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3′-Modified Nucleotides: Substrate Recognition by Phosphohydrolases

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Analogs of thymidine-5′-monophosphate, thymidine-5′-monophosphate-*p*-nitrophenylester, and adenosine-5′-monophosphate with an amino or azido group in the 3′-position have been synthesized by convenient methods. These compounds were tested as substrates for acid phosphatase from potatoes (EC 3.1.3.27), 5′-nucleotidase from snake venom (EC 3.1.3.5), alkaline phosphatase from calf intestine (EC 3.1.3.1), and phosphodiesterase from snake venom (EC 3.1.4.1). The influence of the modification was found to increase with the higher specificity of the enzymes (thus, e.g., 5′-nucleotidase does not accept the 3′-modified thymidine derivatives).

INTRODUCTION

In many organs one can find enzymes with phosphohydrolytic activities of different specifiities such as acid and alkaline phosphatases, nucleotidases, phosphodiesterases, etc. (*1–5*). This phenomenon seems to play a certain role in the differentiation of cells. Thus there is an interplay of alkaline phosphatase and 5′-nucleotidase in human bone changing during development. In Paget's disease this change is disturbed (*2*).

In order to investigate different enzyme's requirements for their substrates, we synthetized several modified nucleotides. The 3′-OH group was chosen as the site of modification since this position is close to that of the phosphate, viz., the site of enzyme action. The 3′-OH was replaced by either an azido or an amino group, and the compounds were studied with regard to their ability to act as substrates for several phosphohydrolases. The newly introduced 3′-group should give a slower reaction with enzymes requiring the 3′-OH group for substrate recognition. Under acidic conditions the 3′-amino group could also interfere with the cleavage of the 5′-phosphate residue by forming an inner salt.

Chemical Synthesis of the Nucleotide Analogs

The 3′-azido derivatives of thymidine-5′-phosphate (**2**) and thymidine-5′-phosphoric acid *p*-nitrophenylester (**4**) were synthesized by phosphorylation of 3′-azidothymidine (**1**) (*6*) with $POCl_3$ in acetone and with *p*-nitrophenylphosphorodichloridate (*7*) in pyridine, respectively. 3′-Aminothymidine-5′-phosphate (**3**) was prepared from (**2**) as described by Glinski *et al.* (*8*). As the 3′-amino derivative **7** could not be synthesized by

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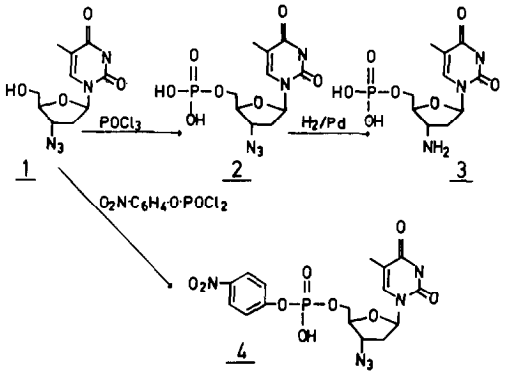
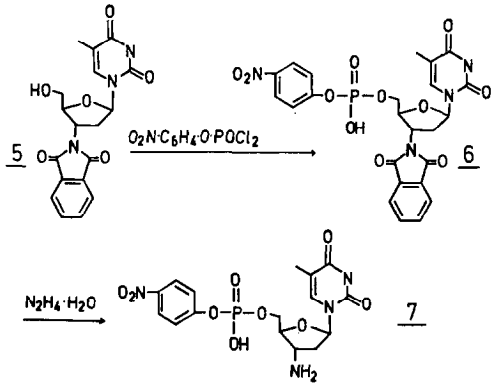
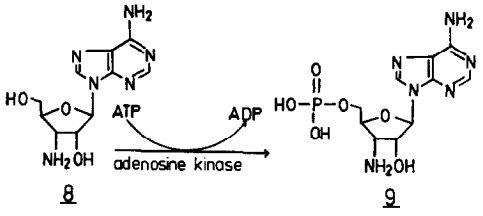


hydrogenation of (**4**), it was prepared by phosphorylation of 3′-phthalimide thymidine (**5**) (*9*) with *p*-nitrophenylphosphorodichloridate in pyridine and subsequent cleavage of the protecting group using hydrazine hydrate (*9*).



