NONGLYCOSIDE ANALOGS OF NUCLEOTIDES

I. 2,3-DIHYDROXYPROPYL DERIVATIVES OF NUCLEIC BASES

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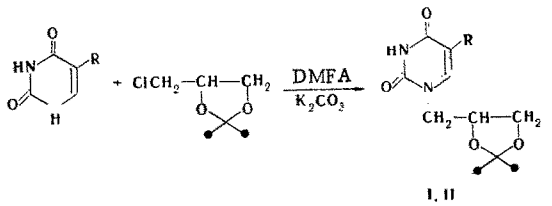
N^1-(2',3'-Dihydroxypropyl)uracil, -thymine, -cytosine, and N^9-(2',3'-dihydroxypropyl)adenine were synthesized by alkylation of nucleic bases with 2,3-O-isopropylideneglycerol chlorohydrin, subsequent separation of the resulting mixtures, and removal of the protective groupings. Phosphorylation of these compounds or of their selectively substituted derivatives gave 2'(3')-monophosphates, which were converted to 2',3'-cyclophosphates by reaction with N,N'-dicyclohexylcarbodiimide. Thionation of the corresponding cytosine derivatives gave N^1-(2',3'-dihydroxypropyl)-4-thiouracil and its 2'(3') phosphate.

The principal method for the creation of inhibitors for enzyme systems consists in modification of the important functional groups of natural substrates. However, the study of compounds in which the principal groups necessary for bonding and reactivity of the substrate are retained but which differ from the natural compounds with respect to "rigidity" or, on the other hand, with respect to great "lability" of the molecule seems just as reasonable. The present communication is the first of a series of papers devoted to the synthesis of "labeled" analogs of nucleotides in which the furanose ring is replaced by hydroxyalkyl substituents.

N^9-(2',3'-Dihydroxypropyl)adenine, N^1-(2',3'-dihydroxypropyl)uracil, and N^1-(2',3'-dihydroxypropyl)-cytosine were previously synthesized [1] by alkylation of the appropriate nucleic bases with glycidol. In addition, an adenine derivative was obtained by reaction of the sodium salt of adenine with glycerol α-chlorohydrin. A year ago [2] N^9-(2',3'-dihydroxypropyl)adenine was resynthesized and converted to a hypoxanthine derivative by deamination. Both compounds were converted to 3'-phosphates by reaction with phosphorus oxychloride in triethyl phosphate.

The method that we developed for the synthesis of dihydroxypropyl derivatives of nucleic bases consists in alkylation of the appropriate bases with 2,3-O-isopropylideneglycerol chlorohydrin. The selection of precisely a protected alkylating agent made it possible, at a yield level close to that obtained in preceding studies, to develop convenient methods for the isolation of the products and convenient methods for the synthesis of amino-group protected derivatives:



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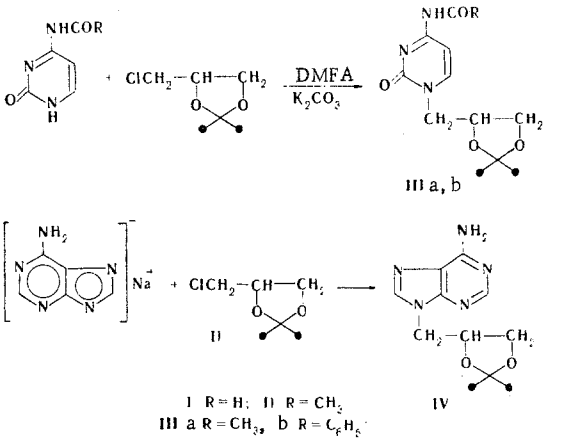
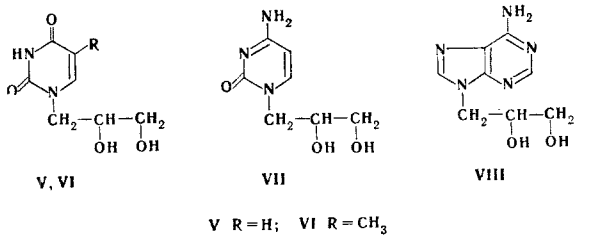


In all cases, alkylation gives complex mixtures of reaction products, from which the necessary compounds were isolated either by crystallization or chromatography.

