therefore considerably inferior to the latter with respect to their electrophoretic mobilities. In acidic media, the secondary dissociation of the phosphate group is suppressed in VII and VIII, and their electrophoretic mobilities therefore do not differ from the mobilities observed for adenosine 3',5'-cyclophosphate and IX.



Oligomers containing both pyrophosphate and phosphate diester bonds were obtained in 30% yield by polycondensation of II with V and III with VI under the influence of triisopropylbenzensulfonyl chloride in pyridine [4]. Considering the fact that the pyrophosphate bonds of the oligomers are cleaved by acid hydrolysis by refluxing with 0.1 N hydrochloric acid for 3.5 h, we subjected them to this sort of treatment and were able to show that ∿12% of the phosphate bonds, which consequently should correspond to phosphate diester bonds, remain intact in the oligomers after hydrolysis. To estimate the molecular weights of the oligomers we determined their distribution coefficients on Sephadex G-25 before and after hydrolysis [5]. The synthesized oligomers were isolated by dialysis against water and an 2 N sodium chloride solution.

EXPERIMENTAL

The methods used to prepare the standard solutions of 2-cyanoethyl phosphate, dicyclohexylcarbodiimide, triisopropylbenzesulfonyl chloride, the purification and absolutizing of

the pyridine, and the method used to prepare the ion-exchange resins were the same as those previously described in [3,4]. Anion-exchange chromatography was carried out with a stepwise ammonium carbonate buffer. Gel filtration was carried out with a 1 N ammonium carbonate buffer solution. A column ($V_c = 74.5$ cm^3, $H = 65$ cm) filled with Sephadex G-25 was used to determine the distribution coefficients. The distribution coefficient (K_d) in gel filtration was calculated from the formula $K_d = V_e - (V_o/V_i)$, where V_e is the elution volume of the substance, V_i is the inner volume, and V_o is the outer volume of the column.

Column chromatography was monitored by means of an LKB Uvicord-2 flow UV adsorption meter at 254 nm.

Electrophoresis was carried out on sheets of FN3 paper in a phosphate buffer (pH 7.5) in 0.01 N HCl solution at a voltage gradient of 760 V.

Ascending paper chromatography was carried out on Whatman 3-mm paper in the following systems: 1 M ammonium acetate—ethanol (1:1) (A) and 0.5 N NH_4OH—isobutyric acid (6:10) (B).

6-Amino-9-(1,5-dihydroxy-3-pentyl)purine 1',5'Diphosphate (IV). Pyridine solutions of purine I [1.42 g (6 mmole) in 40 ml], 2-cyanoethyl phosphate (36 mmole in 36 ml), and dicyclohexylcarbodiimide [14.8 g (72 mmole) in 60 ml] were mixed, and the mixture was allowed to stand for 60 h with periodic shaking. Water (50 ml) was added, and the mixture was allowed to stand for 3 h, after which the precipitated dicyclohexylurea was removed by filtration. Concentrated ammonium hydroxide (250 ml) was added to the filtrate, and the mixture was refluxed for 3.5 h. The solution was evaporated to half its original volume, the resulting precipitate of dicyclohexylurea was removed by filtration, and the filtrate was evaporated to dryness. The syrupy residue was dissolved in 100 ml of water and introduced into a column filled with 400 ml of Dowex-50W × 4 ion-exchange resin in the H^+ form and eluted successively with water (3000 ml) (until UV absorption vanished) and 4 N ammonium hydroxide (1100 ml). The first 500 ml of the aqueous eluate (pH 1), which contained phosphoric acid, was discarded, and the remainder of the aqueous eluate (pH 3) was made alkaline to pH 7.5 and introduced into a column containing 130 ml of QAE-Sephadex. Elution was effected with an $(NH_4)_2CO_3$ buffer (pH 8.3) containing 10% ethanol while gradually increasing the ionic strength of the solution. The bulk of the substance (68%) was eluted by means of 0.25 N $(NH_4)_2CO_3$. The eluate corresponding to this substance was evaporated several times with water, and the residue was dried by evaporation several times with ethanol and ether. Compound IV was obtained as colorless strongly hygroscopic plates that were insoluble in ether, alcohol, and dimethylformamide but soluble in water and dimethyl sulfoxide. The yield of product with mp 148-152° was 0.56 g (20%); pK_{PO_3H} 6.41 and pK 3.94. Found: C 29.3; H 4.5; N 16.1%; M 415. $C_{10}H_{17}N_5O_8P_2 \cdot H_2O$. Calculated: C 28.9; N 4.6; N 16.9%; M 413.