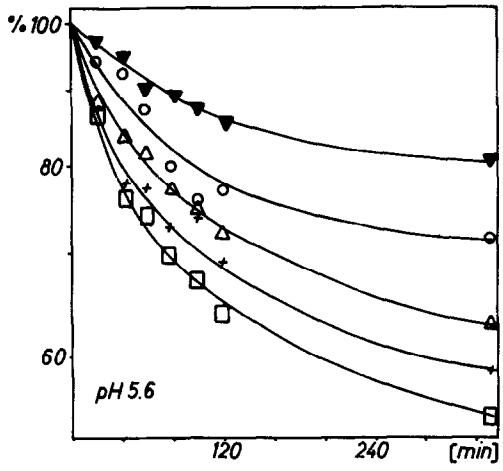
3′-Aminoadenosine (**8**) is a substrate for adenosine kinase (**9**) (*10*), and so its 5′-phosphate could be synthesized enzymatically in 45% yield:



The structure of the new compounds was unambiguously established by their chromatographic behavior (see Table 3), their spectroscopic properties, and by chemical or enzymatical degradation, including chromatographic comparison of the products with authentic compounds. The 5′-monophosphate analogs **2**, **3**, and **9** were dephosphorylated by alkaline phosphatase to form the parent compounds, and the nitrophenylester derivatives **4**, **6**, and **7** were either treated with 1 *N* NaOH to yield the 5′-monophosphates or with phosphodiesterase and alkaline phosphatase to yield the nucleoside analogs.

Biochemical Studies

We compared the substrate behavior of the 5'-monophosphate analogs **2**, **3**, and **9** with that of thymidine-5'-monophosphate (5'-TMP) and adenosine-5'-monophosphate (5'-AMP), respectively, for acid and alkaline phosphatase and for 5'-nucleotidase. The substrate behavior of the diester analogs **4** and **7** was compared with thymidine-5'-*p*-nitrophenylphosphate (5'-NO_2phe-TMP) for the enzyme snake venom phosphodiesterase. All enzymes were obtained from commercial sources. The hydrolysis of 5'-phosphates can be monitored by various methods (*11*, *12*). In order to detect side reactions we followed the reaction by quantitative thin-layer chromatography, taking samples every 20 min and determining the rate of hydrolysis from the nucleoside/nucleotide ratio. The hydrolysis of the *p*-nitrophenylester bond by snake venom phosphodiesterase could easily be monitored by measuring the absorbance at 405 nm.



FIGS. 1 AND 2. Hydrolysis by acid phosphatase from potatoes at various pH values. Ordinate: percentage of unreacted nucleotide, determined as described in the Experimental section. ○, 3'-N_3-5'-TMP (**2**); △, 3'-NH_2-5'-TMP (**3**); +, 5'-TMP; ▼, 3'-NH_2-5'-AMP (**9**); □, 5'-AMP.

Acid Phosphatase Studies

The acid phosphatase from potatoes is an unspecific phosphate monoester hydrolase with a broad pH optimum. Its maximum activity is at pH 5.6, but it still cleaves phosphate esters in the range of pH 3–6 at a reasonable rate. In order to see whether the protonation of the 3'-amino group in **3** and **9** influences the reaction rate, assays at pH 3 and pH 5.6 were performed (Figs. 1 and 2).

All analogs were cleaved by the enzyme at both pH values, the rate of cleavage for all compounds being higher at pH 5.6. The natural compounds 5'-AMP and 5'-TMP were better substrates; 5'-AMP is the substrate with the highest reaction rate. In order to compare their cleavage rates at different pH values, the initial velocity for all compounds was determined and calculated relative to 5'-AMP (Table 1). The results show that protonation of the amino group seems to have no influence on the rate of hydrolysis. At pH 5.6 the amino compound **3** is cleaved faster than the azido compound **2**; at pH 3 both are cleaved very slowly.

