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Studies of Phosphorylation. II. Reaction of 2′, 3′-*O*-Isopropylidene-inosine and -guanosine with Phosphoryl Chloride[1]

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Several reactions accompanied by the phosphorylation of 2′, 3′-*O*-isopropylidene nucleosides with phosphoryl chloride in the absence of an organic solvent were investigated. When the reaction temperature exceeded 30°C, 2′, 3′-*O*-isopropylideneinosine gave 5′-chloro-5′-deoxy-2′, 3′-*O*-isopropylideneinosine and 5′-deoxy-2′, 3′-*O*-isopropylidene-3, 5′-cycloinosine. This cycloinosine, was then further subjected to rapid cleavage in a pyrimidine ring to give 5-amino-4-carbamoyl-N^5, 5′-cyclo-1-β-D-(5′-deoxy-2′, 3′-*O*-isopropylideneribofuranosyl)-imidazole, 5-amino-4-cyano-N^5, 5′-cyclo-1-β-D-(5′-deoxy-2′, 3′-*O*-isopropylideneribofuranosyl)-imidazole, and the N^5-formyl derivative of the latter. Similar sequences of the chlorination at the 5′-position and the cyclization between 3 and 5′-positions were observed in the case of 2′, 3′-*O*-isopropylideneguanosine, but the resulting 5′-deoxy-2′, 3′-*O*-isopropylidene-3, 5′-cycloguanosine was not susceptible to ring cleavage under the conditions used. The factors affecting these reactions were described, and possible mechanisms were proposed.

A previous paper[2] reported a successful method for the preparation of 5′-nucleotides by a simple procedure. This method, involving the reaction of 2′, 3′-*O*-isopropylidene nucleosides with a large excess of phosphoryl chloride in the absence of any organic solvent, has given the maximum yields of the corresponding 5′-nucleotides at 0—15°C. A slight elevation of the reaction temperature or a prolongation of the reaction time, however, resulted in a gradual decrease in the yield as a result of several side reactions and the glycosidic cleavage. The present paper deals with the identification of all of the side products obtained by the reactions of 2′, 3′-*O*-isopropylidene-inosine and -guanosine with phosphoryl chloride, and discuss possible mechanisms.

Results and Discussion

Identification of the Products. The reaction mixtures were treated with ether to precipitate the products, which were then hydrolyzed under mild acidic conditions. The two-dimensional paper chromatographies of the hydrolyzates using solvent 1 and then solvent 2 are shown in Figs. 1 and 2. The products, named H-1, H-2,··· and H-6, and G-1, G-2,··· and G-4, are represented in the figures. The R_f values of the products with three kinds of solvents are summarized in Table 1.

The spots of H-1 and G-1 were positive to the Hanes-Isherwood reagent[3] and negative to the periodate-benzidine test.[4] These properties indicate that both products are nucleotides substituted at one or both hydroxyl groups at 2′ and 3′-positions. Their R_f values and electrophoretic mobilities were in good agreement with those of authentic 2′(or 3′), 5′-diphosphoric esters of inosine and guanosine.

All spots of the other side-products were negative to the Hanes-Isherwood reagent.