6-Dimethylamino-9-(1,5-dihydroxy-3-pentyl)purine 1',5'-Diphosphate (V). A solution of 0.9 g (3 mmole) of the hydrochloride of II in 25 ml of pyridine was combined with pyridine solutions of 2-cyanoethyl phosphate (18 mmole in 18 ml) and dicyclohexylcarbodiimide [7.42 g (36 mmole) in 30 ml], and the mixture was allowed to stand for 48 h. Water (20 ml) was added, and the mixture was worked up as described for IV. An aqueous solution of the reaction products (80 ml) was introduced into a column filled with 300 ml of Dowex-50W × 4 resin in the H^+ form, and the mixture was eluted successively with water and 4 N NH_4OH as described above. The first 250 ml of the aqueous solution (pH 1) was discarded, and the remainder was evaporated to dryness. According to data from anion-exchange chromatography on QAE-Sephadex, the chromatographic degree of purity of the product was 80%. This substance was dissolved in 40 ml of water and precipitated in the form of the barium salt by the addition of an aqueous solution of barium acetate (10.5 g per 50 ml). The precipitate was separated by centrifugation, washed successively with ethanol and ether, and dissolved in water while stirring with Dowex-50W × 4 resin in the H^+ form (10 ml); the solution was filtered into the column and eluted with water until the wash no longer had UV absorption. The eluate was evaporated to dryness, and the residue was dried by evaporation with ethanol and ether. The chromatographic degree of purity of V was 98%, and its solubility was similar to that of IV. The yield of product with mp 154-157° was 0.1 g (7%). Found: C 31.9; H 5.2; N 14.6%; M 161. $C_{12}H_{21}N_5O_8P_2 \cdot 2H_2O$. Calculated: C 31.2; H 5.5; N 15.2%; M 459.

6-Oxo-9-(1,5-dihydroxy-3-pentyl)purine 1',5'-Diphosphate (VI). A 1.43-g (6 mmole) sample of purine III was dissolved by gentle heating in 200 ml of pyridine, after which the so-

lution was cooled to room temperature, and 18.5 g (90 mmole) of dicyclohexylcarbodiimide and 36.0 ml (36 mmole) of a solution of 2-cyanoethylphosphate in pyridine were added. The mixture was then allowed to stand for 48 h with periodic shaking. The reaction was stopped by the addition of 70 ml of water, and the aqueous solution (100 ml) remaining after the standard operations of removal of the dicyclohexylurea, the cyanoethyl protective group, and pyridine was applied to a column with 300 ml of Dowex-50W × 4 resin in the H^+ form. Elution was carried out in accordance with the method described above for IV and V. The aqueous solution, which was free of phosphoric acid, was evaporated to dryness. The residue was rendered anhydrous by evaporation with ethanol and ether to give VI with 88% purity (according to the results of anion-exchange chromatography). Additional purification of this substance by reprecipitation in the form of the barium salt proved to be inefficient. Chromatographically pure diphosphate VI was obtained by washing it several times with hot ethanol. The solubility of VI was similar to that of IV. The yield of product with mp 206-208°, pK_{PO_3H} 6.46, and pK 9.12, was 1.55 g (65%). Found: C 30.8; H 4.5; N 13.5%; M 397. $C_{10}H_{15}N_4O_9P_2$. Calculated: C 30.2; H 4.6; N 14.1%; M 395.

Isolation of the Monophosphorylation Products. The products of phosphorylation of purines I-III, which were eluted from columns filled Dowex-50W × 4 resin by means of 4 N ammonium hydroxide, were freed of ammonia by evaporation and rechromatography with columns filled with QAE-Sephadex. The starting compounds were eluted with 0.01 N $(NH_4)_2CO_3$ solution containing 10% ethanol. The bulk of the substance retained in the columns was eluted with 0.1 N $(NH_4)_2CO_3$ solution. The chromatographic and electrographic and electrophoretic constants of the monophosphorylation products are presented in Table 1.

Polycondensation of III and VI. A 0.4-ml sample of trioctylamine was added to an aqueous pyridine (1:1) solution of 0.09 g (0.23 mmole) of diphosphate VI, after which the mixture was evaporated to give a viscous mass. The viscous material was dissolved in dry pyridine, and the solution was combined with a solution of 0.053 g (0.23 mmole) of purine III in dry pyridine. The mixture was dried by repeated evaporation with dry pyridine. A solution of 0.362 g (1.13 mmole) of triisopropylbenzenesulfonyl chloride in 1 ml of pyridine was added to a solution of the residue in 2 ml of dry pyridine, and the mixture was allowed to stand at room temperature for 7.5 h, after which 25 ml of water was added, and the mixture was allowed to stand for 14 h. The resulting precipitate was removed by filtration and washed with 5 ml of water. The solution was evaporated, refiltered, and dialyzed in a cellophane tube against 200 ml of water twice for 12 h and then against an 2 N NaCl solution twice for 24 h each time. The products that passed into the 2 N NaCl solution were purified to remove the salts by dialysis against water. They constituted 30% of the entire reaction mixture. The K_d value of the resulting oligomer was 0.07.