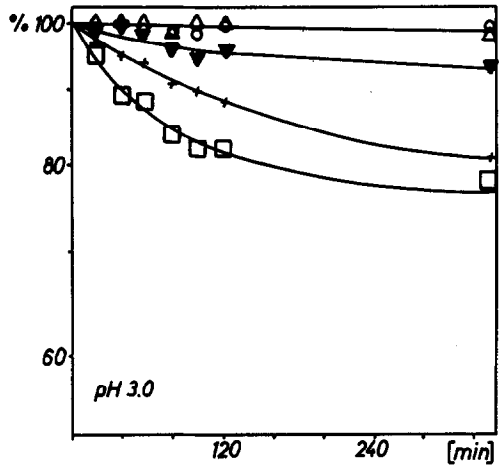


FIGURE 2

5'-Nucleotidase Studies

The 5'-nucleotidase from snake venom is an enzyme that specifically cleaves 5'-nucleotides; 2'- and 3'-nucleotides are not hydrolyzed. As Fig. 3 shows, nucleotides with an unmodified 3'-OH group are much better substrates for this enzyme. Compounds **2** and **3** are very poor substrates. After 5.5 hr only 1.1 and 2.8%, respectively, of these analogs were cleaved [these results may be due to unspecific phosphatases in the enzyme preparation (*13*)].

Examples in the literature support this sensitivity of nucleotidases toward modification in the sugar moiety. Thus 2'-*O*-methylated nucleotides are no longer substrates (*14*, *15*). By contrast a 5'-nucleotidase from rat liver is able to cleave 3'-deoxythymidine-5'-phosphate (*16*). Naito *et al.* described the properties of a 5'-nucleotidase from chicken liver that has an optimum at pH 6.5 in nucleotide cleavage. With *p*-nitrophenylphosphate as substrate, the maximum activity was found to be in the acidic range (*17*). It is therefore possible that some acid phosphatases, which have only been tested with *p*-nitrophenylphosphate as a substrate (*1*), act as nucleotidases under physiological conditions.

TABLE 1

INITIAL VELOCITIES V_0 OF THE HYDROLYSIS BY DIFFERENT PHOSPHOHYDROLASES

Compound	No.	Acid phosphatase V_0 (%)[a] at pH 3	Acid phosphatase V_0 (%)[a] at pH 5.6	Alkaline phosphatase V_0 (%) at pH 10.5	5'-Nucleotidase V_0 (%) at pH 8.4
5'-AMP	—	100	100	100	100
3'-NH_2-5'-AMP	**9**	12	20	23	7
5'-TMP	—	50	83	46	68
3'-N_3-5'-TMP	**2**	<1	38	58	<1
3'-NH_2-5'-TMP	**3**	<1	65	35	<1

[a] Referred to V_0 of 5'-AMP = 100%.

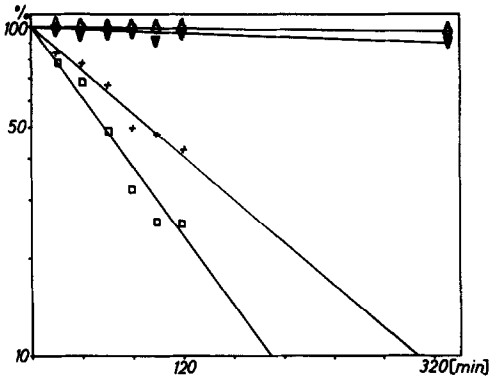


FIG. 3. Hydrolysis by 5′-nucleotidase from snake venom. Ordinate: percentage of unreacted nucleotide, determined as described in the Experimental section. O, 3′N_3-5′-TMP (**2**); △, 3′-NH_2-5′-TMP (**3**); + 5′-TMP; ▼, 3′-NH_2-5′-AMP (**9**); □, 5′-AMP.

Alkaline Phosphatase Studies

Alkaline phosphatase from calf intestine is an unspecific phosphate monoester hydrolase which is not as sensitive against modification at the 3′-position as the ones described before. All analogs are cleaved at a reasonable rate (Table 1) as compared to 5′-AMP.

Interestingly the 3′-azido derivative of 5′-TMP appears to be a better substrate than the unmodified compound. (Fig. 4 and Table 1). The influence of the 3′-amino group is more important for the adenosine derivative than for the thymidine compound.