Removal of the protective groups gives the known analogs of nucleosides (V-VIII).



In order to phosphorylate the compounds we used a classical method [3] that was successfully modified in [4]. The phosphoric acid diester obtained by reaction of the nucleoside analog with dicyclohexylcarbodiimide and cyanoethyl phosphate was isolated by chromatography on DEAE-cellulose (HCO_3^- form) and only then was the cyanoethyl group removed by hydrolysis with 2 N lithium hydroxide in the course of precisely 2 min. Neutralization, evaporation, and washing of the residue with alcohol–acetone made it possible to isolate the electrophoretically pure lithium salts of phosphates, free of inorganic phosphate, in up to 75% yields.

In connection with the necessity for the protection of the amino group in the case of cytosine and adenine derivatives, IIIa,b, after selective removal of the isopropylidene grouping, were converted to N^1-(2',3'-dihydroxypropyl)-N^4-acetyl- and -N^4-benzoylcytosine (XIa and XIb, respectively). Benzoylation of IV and subsequent acid hydrolysis of the resulting N^6-benzoyl derivative (XII) made it possible to obtain N^9-(2',3'-dihydroxypropyl)-N^6-benzoyladenine (XIII). Phosphorylation of these compounds by the above-described methods gives N^1-(2',3'-dihydroxypropyl)-N^4-acetyl- and -N^4-benzoylcytosine 2'(3')-phosphates (XIVa and XIVb, respectively) and N^9-(2',3'-dihydroxypropyl)-N^6-benzoyladenine 2'(3') phosphate (XV),

TABLE 1. Alkylation of Pyrimidine Bases

Alkylation product	mp, °C	Empirical formula	Found, %		Calc., %		UV spectrum (C_2H_5OH), λ_{max}, nm (ε)	Yield, % (isolation method)
			C	H	C	H		
2',3'-O-Isopropylidene-N^1-(2',3'-dihydroxypropyl)uracil	148—149	$C_{10}H_{14}N_2O_4$	52,8	6,5	53,1	6,2	266 (7900)	24 (A)
2',3'-O-Isopropylidene-N^1-(2',3'-dihydroxypropyl)thymine (II)	161—162	$C_{11}H_{16}N_2O_4$	55,1	6,9	55,0	6,7	271 (6600)	32 (A)
2',3'-O-Isopropylidene-N^1-(2',3'-dihydroxypropyl)-N^4-acetylcytosine (IIIa)	202—203	$C_{12}H_{17}N_3O_4$	53,6	6,5	53,9	6,4	248 (12 800) 302 (6100)	25 (B)
2',3'-O-Isopropylidene-N^1-(2',3'-dihydroxypropyl)-N'-benzoylcytosine (IIIb)	217—219	$C_{17}H_{19}N_3O_4$	61,7	6,0	62,0	5,8	254 (19 600) 304 (10 800)	30 (B)

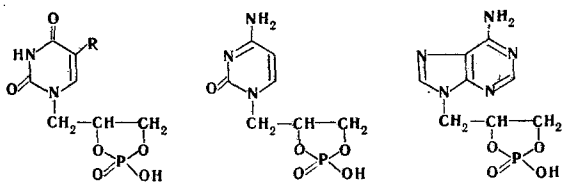
TABLE 2. N-(2',3'-Dihydroxypropyl) Derivatives of Nucleic Bases

