solutions. These solutions were filtered and poured into 1 l. of cold ethanol (for glycine and DL-alanine) or 1 l. of cold butanol (for sarcosine). The blue precipitates were collected, washed with ethanol and ether, and then dried in a vacuum oven (80°) for 24 hr. Further purification was not necessary.

Bis(sarcosino)copper(II) was obtained in 80% yield. *Anal.* Calcd for $C_6H_{12}CuN_2O_4$: C, 30.06; H, 5.04; Cu, 26.51; N, 11.69. Found: C, 30.3; H, 5.17; Cu, 26.60; N, 11.89.

Bis(glycino)copper(II) was obtained in 85% yield. *Anal.* Calcd for $C_4H_8CuN_2O_4$: C, 22.70; H, 3.81; Cu, 30.02; N, 13.24. Found: 22.91; H, 3.88; Cu, 30.00; N, 13.18.

Bis(DL-alanino)copper(II) monohydrate was obtained in 81% yield. *Anal.* Calcd for $C_6H_{12}CuN_2O_4 \cdot H_2O$: C, 27.96; H, 5.48; Cu, 24.65; N, 10.87. Found: C, 28.23; H, 5.49; Cu, 24.70; N, 11.07.

Reaction of Phosgene with Amino Acid–Copper(II) Complexes.—The following general procedure was used. Phosgene was bubbled at a moderate rate for 1 hr into rapidly stirred suspensions of the finely ground copper(II)–amino acid complexes (5 g) in dry tetrahydrofuran (500 ml). The color of the suspensions changed to green, and then to orange-brown as copper(II) chloride precipitated. Rapid stirring was continued for 1 more hr, after which the solvent was evaporated (<50°) under reduced pressure. The residues were extracted with warm benzene, ethyl acetate, and chloroform (for sarcosine, glycine, and DL-alanine, respectively), and the resulting extracts were pressure-filtered under nitrogen through glass wool and coarse fritted glass. The solvents were then removed under reduced pressure (<50°), leaving the crude N-carboxyanhydrides.

Sarcosine N-carboxyanhydride was obtained in 88% yield as an oil, which solidified upon standing. It was recrystallized from chloroform–petroleum ether (bp 30–60°) and had a melting point of 105° (lit.[10] mp 105°). *Anal.* Calcd for $C_4H_5NO_3$: C, 41.74; H, 4.38; N, 12.17. Found: C, 42.13; H, 4.51; N, 12.51.

Glycine N-carboxyanhydride was obtained in 78% yield and was recrystallized twice from acetic anhydride. It decomposed above 100° (lit.[5a] dec pt >100°). *Anal.* Calcd for $C_3H_3NO_3$: C, 35.65; H, 2.99; N, 13.86. Found: C, 35.99; H, 3.01; N, 13.80.

DL-Alanine N-carboxyanhydride was obtained in 90% yield. It was recrystallized from chloroform at −40° and had a melting point of 46° (lit.[11] mp 46°). *Anal.* Calcd for $C_4H_5NO_3$: C, 41.74; H, 4.38. Found: C, 41.81; H, 4.47.

Registry No.—Phosgene, 75-44-5; bis(sarcosino)-copper(II), 18253-88-8; bis(glycino)copper(II), 13479-54-4; bis(DL-alanino)copper(II), 15274-59-6; sarcosine N-carboxyanhydride, 5840-76-6; glycine N-carboxyanhydride, 2185-00-4; DL-alanine N-carboxyanhydride, 1192-73-0.

Acknowledgment.—This research was supported in part by the National Institute of Arthritis and Metabolic Diseases under Contract No. PH 43-66-493.