Phosphodiesterase Studies

Phosphodiesterase from snake venom cleaves nucleotide diesters to yield nucleotide-5′-monophosphates. Kinetic studies (see Fig. 5 and Table 2) show that all analogs are substrates for the enzyme. The azido compound **4** is cleaved with a higher rate and the amino compound **7** with a lower rate than the unmodified thymidine derivative.

Not only the reaction rate but also the binding is altered by modification (Table 2). The most polar compound **7** is much better bound by the enzyme than the least polar one **4**.

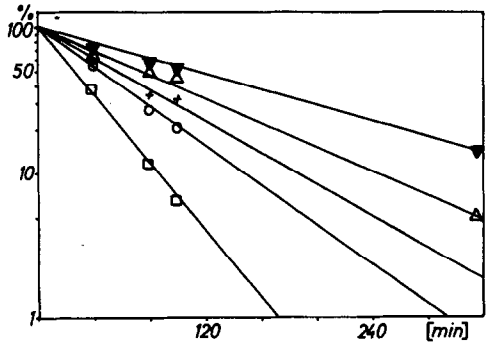


FIG. 4. Hydrolysis by alkaline phosphatase from calf intestine. Ordinate: percentages of unreacted nucleotide, determined as described in the Experimental section. O, 3′-N_3-5′-TMP (**2**); △, 3′-NH_2-5′-TMP (**3**); + 5′-TMP; ▼, 3′-NH_2-5′-AMP (**9**); □, 5′-AMP.

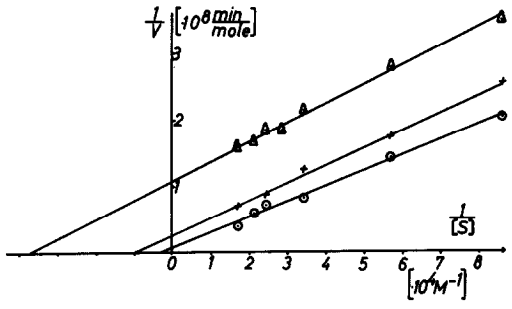


FIG. 5. Lineweaver–Burke plot of the hydrolysis by snake venom phosphodiesterase. Incubation conditions are described in the Experimental Section. ▲, 3′-NH_2-5′-NO_2phe-TMP (**7**); ×, 5′-NO_2phe-TMP; ⊙ 3′-NO_2phe-TMP (**4**).

TABLE 2

KINETIC DATA FOR THE HYDROLYSIS OF DIESTER DERIVATIVES BY SNAKE VENOM PHOSPHODIESTERASE

Compound	No.	K_m [M]	V_{max} [$\frac{mole}{min}$]
5′-NO_2phe-TMP	—	1.1×10^{-4}	4.0×10^{-8}
3′-N_3-5′-NO_2phe-TMP	**4**	4.3×10^{-4}	3.3×10^{-7}
3′-NH_2-5′-NO_2phe-TMP	**7**	2.7×10^{-5}	8.1×10^{-9}

The influence of other modifications at the sugar moiety on the substrate specificity for snake venom phosphodiesterase has been studied previously. Ribo- and deoxyribonucleotides are cleaved at about the same rate (*18*). Differently linked thymidine dinucleotides (5′–5′ and 3′–5′) were also cleaved, the K_m values differing by a factor of 130 (*19*). 2′-*O*-methyl (*20*) as well as 2′-azido (*21*) and 2′-amino (*22*) groups do not prevent enzymatic reaction but decrease the reaction rates to a certain extent. Richards *et al.* studied several (2′–5′, 3′–5′, 5′–5′) arabino- and ribonucleotides. They were also cleaved and had K_m values differing by a factor of 20 (*23*). A P–N internucleotide bond, described by Letsinger *et al.* (*24*) and Jastorff *et al.* (*25*) is not stable against enzymatic degradation, but the reaction rate is slightly decreased.