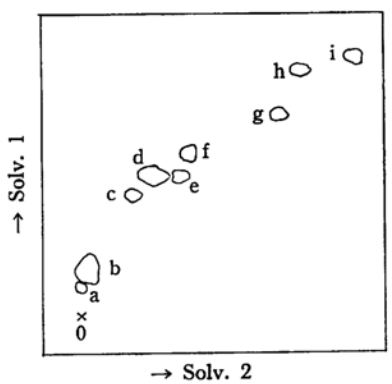
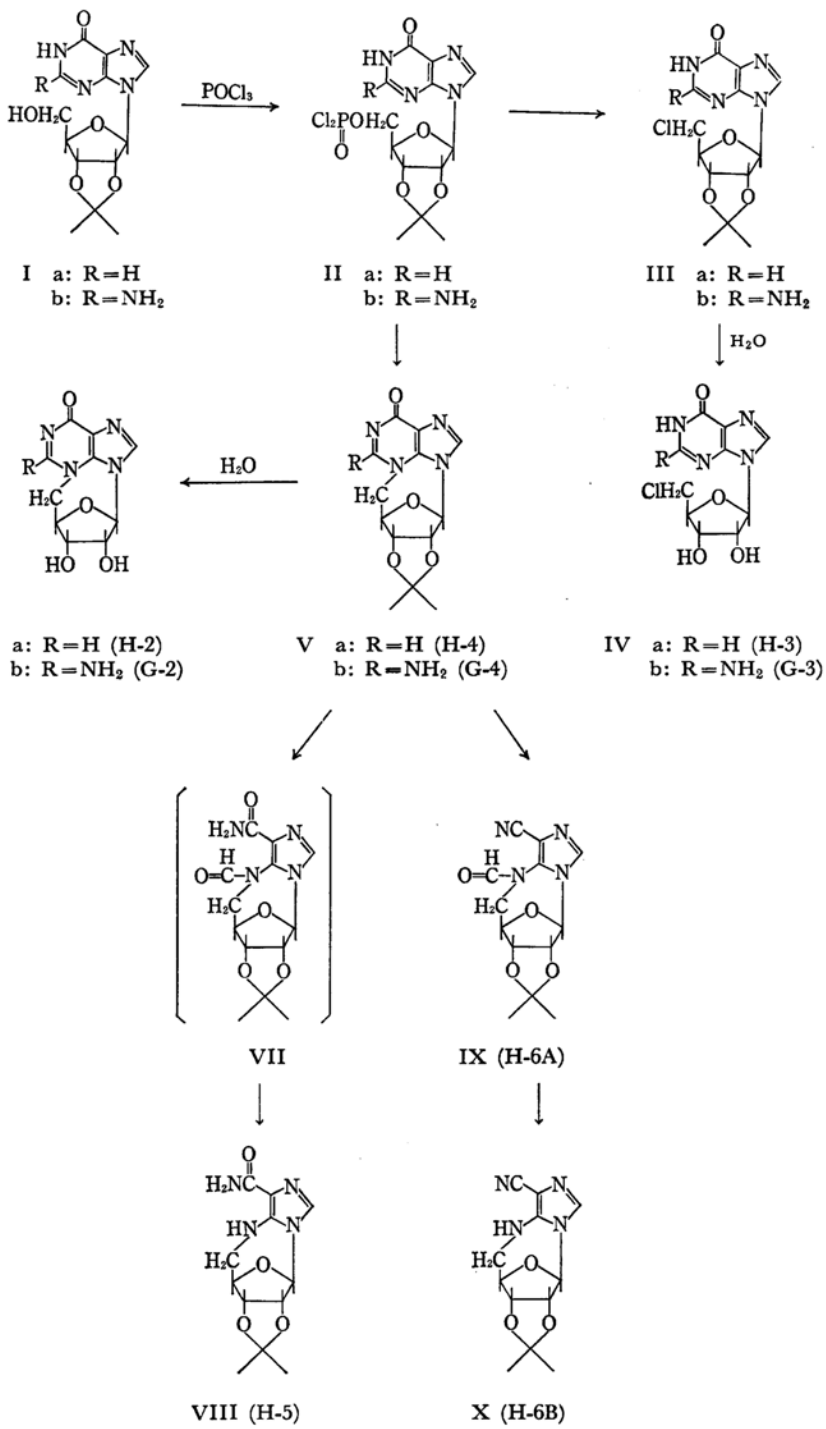


Fig. 1. Two-dimensional paper chromatography of reaction mixture of Ia.
0: original point
a: H-1, b: 5′-IMP, c: H-2, d: inosine
e: hypoxanthine, f: H-3, g: H-4
h: H-5, i: H-6

1) Presented at the Hokkaido Meeting of the Chemical Society of Japan, Sapporo, July 19, 1965.
2) M. Yoshikawa and T. Kato, This Bulletin, **40**, 2849 (1967).
3) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).
4) M. Viscontini, D. Hoch and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).



The spot of H-3 gave a positive periodate-benzidine test, and the ultraviolet absorption spectra proved to be similar to those of inosine. These results show the absence of the isopropylidene group and the presence of the unchanged base. The treatment of H-3 in 0.1 N sodium hydroxide under reflux afforded inosine. 5′-Chloro-5′-deoxy-2′,3′-*O*-isopropylideneinosine (IIIa) was isolated from the chloroform extract of the reaction products and was entirely identical with the compound synthesized by the nucleophilic replacement of 2′,3′-*O*-isopropylidene-5′-methane-sulfonylinosine with lithium chloride. The R_f values of its acid hydrolyzate were in good agreement with those

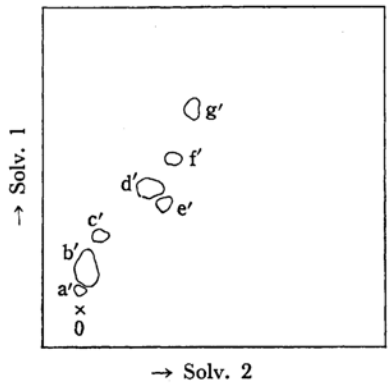


Fig. 2. Two-dimensional paper chromatography of reaction mixture of Ib.