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Nucleic Acids. VIII.[1] Synthesis and Chemistry of *ara*-Cytidine 2′,5′ Cyclic Phosphate. Phosphate Anisotropy

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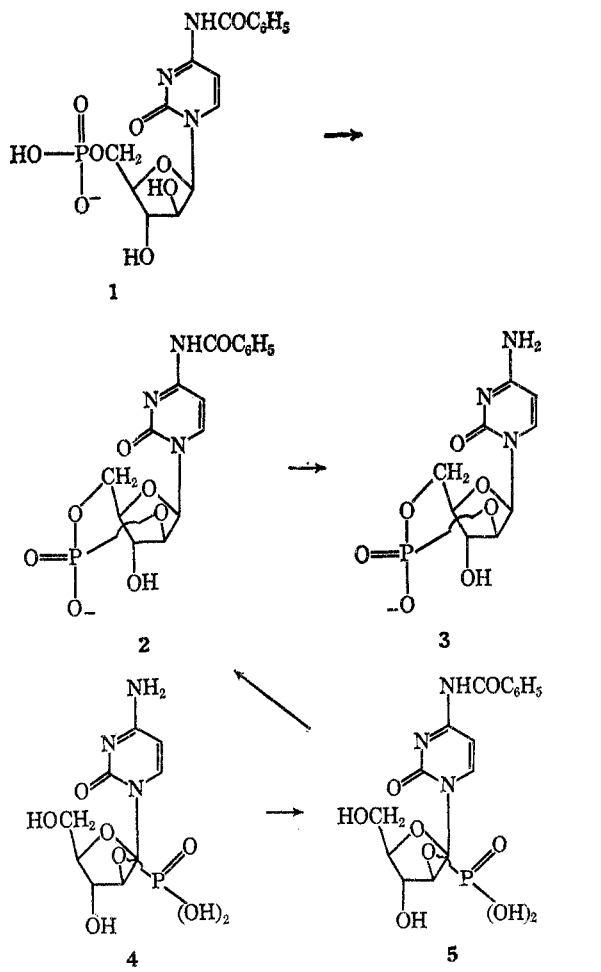
The biological importance of nucleoside 3′,5′ cyclic phosphate esters, particularly of adenosine and uridine,[2] prompted us to investigate the synthesis and chemistry of the cyclic phosphates derived from the cytotoxic[3a,b] antiviral[3c–e] nucleoside *ara*-cytidine[3f] as part of our continuing effort to assess the biodynamic effects of nucleotides and their derivatives.[4] It was also of interest to assign the structure of *ara*-adenosine cyclic phosphate[5] by employing *ara*-cytidine chemistry as a model. The work described herein contributed to the first goal, clarified the structural problem with regard to *ara*-adenosine and led to a further understanding of the contribution of phosphate anisotropy to the nmr spectra of organophosphorus compounds, particularly nucleotides and oligonucleotides.

N^4-Benzoyl-*ara*-cytidine 5′-phosphate[4] (**1**), when cyclized employing dicyclohexylcarbodiimide (DCC), afforded a single crystalline cyclic phosphate (Scheme I). This material, **2**, after removal of the N^4 protecting group, proved to be *ara*-cytidine 2′,5′ cyclic phosphate (**3**), a molecule uniquely unreactive toward acid, base, and the multitude of nucleases present in crude snake venom.[6] Spin decoupling experiments and analysis of