Compound	mp, °C	Empirical formula	Found, %		Calc., %		UV spectrum, λ_{max}, nm (ε)			Yield, %
			C	H	C	H	pH 1	pH 7	pH 13	
N^1-(2',3'-Dihydroxypropyl)uracil (V)	142—143*	$C_7H_{12}N_2O_4$	47,8	6,2	48,0	6,0	266 (9900)	267 (9800)	265 (6900)	80
N^1-(2',3'-Dihydroxypropyl)thymine (VI)	151—152	$C_8H_{14}N_2O_4$	47,7	5,9	47,6	5,8	272 (9900)	270 (7800)	270 (7400)	78
N^1-(2',3'-Dihydroxypropyl)-N^4-acetylcytosine (XIIa)	207—209	$C_9H_{13}N_3O_4$					246 (6700)	249 (12 900)	316 (12 700)	91
N^1-(2',3'-Dihydroxypropyl)-N^4-benzoylcytosine (XIb)	219—221	$C_{14}H_{15}N_3O_4$	58,0	5,4	58,1	5,2	309 (13 300)	306 (5050)	314 (20 200)	93
							251 (12 600)	258 (21 300)		
							316 (20 100)	305 (12 100)		
N^9-(2',3'-Dihydroxypropyl)adenine (VIII)	210—212†	$C_{15}H_{15}N_5O_3$	57,3	5,0	57,5	4,8	259 (13 300)	260 (14 000)	260 (11 500)	84
N^9-(2',3'-Dihydroxypropyl)-N^6-benzoyladenine (XIII)	196—198						255 (14 500)	279 (17 500)	305 (11 900)	52
							285 (17 800)			

* Literature mp 143-144° [1].

† Literature mp 207-209° [1].

which were converted to N^1-(2',3'-dihydroxypropyl)cytosine 2'(3')-phosphate (XVI) and N^9-(2',3'-dihydroxypropyl)adenine 2'(3')phosphate. The resulting mixtures of monophosphates were converted to the corresponding 2',3'-cyclophosphates (XVIII, XXI) by reaction with dicyclohexylcarbodiimide:



XVIII, XIX XX XXI

XVIII R = H; XIX, R = CH_3

Using a recently described method for the conversion of the 4-amino group of cytidine to a thione [5] group, we synthesized 4-thiouracil derivatives – N^1-(2',3'-dihydroxypropyl-4-thiouracil (XXII) and its 2'(3')-phosphate (XXIII).

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EXPERIMENTAL

The UV spectra were recorded with a Specord UV-vis spectrophotometer, and the PMR spectra were recorded with an XL-100 spectrometer. Thin-layer chromatography (TLC) was carried out either on Silufol UV-254 plates or on plates with a layer of FND cellulose. In the first case, chloroform containing from three to 30% (by volume) methanol was used as the solvent system, whereas in the second case isopropyl alcohol–ammonia–water (7:3:2) was used. Electrophoresis on Whatmann 3-mm paper in a pH 9 volatile buffer [a 0.1% solution of $(NH_4)_2CO_3$] was carried out with a voltage gradient of 35 V/cm.

Alkylation of Pyrimidine Bases. A mixture of 100 mmole of the base, 15 g (100 mmole) of 2,3-O-isopropylideneglycerol chlorohydrin, and 27.6 g (200 mmole) of anhydrous K_2CO_3 in 500 ml of dry DMFA was heated at 90° for 30 h. The mixture was filtered, the solid material was washed with DMFA (three 20-ml portions), and the combined filtrates were vacuum evaporated to dryness. The residue was dissolved in 30 ml of water, the pH of the mixture was brought up to 7, and the mixture was extracted with chloroform. The extract was dried and evaporated to dryness. The following two methods were used for the subsequent workup.

A) The solid material was dissolved in the minimum amount of chloroform, and the solution was applied to a column containing silica gel (1 liter). The column was eluted with $CHCl_3$–CH_3OH (95 : 5 by volume). The eluant was monitored from the absorption at 254 nm. The second fractions were combined and evaporated to dryness, and the residue was evaporated twice with alcohol (20 ml each time) and recrystallized from alcohol (see Table 1).