0: original point
a′: G-1, b′: 5′-GMP, c′: G-2
d′: guanosine, e′: guanine, f′: G-3
g′: G-4

TABLE 1. R_f VALUES OF PRODUCTS

Compound	Solv. 1	Solv. 2	Solv. 3
H-1	0.10	0.00	0.68
5′-IMP*	0.15	0.02	0.06
H-2	0.40	0.17	—
Inosine	0.46	0.24	0.48
Hypoxanthine	0.46	0.32	0.30
H-3	0.53	0.35	0.32
H-4	0.76	0.65	0.22
H-5	0.81	0.72	0.14
H-6A	0.85	0.90	0.29
H-6B	0.85	0.90	0.21
G-1	0.06	0.00	0.58
5′-GMP*	0.14	0.02	0.49
G-2	0.24	0.06	—
Guanosine	0.40	0.22	0.36
Guanine	0.35	0.27	0.20
G-3	0.50	0.30	0.18
G-4	0.66	0.37	0.22

* 5′-IMP and 5′-GMP represent inosine 5′-phosphate and guanosine 5′-phosphate respectively in tables and figures.

of H-3. In view of the above facts, it may be concluded that H-3 is 5′-chloro-5′-deoxyinosine (IVa).

The isopropylidene derivative of G-3 was readily separated from the aqueous solution of the reaction products. The acidic removal of the protective group gave G-3. The composition of G-3 satisfied $C_{10}H_{12}O_4N_5Cl$, corresponding to formula IVa. G-3 gave a positive periodate-benzidine test and the same ultraviolet absorption spectra as those of guanosine. After refluxing in 0.1 N sodium hydroxide, G-3 afforded guanosine. From these observations, G-3 was identified as 5′-chloro-5′-deoxyguanosine (IVb).

The infrared and ultraviolet spectra, melting point, and R_f values of H-4 proved to be the same as those[5] of the 5′-deoxy-2,′ 3′-*O*-isopropylidene-3, 5′-cycloinosine (Va) prepared *via* 2′, 3′-*O*-isopropylidene-5′-*O*-(*p*-toluenesulfonyl)-inosine according to the Robins method.[6] The structure was characterized by a single peak at 3.64 τ assigned to a proton at the 1′-position in the NMR spectrum.[7]

Such a characteristic peak of a proton at the 1′-position was also observed in the other 3, 5′-cyclonucleoside derivatives described below, which are summarized in Table 2.

The yield of G-4 was increased nearly 50% by elevating the reaction temperature to 40°C. The ultraviolet absorption spectra were very similar to those of 5′-deoxy-2′, 3′-*O*-isopropylidene-3, 5′-cycloguanosine reported by Chambers *et al.*[8] 3, 5′-(5′-Deoxy-D-ribofuranosyl)-guanine and 5′-deoxy-2′, 3′-*O*-isopropylidene-3, 5′-cycloxanthosine were formed when G-4 was refluxed in 1 N hydrochloric acid and in 1 N sodium hydroxide respectively. These compounds were confirmed by the ultraviolet spectra measurement of the spots on a paper chromatogram according to the Robins method.[6] Also, the NMR spectrum of G-4 supported the 2′, 3′-*O*-isopropylidene cyclonucleoside structure. On the basis of these results, G-4 was concluded to be 5′-deoxy-2′, 3′-*O*-isopropylidene-3, 5′-cycloguanosine (Vb).

The compound Vb could also be prepared directly by the reaction of Ib with methanesulfonyl chloride in pyridine.

The minor products, H-2 and G-2, gave positive periodate-benzidine tests. Their respective ultraviolet spectra were identical with those of Va and Vb, convincing evidence that they were 5′-deoxy-3, 5′-cycloinosine (VIa) and -guanosine (VIb). In fact, the R_f values of the acid hydrolyzates of Va and Vb were in complete agreement with those of H-2 and G-2 respectively.

The ultraviolet absorption spectra of H-5 were very different from those of the starting nucleoside. The NMR spectrum showed a single peak at 3.65 τ due to –NH–, in addition to the peaks indicating a 2′, 3′-*O*-isopropylidene cyclonucleoside structure, and the infrared spectrum exhibited an amide band at 1647 cm^{-1}. These spectral results and the composition supported the theory that H-5 was 5-amino-4-carbamoyl-N^5, 5′-cyclo-1-β-D-(5′-deoxy-2′, 3′-*O*-isopropylideneribofuranosyl)-imidazole (VIII).

5) The absorption maximum at pH 1 was in disagreement with their description.

6) R. E. Holmers and R. K. Robins, *J. Org. Chem.*, **28**, 3483 (1963).

7) The dihedral angle between the $H_{1'}C_{1'}C_{2'}$ plane and the $H_{2'}C_{2'}C_{1'}$ plane becomes nearly 90° upon the 3, 5′-cyclization in purine nucleoside.

8) R. W. Chambers, J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3747 (1957).