SCHEME I



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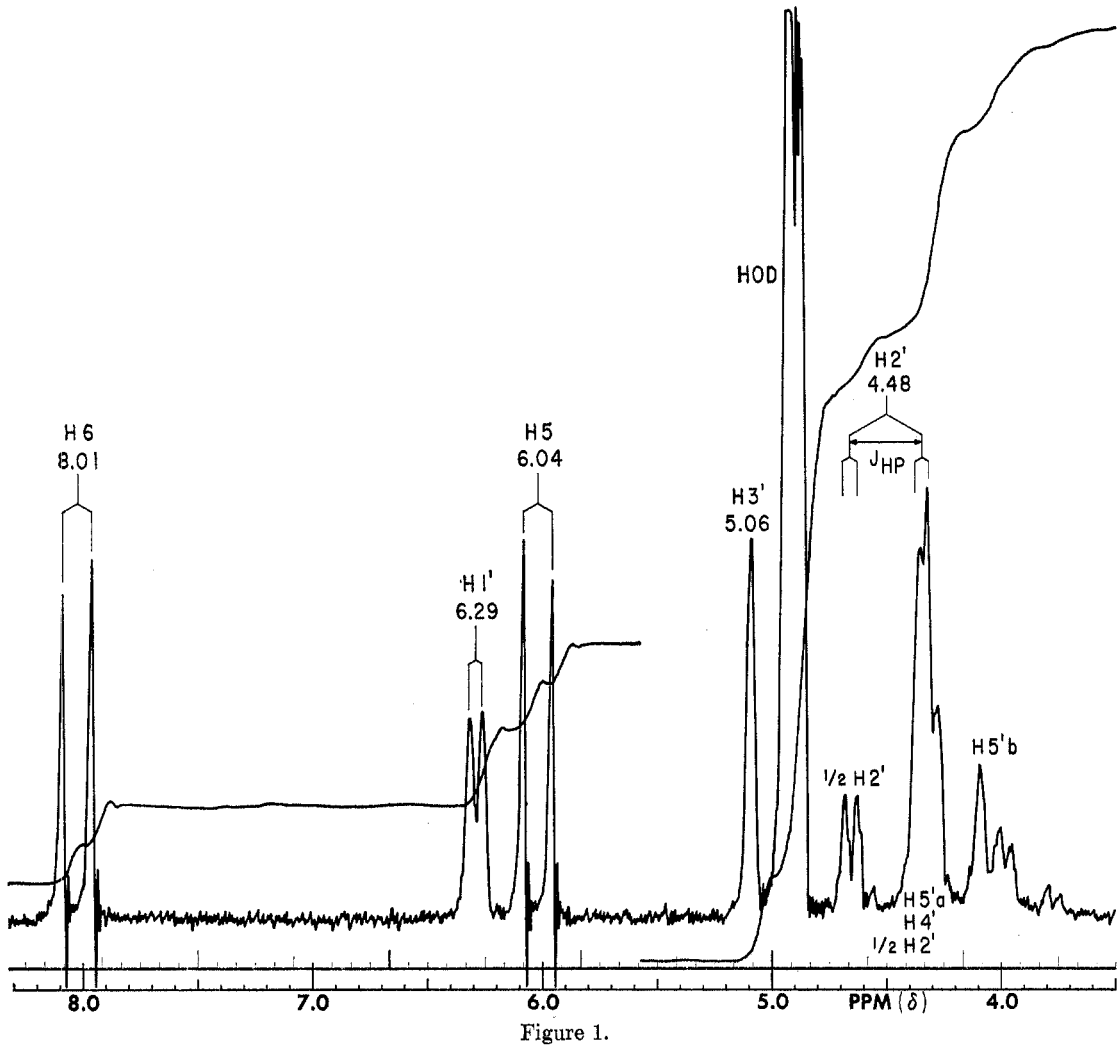

Figure 1.

TABLE I[a,b]

	Solvent	Std	H-6	H-5	H-1'	H-2'	H-3'
ara-Cytidine (**6**)	D_2O	SDSS	7.80 [8]	6.01 [8]	6.19 [5]	4.41 q [5, 3.5]	~4.09 m
	DMF-d_7	TMS	7.97 [7]	6.23 [7]	5.90 [3–3.5]		
ara-Cytidine 2',5' cyclic phosphate (**3**)	NaOD, D_2O	SDSS	8.01 [8]	6.04 [8]	6.29 [3]	4.48 [J_{HP} = 18, $J_{1,2}$ = 3]	5.06 [<1]
N^4-Benzoyl-*ara*-cytidine 2',5' cyclic phosphate 4-morpholine-N,N'-dicyclohexylcarboxamidine salt (**2**)	DMF-d_7	TMS	8.58 [7]	8.50 [7]	6.38 [2–3]		5.72
ara-Cytidine 2'-phosphate (**4**)	D_2O	SDSS	8.05 [8]	6.31 [8]	6.34 [4]		
ara-Cytidine 5'-phosphate[c] (**7**)	D_2O, NH_4OH	SDSS	7.95 [8]	6.10 [8]	6.22 [5]	~4.45 m	