DISCUSSION

The unspecific phosphomonoesterhydrolases alkaline and acid phosphatase do accept the modification at the 3′-position. In principal one cannot see any significant difference between the influence of the rather polar amino and the less polar azido group on the enzyme reaction. Thus the 3′ position seems not to be essentially involved in binding and/or catalytic reaction. Quite different is the more specific 5′-nucleotidase: The 3′ position seems to be in an essential contact with the active site of the enzyme. The catalytic activity is markedly reduced by substitution of the 3′-OH groups by an amino and an azido group as well. In a further study we will try to establish if, and in which way, these substrate analogs are bound to the enzyme.

In the case of the 5'-phosphodiesterhydrolase, phosphodiesterase, the 3' position seems to be involved in binding and enzymatic reaction. By substitution of the 3'-OH group by an amino or an azido group, the binding constants as well as the reaction rates are significantly influenced. The meaning of these data for the mechanism of molecular interaction between substrate and enzyme shall be investigated further.

EXPERIMENTAL

General Procedures

Ultraviolet spectra were recorded using a Shimadzu spectrometer UV-200; the ir spectra were measured with a Perkin–Elmer Infracord spectrometer using the KBr method. Thin-layer chromatography was performed on Riedel–de Haen silica gel F 254 or Merck PF 254 using solvent systems A (*n*-propanol–conc. ammonia–water, 7 : 1 : 2, v/v/v) and B (acetone–benzene–water, 8 : 2 : 1, v/v/v) and on Macherey & Nagel PEI-cellulose using the solvent system C (0.1 *M* triethanolamine–formic acid, pH 3.3).

Paper chromatography was performed by the descending technique on Schleicher & Schüll 2043 b paper with solvent system A. Paper electrophoresis was also carried out on Schleicher & Schüll 2043 b paper using the solvent systems D (0.05 *M* ammonium formate, pH 3.3), E (0·05 *M* ammonium formate, pH 4) and F (0.1 *M* triethylammonium bicarbonate, pH 7.4).

Chromatographic data and uv and ir spectra of all compounds synthesized are given in Table 3.

Materials

DEAE-cellulose DE 52 was purchased from Whatman and DEAE-Sephadex A25 from Pharmacia Fine Chemicals. Adenosine kinase from Baker's yeast (EC 2.7.1.20) used in this study was generously given by Dr. H. Sternbach, Göttingen. 3-Amino-3'-deoxyadenosine (**8**) was a generous gift from Drs. R. M. Kula and M. Morr, Stöckheim. 3'-Azido-3'-deoxythymidine (**1**) and 3'-phthalimido-3'-deoxythymidine (**5**) were prepared according to literature procedures (*6*) and (*9*), respectively. Snake venom phosphodiesterase (EC 3.1.4.1) and alkaline phosphatase (EC 3.1.3.1) were purchased from Boehringer–Mannheim.

Preparation of the Nucleotide Analogs

3'-Azido-3'-deoxythymidine-5'-phosphate (3'-N_3-TMP) (**2**). 3'-Azido-3'-deoxythymidine (**1**) (267 mg, 1 mmol) was stirred with dry acetone (6 ml), pyridine (1.6 ml; 20 mmol), and $POCl_3$ (0.11 ml, 1.2 mmol) for 2.5 hr at −75°C. Water (2.5 ml) was added. After warming up to room temperature the mixture was evaporated to dryness, the residue was dissolved in water, and the pH was brought to 7.5. Compound **2** was isolated by column chromatography on DEAE-cellulose (4 × 35-cm) column with a linear gradient of 0.01 to 0.2 *M* triethylammonium bicarbonate buffer, pH 7.5 (3 liters each). Compound **2** was eluted at a concentration of 0.15 *M*. The yield was 320 mg (92%).

3'-Amino-3'-deoxythymidine-5'-phosphate (**3**). This compound was synthesized from **2** as previously described (*8*). Purification was performed as described for compound **2**. The yield was 90%.

3'-Azido-3'-deoxythymidine-5'-p-nitrophenylphosphate (**4**) (*3'-N_3-5'-NO_2phe-TMP*).