TABLE 2. PROPERTIES OF THE PRODUCTS

Compound	max (mμ) pH 1	max (mμ) pH 11	Solvent	NMR (τ) $H_{1'}$	$J_{1'2'}$ (cps)	Isopropylidene group		Characteristic group
IIIa	252	254	DMSO	3.78	2.5	8.48	8.65	
Va	258—263	271	C_5H_5N	3.64	0	8.50	8.76	
IIIb	258	258—267	DMSO	3.93	2.5	8.46	8.65	
Vb	251—257	218, 267	D_2O	3.51	0	8.42	8.68	
VIII	255—264	273	$CDCl_3$	4.24	0	8.49	8.69	3.65(-NH-)
IX	251	252	$CDCl_3$	4.11	0	8.49	8.70	1.52(-CHO)
X	251	252	DMSO	3.90	0	8.57	8.72	3.24(-NH-)

The compound H-6 was too small to isolate, but it was increased in the reaction with a mixture of phosphoryl chloride and pyrophosphoryl tetrachloride. Two compounds (named H-6A and H-6B, as is shown in Table 1) were separated by the careful treatment of the crude H-6 precipitates on an alumina column. From the assignment of the NMR spectra, both H-6A and H-6B were found to be isopropylidene cyclonucleosides.

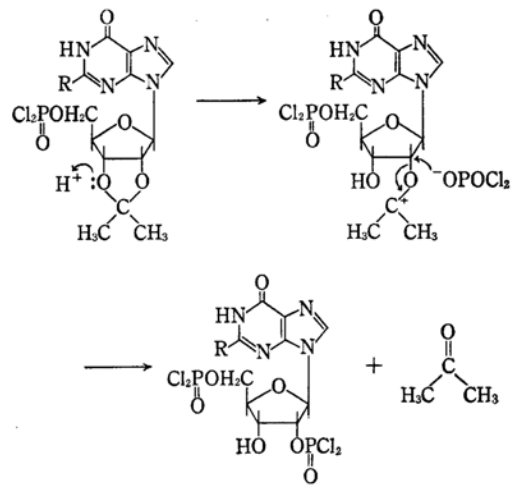
H-6B had a sharp band due to a conjugated cyano group at 2225 cm^{-1} in the infrared spectrum and a broad peak due to –NH– at 3.24 τ in the NMR spectrum. These spectral observations and the composition led us to conclude that H-6B was 5-amino-4-cyano-N^5,5′-cyclo-1-β-D-(5′-deoxy-2′,3′-*O*-isopropylideneribofuranosyl)-imidazole (X).

The infrared spectrum of H-6A showed a new band due to a formyl group at 1705 cm^{-1} in addition to a cyano group at 2230 cm^{-1}. The NMR spectrum showed the presence of a formyl proton at 1.52 τ and the absence of the –NH– group. These results indicate that H-6A is the N^5-formyl derivative of X (IX).

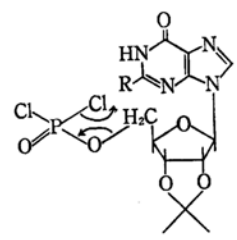
Discussion of the Mechanism. One possible mechanism for the formation of the nucleoside 2′(or 3′),5′-diphosphoric ester from 2′,3′-*O*- isopropylidene nucleoside is illustrated by the reaction of the intermediate, 2′,3′-*O*-isopropylidenenucleoside 5′-phosphorodichloridate (II), with dichlorophosphoric acid according to the following equation:

TABLE 3. INFLUENCE OF WATER FOR PREPARATION OF IVa

Molar ratio H_2O/Ia	Time hr	Yield of product (mol%) IVa	5′-IMP
0	2	7	75
0	16	22	42
0.5	2	2	76
0.5	16	10	53
1.0	2	trace	77
1.0	16	4	67
2.0	2	nothing	52
2.0	16	nothing	46



The 5′-chloro derivative IVa, which was a major side product from Ia, decreased with an increase in the amount of water added to the reaction mixture, as is shown in Table 3. This fact indicates that the formation of 5′-chloro-5′-deoxy-2′,3′-*O*-isopropylideneinosine (IIIa) is not due to the reaction of 2′,3′-*O*-isopropylideneinosine (Ia) with the hydrogen chloride produced. In the reaction without the addition of water, the amount of IVa increased with a decrease in 2′,3′-*O*-isopropylideneinosine 5′-phosphorodichloridate (IIa) (analyzed as inosine 5′-phosphate) even after the starting nucleoside Ia (analyzed as inosine) had phosphorylated, as is shown in Fig. 3. From these results, it seems reasonable to assume that the compound III is formed by the intramolecular rearrangement of the compound II.