[a] All spectra were obtained on a Varian A-60A nmr spectrometer. All chemical shifts are reported downfield from an internal standard taken as 0 ppm (TMS, tetramethylsilane; SDSS, 2,2-dimethyl-2-silapentane-5-sulfonic acid). [b] Coupling constants are enclosed in brackets and are in cycles per second. [c] See ref 4.

the nmr spectrum of compound **3** (Figure 1) allows the structure determination and the quantitative assignment of phosphate anisotropy acting on the 3'-H of this molecule. In Table I are summarized the nmr data of the cyclic phosphate **3** and related compounds. Owing to the different conditions of solvent, concentration, and pH under which the spectra were run no meaningful conclusions can be drawn from small differences in the chemical shifts of the majority of the protons. It is sufficient to note that, over the range of structures, the chemical shifts of H-6, H-5, H-1', and H-2' do not vary greatly except in the case of the N^4-benzoyl derivative **2**. Thus, as can be noted in earlier work,[4] phosphorylation of an hydroxyl group does not produce a paramagnetic shift of the carbinol protons characteristic of the acylation of alcohols[7] with carboxylic acids. A study of molecular models (Dreiding) suggests little change in bond angles of the H-1' and H-2' protons of cyclic phosphates **2** and **3** as compared with the simple nucleotides **4** and **7** which is reflected experimentally in the small change in $J_{1',2'}$ in going from **3** to **7**.

Structurally conclusive nmr evidence for **3** is provided by identification of the H-2' proton owing to its coupling to H-1' and phosphorus by decoupling experiments. Decoupling experiments allowed the portion of the H-2' quartet hidden under the H-4' and H-5' resonances to be detected and its approximate chemical

(7) L. M. Jackman, NMR Spectroscopy in Organic Chemistry, Pergamon Press, Long Island City, N. Y., 1959, p 55.

TABLE II

ACID AND BASIC HYDROLYSIS OF *ara*-CYTIDINE 2′,5′-CYCLIC PHOSPHATE

Substrate	Hydrolysis mix[a]	Time, hr	Products[b]
pCa N,Bz	1 *N* $HClO_4$[c]	0.5	No appreciable hydrolysis
pCa N,Bz	1 *N* $HClO_4$[c]	1.5	pUa, pCa, trace of nucleotide
pCa N,Bz	0.2 *N* $Ba(OH)_2$[d]	0.5	$C_6H_5CO_2H$ + pCa
pCa	1 *N* $HClO_4$	0.5	Starting material + some nucleotide
pCa	1 *N* $HClO_4$	2.0	94% starting material + 6% mixed nucleotides
pCa	0.4 *N* $Ba(OH)_2$	pCa 0.5	No hydrolysis
	Literature[e] Comparisons		
pC	1 *N* HCl	$t_{1/2}$ 26 sec (complete 2 hr)	Cp + C[p] (80%), pC (20%)
pC	0.2 *N* $Ba(OH)_2$	0.5	Complete hydrolysis Cp + Up (major) pC + pU (minor)

[a] All experiments were carried out at 97°. [b] Isolated and identified by preparative paper chromatography (3MM paper, solvent system A). [c] In the perchloric acid experiments the majority of the salt was precipitated by the addition of 1 *N* KOH. [d] To rid the solution of excess Ba^{2+}, Dowex 50W-X8 (H^+) was added to neutrality. [e] See ref 9.