TABLE 3

CHROMATOGRAPHIC DATA AND UV AND IR SPECTRA[a]

Compound	No.	Tlc solvents[b] A	Tlc solvents[b] B	PEI-Tlc solvent C	PC solvent[b] A	Electrophoresis solvents[c] D	Electrophoresis solvents[c] E	Electrophoresis solvents[c] F	uv in methanol δ_{max} (nm)	ir (cm^{-1})
3′-N_3-5′-TMP	**2**	0.18	0	0.33	0.18	1.0	1.0	1.0	267 ($\varepsilon = 10\,000$)	2110
3′-NH_2-5′-TMP	**3**	0.08	0	0.52	0.08	0	0.4	1.0	267 ($\varepsilon = 10\,000$)	—
3′-N_3-5′-NO_2-phe-TMP	**4**	—	0.6	0.47	0.76	0.4	0.4	0.4	272 ($\varepsilon = 12\,300$); 300 (shoulder)	2110
3′-Phth-5′-NO_2phe-TMP	**6**	—	0.5	0.46	0.43	0.4	0.4	0.4	272; 300 (shoulder)	1380 1390
3′-NH_2-5′-NO_2phe-TMP	**7**	—	—	0.48	0.52	0.0	—	0.4	272 ($\varepsilon = 12\,500$); 300 (shoulder)	—
3′-NH_2-5′-AMP	**9**	0.11	0	0.34	0.10	0.0	0.5	1.0	260 ($\varepsilon = 15\,000$)	—

[a] For solvent systems, see Experimental section, General Procedures.
[b] Referred to thymidine = 1.
[c] Referred to 5′-TMP = 1.

Into a stirred solution of 3'-azido-3'-deoxythymidine (**1**) (267 mg, 1 mmol) in dry pyridine (4 ml) at 0°C was slowly dropped a solution of *p*-nitrophenylphosphorodichloridate (*8*) (303 mg, 1.2 mmol) in dry pyridine (1 ml). After 3 hr, water (3 ml) was added, and the solution was evaporated to dryness. The residue was dissolved in water–methanol (3:1, v/v), and the pH was brought to 7.5. Compound **4** was isolated at 4°C by column chromatography on DEAE-cellulose (3 × 50-cm column) with a linear gradient of 0.01–0.03 *M* triethylammonium bicarbonate buffer, pH 7.5 (3 liters each) and identified by its typical uv-spectrum. The yield was 290 mg (62%).

3'-Phthalimido-3'-deoxythymidine-5'-p-nitrophenylphosphate (3'-Phth-5'-NO_2phe-TMP (**6**). A solution of 3'-phthalimido-3'-deoxythymidine (**5**) (85.3 mg, 0.23 mmol) in dry pyridine (2 ml) was slowly dropped into a solution of *p*-nitrophenylphosphorodichloridate (74.3 mg, 0.29 mmol) in dry pyridine (1 ml) at −10°C. After 3 hr at −10°C the reaction was stopped by adding water (2 ml). The solvent was evaporated, the residue was dissolved in methanol, and **6** was isolated by preparative thin-layer chromatography (two plates, 20 × 40 cm) in solvent system B. It was identified on the plate by its absorption at 254 and 365 nm and its typical uv spectrum. Compound **6** was eluted from silica gel with solvent system A. The yield was 88 mg (67%).

3'-Amino-3'-deoxythymidine-5'-p-nitrophenylphosphate (3'-NH_2-5'-NO_2phe-TMP (**7**). To a solution of 3'-phthalimido-3'-deoxythymidine-5'-*p*-nitrophenylphosphate (**6**) (48 mg, 0.08 mmol) in methanol–water, 6:4 v/v, 5 ml) was added a 10% ethanolic hydrazine hydrate solution (80 μl, 0.16 mmol). The mixture was stirred for 1 hr at 45°C. The reaction was stopped with 1 *N* HCl (0.5 ml). After 20 min of stirring, the mixture was adjusted to pH 7.5 with triethylamine and diluted with water to 30 ml. Compound, **7** was isolated by column chromatography on DEAE-cellulose (2 × 50-cm column) with a linear gradient of 0.05–0.25 *M* triethylammonium bicarbonate buffer, pH 7.5 (1.5 liters each), and identified by its typical uv-spectrum. The yield was 12 mg (34%).