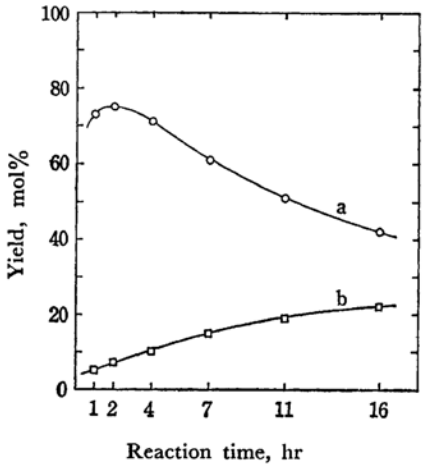


Fig. 3. Influence of reaction time for preparation of IVa.

Experimental condition; Ia: 10 mmol, $POCl_3$: 170 mmol, 20°C
a: 5′-IMP, b: IVa

The formation of the 3, 5′-cyclonucleoside derivative V is considered to be caused by the elimination of the dichlorophosphoryl group from the compound II. The possibility of this mechanism is supported by the increase in the cycloguanosine derivative Vb and the concurrent decrease in the compound IIb with the prolongation of the reaction period, as is shown in Fig. 4.

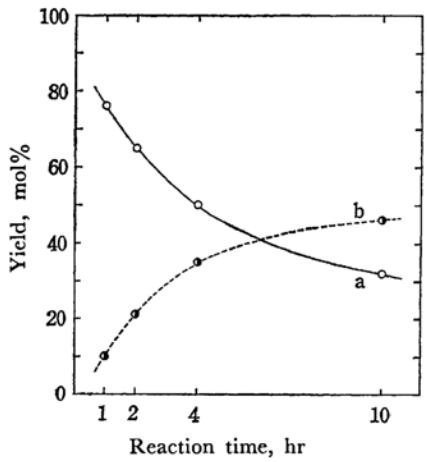
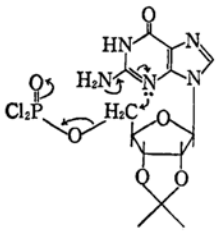


Fig. 4. Influence of reaction time for the preparation of Vb.

Experimental condition; Ib: 10 mmol, $POCl_3$: 170 mmol, H_2O: 5 mmol, 40°C
a: 5′-GMP, b: Vb

3, 5′-Cyclonucleoside has been synthesized *via* 2′, 3′-*O*-isopropylidene-5′-*O*-(*p*-toluenesulfonyl)-nucleoside.[6,9] It can be considered that the reaction is dependent on the inductive effect of the tosyl group, and a similar effect can also be expected for the dichlorophosphoryl group. The 3, 5′-cyclization of 2′, 3′-*O*-isopropylidene-5′-*O*-(*p*-toluenesulfonyl)-inosine has been more difficult than that of the same guanosine derivative, and the ease of the formation of such cyclonucleosides has been reported to be a function of the basicity of their purine bases.[9] A similar tendency was observed in the present studies. The cycloderivative Vb was more easily produced than the chloro-derivative IIIb in the reaction of Ib, in contrast to the case of Ia. The ease of the cyclization of the compound IIb seems to be due to an increase in the electron density of the nitrogen atom at the 3-position because of the electromeric effect of the 2-amino group in the guanine base in addition to the decrease in the electron density of the carbon atom at the 5′-position because of the inductive effect of the dichlorophosphoryl group.



The isopropylidene group of Vb resisted mild acidic hydrolysis, as is shown in Fig. 5, which also exhibits the difference in the elimination rate from those of Ib and Vb. The increase in the stability of the dioxolane ring in cyclonucleoside seems to be due to a decrease in the strain as a result of the cyclization between the ribose and the purine base.

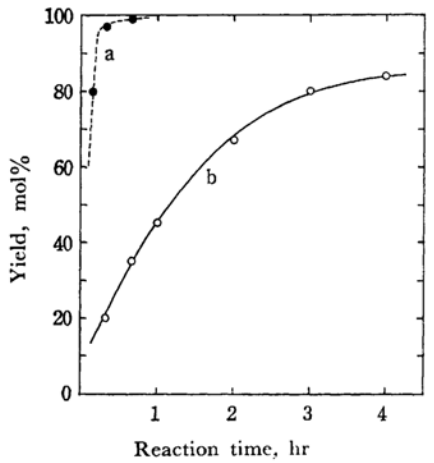
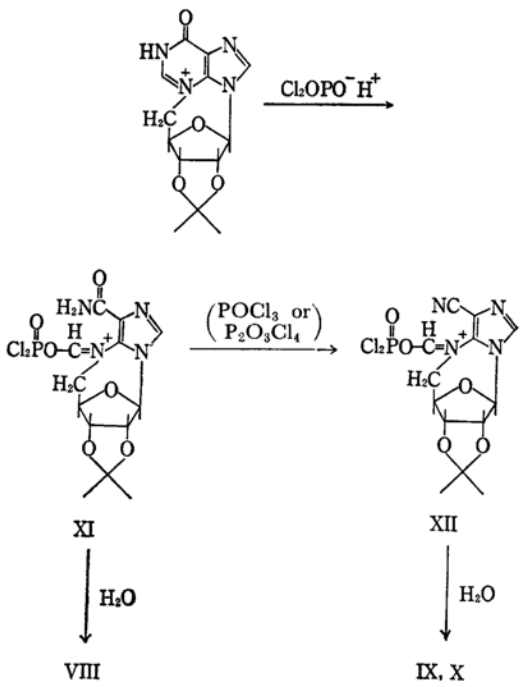