shift determined. Employing field sweep decoupling the doublet at 278 Hz (area one-half proton, J = 3 Hz) proved to be coupled to H-1′ (δ 6.29, J = 3 Hz) and thus must be a part of the H-2′ resonance. The other half of this pattern (split owing to coupling with phosphorus) was established by a similar decoupling experiment to be at about 260 Hz. Thus the chemical shift of the H-2′ proton is *ca.* δ 4.48 and the $J_{H\text{-}2'-P}$ coupling is about 18–19 cps. Phosphorus is also coupled to the 5′ CH_2 but the pattern was not analyzed. Such coupling is in good agreement with the J_{HP} coupling of 21 cps found for the methyl group in *ara*-cytidine methyl phosphate ester.[4] Therefore, the remaining broad singlet at δ 5.06 is the H-3′ proton. (Suitable shift of the HOD resonance at δ 4.9 proved that it hid no spectral lines.) Its small coupling (<1 cps) to H-2′ was confirmed by a third double resonance experiment. The unique line position of this carbinol proton results from its close proximity to the phosphate residue (Courtauld models indicate that they are within van der Waal distance). Phosphate anisotropy has a small effect on the directly bound H-2′ and H-5′ protons as noted above. Yet, phosphate strongly deshields H-3′ resulting in a paramagnetic shift of about 60 cps when compared with C-3′ of *ara*-cytidine itself. We believe this to be the most dramatic effect yet evidenced by phosphate anisotropy and clearly indicates the importance of such effects when phosphorus-containing structures are examined by nmr. In an effort to confirm the phosphorus coupling the ^{31}P nmr spectrum of compound **3** was run at 40.5 MHz in basic phosphate buffer.[8] This spectrum exhibited two resolved but complex multiplets centered at ~0.7 ppm upfield from phosphoric acid, from which one coupling of about 19 cps could be deduced. This coupling is consistent with those observed in the nmr spectrum (Figure 1).

The structure of the 2′,5′-cyclic phosphate ester **3** was also proved chemically by synthesis from *ara*-cytidine 2-phosphate (**4**).[4] Benzoylation of **2** followed by mild alkaline hydrolysis afforded the N^4-benzoate **5**. Cyclization of this material under the same conditions as with the 5′-phosphate **1** afforded, after base hydrolysis and acidification, the crystalline *ara*-cytidine 2′,5′-cyclic phosphate **3**. This material was identical by uv, nmr, ir, thin layer chromatography, and high voltage electrophoresis with the crystalline cyclization product of the 5′-phosphate. It can be inferred by analogy that the cyclic phosphate obtained by Cohen from *ara*-adenosine[5] is probably *ara*-adenosine 2′,5′-cyclic phosphate.

The cyclic phosphate **3** exhibited unusual chemical and enzymatic stability. When incubated with very large (*i.e.*, 0.5 mg of protein per OD unit of substrate) amounts of venom diesterase (for procedure, see ref 4), unlike the known 3′,5′-cyclic phosphates[9] or adenosine 3′,5′-cyclic phosphate,[5] it did not hydrolyze to any detectable extent. In addition the phosphate ring was not opened under either alkaline or acid conditions which completely hydrolyze 3′,5′-cyclic phosphates.[9,10] Only under strong acid conditions and for a prolonged period at 97° was a small amount of hydrolysis realized. (See Table II for a summary of the hydrolysis experiments.) The stability of this seven-membered cyclic phosphate is consistent with Khorana's[11a] and Cherbuliez's[11b] finding that butane-1,4-diol cyclic phosphate was hydrolytically much more stable than the trimethylene cyclic phosphate. On the other hand, compound **3** should be susceptible to hydrolysis according to Westheimer's[12] hypothesis, since it is stereochemically well situated for "apical" attack by solvent. Possibly in this bicyclic system even the pentacovalent intermediate reeliminates the incoming solvent. This course of reaction might be due to the thermodynamic stability of the bicyclo-[1.2.4] system over the open nucleotide, despite the appreciation in entropy on opening.

The usual enzymatic and chemical stability of this cyclic phosphate was reflected in the fact that the compound is devoid of the usual cytotoxic[13] and antiviral[14] activities associated with the parent nucleotides. It is also not a substrate for cytidine deaminase.[15]

Experimental Section[16]

N^4-Benzoyl-*ara*-cytidine 2′,5′-Cyclic Phosphate, 4-Morpholine-N,N′-dicyclohexylcarboxamidinium Salt (2).—Pyridinium N^4-benzoyl-1-β-D-arabinofuranosylcytosine 5′-phosphate (**1**, 542 mg, 1 mmol) and 4-morpholine-N,N′-dicyclohexylcarboxamidine (293 mg, 1 mmol) were dissolved in 10 ml of anhydrous pyridine and the solution concentrated to a gum *in vacuo*. This process was repeated to ensure anhydrous starting materials. The residual gum was then taken up in 100 ml of dry pyridine and

(8) This spectrum was run by Varian Associates through the courtesy of L. F. Johnson.