B) The solid was evaporated three times with alcohol (20 ml each time) and recrystallized from alcohol (see Table 1).

2',3'-O-Isopropylidene-N^9-(2',3'-dihydroxypropyl)adenine (IV). A 3.75-g (157.5 mmole) sample of sodium hydride was added with cooling and stirring to a suspension of 20.2 g (150 mmole) of

TABLE 3. 2'(3')-Phosphates of Nucleoside Analogs

Compound*	UV spectrum, λ_{max}, nm (ε)			Yield, %
	pH 1	pH 7	pH 13	
N^1-(2',3'-Dihydroxypropyl)uracil-2'(3')-phosphate dilithium salt dihydrate (IX)	266 (9400)	267 (9900)	265 (7400)	74
N^1-(2',3'-Dihydroxypropyl)thymine-2'(3')-phosphate dilithium salt monohydrate (X)	271 (9800)	272 (10 400)	270 (7100)	76
N^1-(2',3'-Dihydroxypropyl)-N^4-acetylcytosine 2'(3')-phosphate dilithium salt monohydrate (XIVa)	245 (6300) 310 (13 100)	250 (12 800) 305 (5600)	318 (12 900)	71
N^1-(2',3'-Dihydroxypropyl)-N^4-benzoylcytosine 2'(3')-phosphate dilithium salt monohydrate (XIVb)	252 (12 200) 318 (19 300)	258 (20 900) 306 (11 300)	314 (19 300)	79
N^9-(2',3'-Dihydroxypropyl)-N^6-benzoyladenine 2'(3')-phosphate dilithium salt monohydrate (XV)	252 (10 000) 292 (25 200)	284 (19 900)	298 (13 400)	74

*All of the compounds obtained were electrophoretically homogeneous.

adenine in 600 ml of dry DMFA. After vigorous hydrogen evolution had ceased, the mixture was heated to room temperature and then to 90°. A 22.5-g sample of 2,3-O-isopropylideneglycerol chlorohydrin was added dropwise to the hot mixture, and the resulting mixture was heated at 90° for 30 h. The solution was cooled and evaporated to dryness in vacuo, and the solid was stirred with 450 ml of water and extracted thoroughly with chloroform. The extracts were dried, the solvent was removed, and the residue was evaporated three times with alcohol (50 ml each time). The solid was recrystallized from alcohol to give 9.1 g (24%) of a product with mp 214-215°. UV spectrum (in alcohol): λ_{max} 261 nm (ε 11,800). Found: C 53.2; H 5.9%. $C_{11}H_{15}N_5O_2$. Calculated: C 53.0; H 6.1%.

2',3'-O-Isopropylidene-N^9-(2',3'-dihydroxypropyl)-N^6-benzoyladenine (XII, Table 2). A 4.2-g (30 mmole) sample of benzoyl chloride was added dropwise with cooling to a suspension of 6.8 g (27 mmole) of IV in 75 ml of dry pyridine, and the mixture was stirred at room temperature for 16 h. Water (27 ml) was then added to the mixture, and it was stirred for another 2 h. The solution was vacuum evaporated to dryness, and the residue was stirred with 75 ml of water. The aqueous mixture was extracted with chloroform, and the extracts were washed with saturated $NaHCO_3$ solution and water, dried, and evaporated to dryness. The residue was evaporated with alcohol (three 20-ml portions), 50 ml of water and 5 ml of ether were added to the residue, and the mixture was allowed to stand overnight in a refrigerator. The resulting crystals were removed by filtration, washed with water, and dried to give 8.4 g (87%) of product. UV spectrum (in alcohol): λ_{max} nm, (ε): 235 (13,600) and 279 (17,100).