3'-Amino-3'-deoxyadenosine-5'-phosphate (3'-NH_2L-A) (**9**). 3'-Amino-3'-deoxyadenosine (**8**) (50 mg, 0.19 mmol) was stirred with adenosine-5'-triphosphate (Na_2 salt) (235 mg, 0.43 mmol) and 0.12 U of adenosine kinase in 0.17 *M* triethanolamine–HCl buffer, pH 7.5, containing 67 m*M* KCl and 16.7 m*M* $MgCl_2$ (20 ml) for 20 hr at 25°C. Water (60 ml) was added and **9** was isolated by column chromatography on DEAE-Sephadex (4 × 40-cm column). After washing with 0.2 *M* triethylammonium bicarbonate buffer, pH 7.5 (1.5 liters), **9** was eluted with 0.22 *M* triethylammonium bicarbonate buffer, pH 7.5. The solution was evaporated, and the residue was crystallized from water–isopropanol in the presence of HCl (0.5 ml). The yield was 29 mg (45%).

Cleavage of compounds **4**, **6**, *and* **7** *with NaOH.* Each compound [6.5 OD (0.5) μmol)] was treated with 1 *N* NaOH (20 μl) for 30 min at 100°C. The reaction products (*p*-nitrophenol and the corresponding monophosphates) were identified by electrophoresis in solvent system F.

Cleavage of compounds **2**, **3** *and* **9** *with alkaline phosphatase.* Compounds **2** and **3** [5 OD each (0.5 μmol)] and **9** [7.5 OD (0.5 μmol)] were incubated in 0.1 *M* Tris–HCl buffer, pH 9.5 (50 μl), with 5 μl of alkaline phosphatase (sp. act., 400 U/ml) for 2 hr at 37°C. The dephosphorylated products were identified by paper chromatography in solvent system A.

Cleavage of compounds **4**, **6**, *and* **7** *with phosphodiesterase and alkaline phosphatase.* Each compound [6.5 OD (0.5 μmol)] was incubated in 0.05 *M* Tris–HCl buffer, pH 8.4 (50 μl), with 5 μl of snake venom phosphodiesterase (sp. act., 1.5 U/ml) and 5 μl of alkaline phosphatase (sp. act.) 400 U/ml) for 2 hr at 37°C. The reaction products (*p*-nitrophenol and nucleoside analogs) were identified by paper chromatography in solvent system A.

BIOCHEMICAL

Materials and Methods

Acid phosphatase from potatoes (EC 3.1.3.2) (sp. act., 60 U/mg), alkaline phosphatase from calf intestine (EC 3.1.3.1) (sp. act., 400 U/mg), and phosphodiesterase from snake venom (EC 3.1.4.1) (sp. act., 1.5 U/mg) from snake venom were purchased from Boehringer–Mannheim. 5′-Nucleotidase (EC 3.1.3.5) (sp. act., 17.4 U/mg) was purchased from Sigma, St. Louis, Mo.

Quantitative determination of nucleotide concentration on thin-layer plates was performed on a Vitratron densitometer Model TLD 200; quantitative spectrophotometric measurements were performed on Shimadzu spectrometer Model UV-200.

Thin-layer chromatography was performed as described before.

Acid phosphatase experiments. Nucleotides **2**, **3**, and 5′-TMP [20 OD each (2 μmol)] and **9** and 5′-AMP [30 OD each (2 μmol)] were incubated with acid phosphatase (0.6 U) in 0.1 *M* Na-citrate buffer, pH 3 of pH 5.6, respectively (50 μl each), at 25°C. Every 20 min, 5-μl samples were separated on silica gel thin-layer plates in solvent system A, and the amount of unreacted nucleotide was estimated densitometrically.

5′-Nucleotidase experiments. Compounds **2**, **3**, and 5′-TMP [20 OD each (2 μl)] and compound **9** and 5′-AMP [30 OD each (2 μmol)] were incubated with 5′-nucleotidase (0.14 U) in 0.05 *M* Tris–HCl, pH 8.4, and 0.05 *M* $MgSo_4$ (50 μl) at 25°C. Every 20 min, 5-μl samples were taken, and the quantities of unreacted nucleotides were determined as described above.