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(12) E. A. Dennis and F. H. Westheimer, *J. Amer. Chem. Soc.*, **88**, 3432 (1966).

(13) C. G. Smith, H. H. Buskirk, and W. L. Lummis, *J. Med. Chem.*, **10**, 774 (1967).

(14) H. E. Renis and C. A. Hallowel, *ibid.*, 777 (1967).

(15) G. W. Camiener, private communication.

(16) Melting points were determined on a Thomas–Hoover apparatus and are uncorrected. Evaporation of solutions was carried out at water pump pressure in a Buchi Roto-vap. Paper and thin layer chromatographic systems were as follows: solvent A, isopropyl alcohol–concentrated NH_4OH–water (7:1:2); solvent B, ethyl alcohol–1 *M* NH_4OAc (pH 7.5) (5:2).

added dropwise during 2 hr to a refluxing solution of dicyclohexylcarbodiimide (DCC, 1.3 g, 5.0 mmol) in dry pyridine. The solution was maintained at reflux for 2 hr after the addition was complete. The solution was concentrated to dryness under reduced pressure giving a gum. This gum was partitioned between water (100 ml) and ether (50 ml) with vigorous shaking. The insoluble urea was filtered and the aqueous layer was concentrated to a small volume (*ca.* 10 ml) after Darco-60 treatment. On standing the product crystallized as a colorless, highly crystalline solid. The product was filtered, washed with a small amount of water, and dried under vacuum to give 510 mg of product. A sample was recrystallized three times from water for analysis: mp 202.5–206.0°; λ_{max} 258 mμ (18,000), 302 (10,800); ν_{max} 3560, 3460, 3200, 3100, 1700, 1650, and 1610 cm^{-1}.

Anal. Calcd for $C_{16}H_{16}O_8N_8P \cdot C_{17}H_{31}N_3O$: C, 56.5; H, 6.76; N, 11.98; P, 4.42. Found: C, 53.69;[17] H, 6.60; N, 11.70; P, 4.54.

***ara*-Cytidine 2′,5′ Cyclic Phosphate (3).**—A 400-mg sample of the above cyclic phosphate (2, 0.76 mmol) was dissolved in 20 ml of cold anhydrous ammonia saturated methanol and allowed to stand at room temperature overnight. The solution was taken to dryness under reduced pressure at 40° and the residue dissolved in 20 ml of distilled water and extracted with carbon tetrachloride (20 ml). The aqueous phase was separated, taken to dryness as above, and redissolved in 4.8 ml of water, filtered free of insoluble material, and then adjusted to pH 1 by the addition of concentrated hydrochloric acid, whereupon the product crystallized. After refrigeration the product was isolated (100 mg), washed with a small volume of 1 *N* HCl, then dried (*in vacuo*, 60°). A sample was recrystallized once for analysis, $\lambda_{max}^{pH\ 2.0}$ 210 mμ (ε10,220), 279 (14,060). This material was homogeneous by paper and thin layer chromatography (cellulose DF and silica gel G) employing solvent A and by high-voltage electrophoresis (HVE) at pH 6.8 and 3.5 and migrated as expected.

Anal. Calcd for $C_9H_{12}N_3O_7P$ (305.18): C, 35.42; H, 3.96; P, 9.54; N, 13.78. Found: C, 35.94; H, 4.07; P, 9.48; N, 14.08.