Hydrolysis of the Products of Polycondensation of III and VI. An aqueous solution of 250 optical units of the products of polycondensation of III and VI was refluxed for 3.5 h in 0.1 N hydrochloric acid, after which the mixture was evaporated to dryness, and the residue was diluted with water. The aqueous solution was introduced into a column filled with QAE-Sephadex (the volume of the column was 2.5 ml), and the products were eluted with an ammonium carbonate buffer while gradually raising the ionic strength of the solution. The distribution coefficient of the highest-molecular-weight fraction (12% of the entire reaction mixture) was 0.27.

Polycondensation of II and V. The polycondensation was carried out under the conditions indicated in the preceding experiment using the following component ratios: 0.046 g (0.1 mmole) of diphosphate V, 0.03 g (0.1 mmole) of purine II, and 0.18 g (0.5 mmole) of triisopropylbenzenesulfonyl chloride. The reaction was stopped after 5 h, and the polycondensation product was obtained in 26% yield after dialysis. The K_d value of the resulting oligomer was 0.083. A portion (9%) of the oligomer did not undergo decomposition during hydrolysis by refluxing in 0.1 N HCl for 3.5 h; the K_d value of this fraction was 0.3.

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HETEROCYCLIC ANALOGS OF PLEIADIENE. XXIV*. UNEXPECTED PRODUCTS OF THE REACTION OF 1-METHYLPERIMIDINE WITH PHENYLMETALLIC COMPOUNDS IN THE PRESENCE OF BENZOPHENONE

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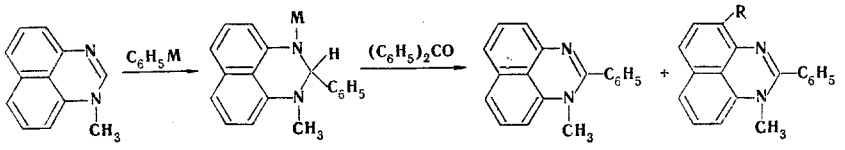
1-Methylperimidine adds phenylsodium and phenyllithium to the C≅N bond to give 1-methyl-2-phenyl-2,3-dihydroperimidines. However, if benzophenone is present in the reaction mixture, a mixture of 1-methyl-2-phenylperimidine and 1-methyl-2,4-diphenylperimidine (when phenylsodium is used) or 1-methyl-2-phenyl-4-(α-hydroxybenzhydryl)perimidine (when phenyllithium is used) is formed. It is assumed that the formation of products involving substitution in the naphthalene ring is associated with the participation of $C_6H_5^{\cdot}$ and $(C_6H_5)_2C^{\cdot}-O^{-}$ radical particles that are formed on reaction of the phenylmetallic compound with benzophenone.

We have previously shown that organomagnesium and organolithium compounds react with 1-methylperimidine (I) to give almost exclusively products of addition to the C=N bond [2]. We have now established that phenylsodium also reacts similarly with I and that the character of the final product depends on the reagents used in the last step. 1-Methyl-2-phenyl-2,3-dihydroperimidine is formed in 90% yield in the case of carbonization with dry ice (to ascertain the possible metallation products). However, completely different compounds — the known 1-methyl-2-phenylperimidine (III, in 54% yield) and the previously undescribed 1-methyl-2,4-diphenylperimidine (IVa, in 14-17% yield) — are obtained on attempts to establish the reaction products by reaction with benzophenone.



I II a,b III IV a,b

II a M=Na; b M=Li; IV a R=C_6H_5; b R=$C(C_6H_5)_2$ (OH)

The structure of IVa is confirmed by the results of elementary analysis, the mass spectrum (molecular weight 334), the absence of bands at 3100-3600 cm^{-1} in the IR spectrum, the yellow-orange color characteristic for 2-arylperimidines [3], and the following features of its PMR spectrum: a) the absence of a quartet at δ 6.87 ppm (Fig. 1) related to the proton attached to the C_4 atom in the spectrum of I [3] and III (Fig. 1); b) the ratio of the signals of the aromatic protons to the protons of the CH_3 group (5:1). In addition, IVa does not form even traces of a methiodide on prolonged heating with excess methyl iodide. The

*See [1] for communication XXIII.

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