Fig. 5. Elimination rate of isopropylidene groups of Ib and Vb.

Experimental condition; pH 1.5, 70°C
a: guanosine produced, b: VIb produced

9) V. M. Clark, A. R. Todd and J. Zussman, *J. Chem. Soc.*, **1951**, 2952.

The degradation of 3, 5′-cyclopurinenucleoside involving ring cleavage has been studied by Baker[10] and by Todd.[11] It has been illustrated that the degradation is due to the attack of the hydroxyl anion on the carbon atom at the 2-position of the original cyclonucleoside. A similar explanation is acceptable in the present case, since the cleaved compounds, VIII and IX, were given when the cycloinosine derivative Va was heated with phosphoryl chloride hydrolized partially by a small amount of water. The ring cleavage of Va to VIII during the phosphorylation can be attributable to the nucleophilic attack of the dichlorophosphate anion on the 2-position, as is illustrated in following equation:



The labile intermediate XI is subjected to the rapid hydrolysis to give VIII.

The dehydration of the intermediate XI by phosphoryl chloride seems to form the compound IX. The addition of a more powerful dehydrating agent, pyrophosphoryl tetrachloride, promoted the formation of IX, as had been expected.

The corresponding ring-opening products of 5′-deoxy-2′, 3′-*O*-isopropylidene-3, 5′-cycloguanosine are not found in the reaction mixture under similar conditions. This may be attributable to the decrease in the positivity of the carbon atom at the 2-position because of the electromeric effect of the amino group. The formyl groups of the compounds VII and IX must be eliminated during the mild acidic hydrolysis.

10) B. R. Baker and J. P. Joseph, *J. Am. Chem. Soc.*, **77**, 15 (1955).
11) A. M. Michelson "The Chemistry of Nucleoside and Nucleotide," Academic Press, London(1963), p. 20.

Experimental

The melting points were determined on a Mitamura Riken hot stage and are uncorrected. The ultraviolet spectra were recorded on Hitachi EPS-2U spectrophotometer, and the infrared spectra on Hitachi EPI-2 and Perkin-Elmer 221 spectrophotometers. The NMR spectra were run on Varian A60 spectrometer, using tetramethylsilane as the internal standard. The paper chromatographies were carried out by the ascending technique on Toyo Roshi No. 51 (40×40 cm), using the following solvent system: Solv. 1, *n*-propyl alcohol - concentrated ammonium hydroxide - water, 20 : 12 : 3; Solv. 2, *n*-butyl alcohol - acetic acid - water, 4 : 1 : 1; Solv. 3, isopropyl alcohol - saturated ammonium sulfate - water, 2 : 79 : 19.

The Isolation of 5′-Chloro-5′-deoxy-2′, 3′-*O*-isopropylideneinosine (IIIa). To phosphoryl chloride (100 m*l*) 2.0 g of 2′, 3′-*O*-isopropylideneinosine (Ia) were added, after which the solution was warmed while being stirred for 5 hr at 40°C. After cooling, the reaction mixture was poured into 500 m*l* of petroleum ether in order to precipitate a syrupy product. The precipitate was centrifuged, washed with petroleum ether, and suspended in 50 m*l* of chloroform, and the solution was poured into an aqueous sodium hydrogen carbonate. After the separated aqueous layer had been extracted with four 50 m*l* portions of chloroform, all the chloroform solutions were collected, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue was recrystallized from methyl alcohol to give 1.0 g (47%) of IIIa, mp 195—196°C.

Found: C, 47.40; H, 4.79; N, 17.17; Cl, 10.70%. Calcd for $C_{13}H_{15}O_4N_4Cl$: C, 47.78; H, 4.59; N, 17.45; Cl, 10.87%.

The ultraviolet spectrum was similar to that of 2′, 3′-*O*-isopropylideneinosine. R_f values: 0.81 (Solv. 1), 0.94 (Solv. 2).