N^4-Benzoyl-*ara*-cytidine 2′-Phosphate (5).—*ara*-Cytidine 2′-phosphate[4] (4, 380 mg, 1.18 mmol) was dissolved in water (100 ml) containing a small amount of pyridine and lyophilized. The finely dispersed powder was taken up in 35 ml of dry pyridine containing 2.5 ml of benzoyl chloride and allowed to stand for 1 hr. Ice–water (100 ml) was added and after the solution had warmed to room temperature it was extracted with three 50-ml portions of chloroform. The combined extracts were backwashed with water, dried and evaporated to dryness. Two-thirds of this material was taken up in pyridine (20 ml) and water (10 ml) and the solution treated with 30 ml of 2 *M* sodium hydroxide. The heterogeneous solution was stirred vigorously for 4 min, then neutralized by the addition of pyridinium Dowex 50W-X8 resin (100 ml). After the pH had fallen to about 7 the resin was filtered and the filtrate taken to dryness (under reduced pressure at 40°) and then suspended in 100 ml of ether–water (1:1). The aqueous portion was separated, freed of ether and percolated through a column of 100 ml of the above resin (100–200 mesh) followed by elution with 250 ml of water. The total eluate was extracted four times with ether and the aqueous solution taken to dryness (*in vacuo*). The residue was taken up in pyridine and examined by tlc (cellulose, solvent B). This material appeared homogeneous and not contaminated with either nucleotide or benzoic acid and its mobility was very similar to that of the known compound 1. This material was employed for the next synthetic step without further purification.

***ara*-Cytidine 2′,5′ Cyclic Phosphate (3) from *ara*-Cytidine 2′-Phosphate.**—The N^4-benzoylated nucleotide above (5, *ca.* 0.68 mmol by uv) was dried by repeated evaporation in anhydrous pyridine. This material was cyclized as described for the 5′-phosphate (1) above giving a crystalline salt which was hydrolyzed in cold ammoniacal methanol as described for compound 2. The crystalline solid produced by acidification of the pyridinium salt was compared with the cyclic phosphate 3 described above and found to be identical by ir, nmr, uv, tlc (cellulose, solvent A) and HVE (6000 V, pH 6.8, 3 hr).

Registry No.—2, 15465-99-3; 3, 15466-01-0; 4, 14433-48-8; 5, 17955-19-0; 6, 147-94-4.

(17) Carbon values in nucleotide analysis are often low because carbon is trapped in the melt which remains in the combustion boat.

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Anomalous Reactions of 4-Methylnicotinic Acids

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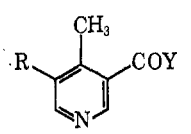
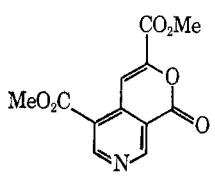
In connection with a problem of alkaloid synthesis, 4-methyl-5-carbomethoxynicotinyl chloride (**1a**) was needed. The dimethyl ester **1b** was prepared by the reported procedure leading to the diethyl ester[1] and was hydrolyzed partially to the acid ester **1c**. Exposure of the latter to oxalyl chloride and triethylamine yielded unexpectedly a mixture whose methanolysis gave the enol lactone **2** and, in trace quantity, the diester **1b**. Thus condensation on the methyl site by the oxalyl moiety appeared to be the preponderant reaction path which even variation of reaction conditions could not avoid.[2]

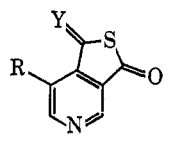




1a, R = CO_2Me; Y = Cl
b, R = CO_2Me; Y = OMe
c, R = CO_2Me; Y = OH
d, R = H; Y = OH

2

Interaction of the acid **1c** with thionyl chloride under a variety of conditions yielded exclusively an anomalous product containing sulfur and chlorine whose instability prevented its elemental analysis. Its facile hydrolysis, the major source of its lability, produced the thioanhydride **3b**. Its mode of formation, spectral properties and comparison with models (*vide infra*) showed it to possess structure **3a**.



3a, R = CO_2Me; Y = Cl_2
b, R = CO_2Me; Y = O
c, R = H; Y = Cl_2
d, R = H; Y = O

(1) R. Lukeš and J. Kuthan, *Collect. Czech. Chem. Commun.*, **25**, 2173 (1960).

(2) *Cf.* N. Ikekawa, *Chem. Pharm. Bull. Jap.*, **6**, 269 (1958), for examples of related condensations of **1d**.