Acid Hydrolysis of 2',3'-O-Isopropylidene Derivatives. A suspension of 10 mmole of 2',3'-O-isopropylidene derivative in 200 ml of 50% acetic acid was heated at 90° for 30 min. The solution was then cooled and vacuum evaporated to dryness at 30°, and the residue was evaporated three times with alcohol (50 ml each time). The residue was recrystallized from alcohol (see Table 2).

Phosphorylation of Analogs of Nucleosides. A mixture of 3 mmole of the nucleoside analog and 6 ml of 1 M solution of cyanoethyl phosphate in pyridine [3] was vacuum evaporated to dryness at 30°, and the residue was re-evaporated with dry pyridine (five 15-ml portions). The residue (protected from air moisture) was dissolved in 30 ml of dry pyridine, 6 g of dicyclohexylcarbodiimide was added, and the mixture was stirred in a hermetic system at room temperature for 3 days. Water (30 ml) was added to the mixture, and it was stirred for another 16 h. The resulting precipitate was removed by filtration and washed with 10% aqueous pyridine (two 10-ml portions). The combined filtrates were extracted with cyclohexane (three 30-ml portions), evaporated at 30° to three-fourths of their original volume, and filtered. The filtrate was applied to a column filled with DE-32 cellulose (HCO_3^- form, 300 ml). The column was washed with water until there was no longer absorption at 254 nm, and it was then eluted with a linear gradient ammonium bicarbonate buffer (pH 7.5) from 0.001 to 0.05 M concentrations (the total volume of eluant was 5 liters). The eluant was monitored from the absorption at 254 nm. The cyanoethyl ester solution disappeared in the presence of an approximately 0.025 M buffer. The fractions were combined and vacuum evaporated to dryness at 30°, and the residue was evaporated four times with 100 ml of water. The residue was stirred for precisely 2 min with 15 ml of 2 N lithium hydroxide solution, during which 15 ml of 2

N hydrochloric acid was added to it slowly. The pH of the solution was brought up to 7 (on a pH-meter) with lithium hydroxide solution, and the resulting precipitate was removed by filtration and washed with 10 ml of water. The combined filtrates were vacuum evaporated to dryness at 30°, and the residue was mixed with 50 ml of absolute methanol–dry acetone (1:5) and centrifuged. The liquid was decanted, the solid was again stirred with 50 ml of methanol–acetone, and the mixture was again centrifuged. This operation was repeated until the supernatant liquid gave a negative test for Cl^-. The solid was transferred with absolute ether to a filter, washed with absolute ether, and dried (see Table 3).

Removal of Alkali-Labile Protective Groupings. A) A suspension of 1 mmole of XIa or XIb in 10 ml of absolute methanol semisaturated at 0° with dry NH_3 was stirred for 20 h. The resulting solution was evaporated to dryness, and the residue was evaporated with alcohol (two 10-ml portions) and recrystallized from alcohol. The yield of N^1-(2',3'-dihydroxypropyl)cytosine (VII) with mp 178-180° was 87-92%. UV spectrum, λ_{max}, nm (ε): 282 (12,300) at pH 1, 274 (8400) at pH 7, and 274 (8100) at pH 13. PMR spectrum (in D_2O), δ, ppm: 7.84 (doublet, J = 8 Hz, 6-H), 6.26 (doublet, 5-H), 3.80-4.42 (multiplet, 1'-, 2'-, and 3'-H); in $(CD_3)_2SO$ 7.72 (doublet, J = 8 Hz, 6-H), 7.22 (broad singlet, NH_2), 5.93 (doublet, 5-H), 5.28 (doublet, J = 4 Hz, 2'-OH), 5.07 (triplet, J = 3 Hz, 3'-OH), 3.40 (doublet, J = 6 Hz, 1'-H).

B) Absolute methanol (10 ml) saturated with dry NH_3 at 0° was added to a solution of XIVa, XIVb, or XV in 5 ml of water, and the mixture was stirred at room temperature for 20 h. Water (10 ml) was added to the mixture, and the resulting precipitate was removed by filtration and washed with 10 ml of water. The combined filtrates were vacuum evaporated to dryness at 30°, and the residue was stirred with 10 ml of absolute alcohol and washed successively on the filter with absolute alcohol (two 10-ml portions) and absolute ether (five 10-ml portions).