The Preparation of 2′, 3′-*O*-Isopropylidene-5′-*O*-methanesulfonylinosine. To a solution of Ia (3.08 g) in 50 m*l* of anhydrous pyridine, 1.25 g of methanesulfonyl chloride were added, after which the solution was stirred for 3 hr at 40°C. After it had stood overnight at room temperature, the reaction mixture was poured into 200 m*l* of water and the aqueous solution was extracted with 150 m*l* of chloroform. The chloroform layer was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The recrystallization of the residue from ethyl alcohol gave 2.0 g of a pure product, mp 172—173°C.

Found: C, 43.67; H, 4.91; N, 14.70%. Calcd for $C_{14}H_{18}O_7N_4S$: C, 43.52; H, 4.66; N, 14.51%.

The Preparation of 5′-Chloro-5′-deoxy-2′, 3′-*O*-isopropylideneinosine (IIIa). 2′, 3′-*O*-Isopropylidene-5′-*O*-methanesulfonylinosine (10 g) and lithium chloride (2.2 g) were added to a mixture of 20 m*l* of acetone and 30 m*l* of ethyl alcohol, and the mixture was heated in a sealed tube for 5 hr at 100°C. The reaction mixture was then concentrated under reduced pressure and poured into ice water to precipitate a colloidal material. After being centrifuged, the product was dried under reduced pressure and recrystallized

from methyl alcohol to yield 1.5 g of an amorphous solid, mp 195—196°C.

Found: C, 47.53; H, 4.85; N, 17.80; Cl, 10.70%. Calcd for $C_{13}H_{15}O_4N_4Cl$: C, 47.78; H, 4.59; N, 17.45; Cl, 10.87%.

The melting point was not depressed on admixture with the IIIa isolated from the phosphorylation mixture; the infrared spectrum was also in complete agreement with that of the isolated IIIa.

The Preparation of 5'-Chloro-5'-deoxyinosine from IIIa. 5'-Chloro-5'-deoxy-2', 3'-*O*-isopropylideneinosine (IIIa) was dissolved in water and hydrolyzed for 1 hr at 70°C after the pH of the solution had been adjusted to 1.5. The hydrolyzed product did not crystallize after concentration owing to its strongly hygroscopic nature, but the product showed the same R_f values (Solv. 1, 0.53; Solv. 2, 0.35) as that of H-3 on paper chromatography. The concentrated residue obtained from the hydrolyzed solution after neutralization afforded hypoxanthine when refluxed in a 0.1 N hydrochloric acid solution and a small amount of inosine when refluxed in a 0.1 N sodium hydroxide solution.

The Preparation of 5'-Deoxy-2', 3'-*O*-isopropylidene-3,5'-cycloinosine (Va). 5'-Deoxy-2',3'-*O*-isopropylidene-3, 5'-cycloinosine was prepared *via* 2', 3'-isopropylidene-5'-*O*-(*p*-toluenesulfonyl)-inosine according to the directions of Holmers and Robins.[6] The melting point, 260—262°C, was slightly lower than their decription, 266—269°C.

The Isolation of 5'-Deoxy-2', 3'-*O*-isopropylidene-3, 5'-cycloinosine (Va). 2' 3'-*O*-Isopropylideneinosine (10 g) was added to a mixture of 46 m*l* of phosphoryl chloride and 0.3 m*l* of water, after which the solution was stirred for 18 hr at 40°C. The reaction mixture was then poured into ether to precipitate the reaction products. After the precipitate had been dissolved in water, the aqueous solution was adjusted to pH 1.5 with a sodium hydroxide solution, heated for 40 min at 70°C, and adjusted again to pH 3. The solution was passed through a Dowex 1-X8 (Cl^- form) (100—200 mesh) column with a bed volume of approximately 500 m*l*, and the ultraviolet absorbing effluent was divided into fraction I and fraction II, each 2 *l* in volume.

The fraction I was neutralized and concentrated to about 50 m*l* under reduced pressure to give a white precipitate. This precipitate was recrystallized twice from water to afford 327 mg (3.3%) of Va, mp 260—262°C, the melting point of which was not depressed by the addition of the authentic sample obtained in the preceding experiment.

Found: C, 50.60; H, 5.44; N, 18.10%. Calcd for $C_{13}H_{14}O_4N_4 \cdot H_2O$: C, 50.64; H, 5.23; N, 18.18%.

The ultraviolet and infrared spectra were entirely superimposable upon those of the corresponding spectra of Va synthesized above.

When this product was heated in an acidic solution of pH 1 for 3 hr at 70°C, the paper chromatography showed the same spot as those of H-2.