Alkaline phosphatase experiments. Compounds **2**, **3**, and 5′-TMP [20 OD each (2 μmol)] and compound **9** and 5′-AMP [30 OD each (2 μmol)] were incubated with alkaline phosphatase (0.2 U) in 0.1 *M* glycine/HCl buffer, pH 10.5, containing 1 m*M* $MgCl_2$ and 1 m*M* $ZnCl_2$ (50 μl) at 25°C. The reaction was followed up as described above.

Phosphodiesterase studies. Different concentrations of compounds **4**, **7**, and 5′NO_2phe-TMP in a 0.33 m*M* Tris–acetate buffer, pH 8.8, containing 30 m*M* of magnesium acetate (3 ml) were incubated with phosphodiesterase (1.5 mU) at 25°C. The extinction was followed at 405 nm. Initial velocity (V_0) was determined for each concentration, and K_m and V_{max} values were determined by means of a Lineweaver–Burke plot.

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REFERENCES

1. D. M. REHKOP AND R. L. VAN ETTEN, *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 1775 (1975).
2. D. M. GOLDBERG AND A. BELFIELD, *Nature (London)* **247**, 286 (1974).
3. A. LEE, K. CHANCE, C. WEEKS, AND G. WEEKS, *Arch. Biochem. Biophys.* **171**, 407 (1975).
4. S. SENESI, G. FALCONE, P. L. IPATA, AND R. A. FELICIOLI, *Biochemistry* **13**, 5008 (1974).
5. A. HOLY AND M. PLSEK, *Coll. Czech. Chem. Commun.* **41**, 2433 (1976).
6. W. OSTERTAG, G. ROESLER, C. J. KRIEG, J. KIND, T. COLE, T. CROZIER, G. GAEDICKE, G. STEINHEIDER, N. KLUGE, AND S. DUBE, *Proc. Nat. Acad. Sci. USA* **71**, 4980 (1974).
7. Y. TSUCHIA, T. TAKENISHI, T. KATO, AND T. ICHIKAWA, Japan. Pat. 108 ('65), cited in *Chem. Abstr.* **62**, P11737g.
8. R. P. Glinski, M. S. KHAN, R. L. KALAMAS, C. L. STEVENS, AND M. B. SPORN, *Chem. Commun.* 915 (1970).
9. N. MILLER AND J. J. FOX, *J. Org. Chem.* **29**, 1772 (1964).
10. J. T. TRUMAN AND H. KLENOW, *Mol. Pharmacol.* **4**, 77 (1968).
11. H. M. KALCKAR, *J. Biol. Chem.* **167**, 445 (1947).
12. J. G. SHAW, *Arch. Biochem. Biophys.* **117**, 1 (1966).
13. Sigma Catalog 1973.
14. M. HONJO, Y. KANAI, Y. FURUKAWA, A. MIZUNO, AND Y. SANNO, *Biochim. Biophys. Acta* **87**, 698 (1964).
15. B. G. LANE, *Biochemistry* **4**, 212 (1965).
16. P. FRITZON, *Eur. J. Biochem.* **1**, 12 (1967).
17. Y. NAITO, R ITOH, AND K. TSUSHIMA, *Int. J. Biochem.* **5**, 807 (1974).
18. M. LASKOWSKI, SR., "The Enzymes" (P. O. Boyer, Ed.), Vol. 4, p. 313. Academic Press, New York, 1971.
19. W. E. RAZZELL AND H. E. KHORANA, *J. Biol. Chem.* **234**, 2105 (1959).
20. M. W. GRAY AND B. G. LANE, *Biochim. Biophys. Acta* **134**, 243 (1967).
21. P. F. TORRENCE, A. M. BOBST, J. A. WATERS, AND B. WITKOP, *Biochemistry* **12**, 3962 (1973).
22. J. HOBBS, H. STERNBACH, M. SPRINZL, AND F. ECKSTEIN, *Biochemistry* **12**, 5138 (1973).
23. G. M. RICHARDS, D. J. TUTAS, W. J. WECHTER, AND M. LASKOWSKI, SR., *Biochemistry* **6**, 2908 (1967).
24. R. L. LETSINGER AND W. S. MUNGALL, *J. Org. Chem.* **35**, 3800 (1970).
25. B. JASTORFF AND H. HETTLER, *Chem. Ber.* **102**, 4119 (1969).