N^1-(2',3'-Dihydroxypropyl)cytosine 2'(3')-Phosphate Dilithium Salt Monohydrate (XVI). This compound was obtained in 84-91% yield. It was electrophoretically homogeneous. UV spectrum, λ_{max}, nm (ε): 282 (11,800) at pH 1, 274 (8600) at pH 7, and 274 (9100) at pH 13. PMR spectrum (in D_2O), δ, ppm: 7.92 and 7.95 (doublets, J = 8 Hz, 6-H of a mixture of 2'- and 3'-phosphates, 2:1), 6.31 (two doublets, 5-H), and 4.01-4.48 (multiplet, 1'-, 2'-, and 3'-H).

N^9-(2',3'-Dihydroxypropyl)adenine 2'(3')-Phosphate Dilithium Salt Monohydrate (XVII). This compound was obtained in 87% yield and was electrophoretically homogeneous. UV spectrum, λ_{max}, nm (ε): 258 (13,000) at pH 1, 262 (13,800) at pH 7, and 262 (13,800) at pH 13, according to the literature data for the 3'-phosphate [2], λ_{max} 259 (13,800) at pH 7.

N^1-(2',3'-Dihydroxypropyl)uracil 2',3'-Cyclophosphate (XVIII) and N^1-(2',3'-Dihydroxypropyl)thymine 2',3'-Cyclophosphate (XIX). A solution of 1 mmole of the lithium salt of V or VI in 10 ml of water was applied to a column filled with Dowex-50 (H^+ form, 10 ml) and eluted with water. The fraction absorbing at 260 nm was vacuum evaporated to dryness at 30° and again evaporated with dry DMFA (three 10-ml portions). With protection from air moisture, 1.2 g of dicyclohexylcarbodiimide and 25 ml of DMFA were added to the residue. The mixture was stirred carefully until the solids had dissolved completely. After 20 min, 120 ml of water was added to the turbid solution, and the precipitate was removed by filtration and washed with two 10-ml portions of water. The combined filtrates were extracted with cyclohexane (two 20-ml portions), evaporated in vacuo at 30° to 150 ml, brought to pH 7 (pH-meter) with 1 N NaOH, and vacuum evaporated at 30° to the consistency of an oil. When dry acetone was added, the residue began to crystallize. The crystals were removed by filtration and washed successively with acetone and dry ether.

The yield of the sodium salt of XVIII was 94%. UV spectrum, λ_{max}, nm (ε): 261 (8,000) at pH 1, 261 (8,800) at pH 7, 262 (6,000) at pH 13. PMR spectrum (in D_2O), δ, ppm: 7.94 (doublet, J = 8 Hz, 6-H), 6.16 (doublet, 5-H), 3.33 (doublet, J = 9 Hz, 1'-H), 4.16-4.63 (multiplet, 2'- and 3'-H).

The yield of the sodium salt of XIX was 96%. UV spectrum, λ_{max}, nm (ε): 270 (7800) at pH 1, 270 (8100) at pH 7, and 269 (6100) at pH 13.

Both compounds were electrophoretically homogeneous and had half the mobilities of the corresponding phosphates.

N^1-(2',3'-Dihydroxypropyl)cytosine 2',3'-Cyclophosphate (XX) and N^9-(2',3'-Dihydroxypropyl)adenine 2',3'-Cyclophosphate (XXI). A solution of 1 mmole of XVI or XVII in 10 ml of water was applied to a column filled with Dowex-50 (H^+ form, 10 ml). The column was eluted successively with 10 ml of water and 2.5% ammonium hydroxide. The fraction absorbing at 260 nm was vacuum evaporated to dryness at 30° and again evaporated with absolute methanol (four 20-ml portions). With protection from air moisture,

1.7 g of dicyclohexylcarbodiimide and 28 ml of absolute methanol were added to the residue, and the mixture was shaken until the solids had dissolved completely, after which the solution was allowed to stand at room temperature overnight. The methanol was removed by vacuum evaporation at 30°, and the residue was stirred with 20 ml of water. The solid was removed by filtration and washed with water (two 10-ml portions). The combined filtrates were extracted with cyclohexane (two 30-ml portions) and vacuum evaporated almost to dryness at 30°. When dry acetone was added, the residue began to crystallize. The crystals were removed by filtration and washed successively with dry acetone and absolute ether.

The yield of the ammonium salt of XX was 86%. UV spectrum, λ_{max}, nm (ε): 282 (10,400) at pH 1, 273 (7500) at pH 7, and 273 (7300) at pH 13. PMR spectrum (in D_2O), δ, ppm: 7.98 (doublet, J=8 Hz, 6-H), 6.35 (doublet, 5-H), and 4.21-4.67 (multiplet, 1'-, 2'-, and 2'-H).

The yield of the ammonium salt of XXI was 83%. UV spectrum, λ_{max}, nm (ε): 259 (13,200) at pH 1, 261 (13,700) at pH 7, and 261 (13,200) at pH 13.

Both compounds were electrophoretically homogeneous and had half the mobility of the corresponding monophosphates.

N^1-(2',3'-Dihydroxypropyl)-4-thiouracil (XXII). A mixture of 0.555 g (3 mmole) of VII, 7.5 ml of water, 7.5 ml of pyridine, and 15 ml of liquid hydrogen sulfide was heated in an autoclave at 75° for 45 h. The mixture was evaporated, and the residue was treated with water. The aqueous mixture was filtered, the filtrate was evaporated to dryness, and the residue was recrystallized from alcohol to give 0.473 g (78%) of XXII with mp 174-175°. UV spectrum, λ_{max}, nm (ε): 245 (3900) and 333 (20,100) at pH 1, 245 (3400) and 333 (18,400) at pH 7, and 317 (17,200) at pH 13. Found: C 41.2; H 5.2%. $C_7H_{10}N_2O_3$. Calculated: C 41.6, H 5.0%.

N^1-(2',3'-Dihydroxypropyl)-4-thiouracil 2'(3')-Phosphate (XXIII). Liquid hydrogen sulfide (10 ml) was added to a solution of 295 mg (1 mmole) of dilithium salt XVI in 5 ml of pyridine and 5 ml of water, and the mixture was heated in an autoclave at 80° for 25 h. It was then vacuum evaporated to dryness at 30°, and the residue was stirred with water. The aqueous mixture was filtered, and the filtrate was evaporated to a small volume. The dilithium salt of XXIII was precipitated by the addition of absolute alcohol, and the precipitate was removed by filtration and washed successively with absolute alcohol and absolute ether. The yield of the dilithium salt of XXIII was 180 mg (61%). UV spectrum, λ_{max}, nm (ε): 245 (4320) and 333 (18,400) at pH 1, 245 (5520) and 333 (18,400) at pH 7, and 314 (15,800) at pH 13. The compound was electrophoretically homogeneous.

LITERATURE CITED

1. N. Ueda, T. Kawabata, and K. Takemoto, J. Heterocycl. Chem., 8, 827 (1971).
2. T. Seita, K. Yamauchi, M. Kinoshita, and M. Imoto, Bull. Chem. Soc. Japan, 45, 926 (1972).
3. G. M. Tener, J. Amer. Chem. Soc., 93, 159 (1961).
4. B. E. Griffin, M. Jarman, C. B. Reese, and J. E. Sulston, Tetrahedron, 23, 2301 (1967).
5. L. I. Kolobushkina, A. M. Kritsyn, and V. L. Florent'ev, Khim. Geterotsikl. Soedin., 996 (1973).