The Isolation of 5-Amino-4-carbamoyl-N^5, 5'-cyclo-1-β-D-(5'-deoxy-2', 3'-*O*-isopropylideneribofuranosyl)-imidazole (VIII). The fraction II obtained by the treatment with Dowex 1 in the preceding experiment was evaporated to dryness under reduced pressure, and the residue was extracted with chloroform. Paper chromatography revealed that the chloroform layer contained VIII, plus small amounts of Va, IX, and X. The chloroform layer was dried over anhydrous sodium sulfate and passed through a column (2×60 cm) of alumina (treated with concd. hydrochloric acid, washed with water, and dried at 100°C). The column was eluted with chloroform, and 50 m*l* fractions were collected. Fractions 12—22 were concentrated to give 550 mg (6%) of VIII as a colorless solid, mp 120—125°C (decomp.).

Found: C, 51.56; H, 5.87; N, 19.98%. Calcd for $C_{12}H_{16}O_4N_4$: C, 51.42; H, 5.75; N, 19.99%.

IR (KBr): ν_{max} 1647 ($-CONH_2$), 1598 (C=C) cm^{-1}.

The compounds IX and X were too small to be isolated in this experiment.

The Isolation of 5-Amino-4-cyano-N^5, 5'-cyclo-1-β-D-(5'-deoxy-2', 3'-*O*-isopropylideneribofuranosyl)-imidazole (X) and Its N^5-Formyl Derivative (IX). To a mixture of 27.6 m*l* of phosphoryl chloride and 10.9 m*l* of pyrophosphoryl tetrachloride, 10 g of Ia were added, after which the solution was worked up according to the method used in the case of the isolation of Va. The fraction II obtained by treatment with Dowex 1 was adjusted to pH 7, concentrated to 100 m*l* under reduced pressure, and extracted with chloroform. The chloroform solution was dried over anhydrous sodium sulfate and evaporated to dryness to give 400 mg of a white solid. Paper chromatography showed that the solid consisted almost entirely of IX (H-6A) and X(H-6B). The solid was dissolved in chloroform and passed through a column (1.5×20 cm) of alumina (treated by the preceding method). The column was then eluted with chloroform, and the eluate was fractionated into 50 m*l* portions. Fractions 3—4 were combined and evaporated to dryness under reduced pressure. The recrystallization of the residue from water gave 50 mg of IX, mp 211°C (decomp.).

Found: C, 54.14; H, 5.17; N, 19.20%. Calcd for $C_{13}H_{14}O_4N_4$: C, 53.79; H, 4.86; N, 19.31%.

IR (KBr): ν_{max} 2230 ($-C\equiv N$), 1705 (–CHO), 1596 (C=C) cm^{-1}.

When fractions 7—8 were also combined and evaporated to dryness under reduced pressure, 150 mg of a white powder were obtained. The powder was recrystallized from methyl alcohol to give white needles, mp 270—271.5°C.

Found: C, 54.80; H, 5.58; N, 20.89%. Calcd for $C_{12}H_{14}O_3N_4$: C, 54.96; H, 5.38; N, 21.36%.

IR (KBr): ν_{max} 2225 ($-C\equiv N$), 1614 (C=C) cm^{-1}.

The Isolation of 5'-Chloro-5'-deoxy-2', 3'-*O*-isopropylideneguanosine (IIIb). A solution of 2.0 g of Ib suspended in 150 m*l* of phosphoryl chloride was warmed for 3 hr at 50—55°C and then poured into ether after cooling. The precipitated product was slowly dissolved in 100 m*l* of water so that the pH of the aqueous solution was maintained at about 7 by the gradual addition of a sodium hydroxide solution, after which the insoluble material was collected on a filter. The insoluble material was then washed with hot water and dissolved in a dilute sodium hydroxide solution. The solution was treated with charcoal and neutralized with hydrochloric acid to precipitate 183 mg (8.7%) of IIIb, mp 275—280°C (decomp.).

Found: C, 45.07; H, 4.80; N, 19.76%. Calcd for $C_{13}H_{16}O_4N_5Cl \cdot \frac{1}{4}H_2O$: C, 45.16; H, 4.81; N, 20.26%.

R_f values: 0.80 (Solv. 1), 0.58 (Solv. 2). The

ultraviolet spectra were similar to those of Ib.

The Preparation of 5-′Chloro-5′-deoxyguanosine (IVb) from IIIb. A solution of 200 mg of IIIb suspended in 50 m*l* of water was adjusted to pH 1.5 and heated for 2 hr at 70°C. The solution was then neutralized with concentrated ammonium hydroxide, concentrated to 7 m*l* under reduced pressure, and cooled to afford a syrupy product. Recrystallization from water gave 120 mg of a white solid, mp 240°C, with a gradual decomposition.

Found: C, 39.18; H, 4.10; N, 22.85; Cl, 11.86%. Calcd for $C_{10}H_{12}O_4N_5Cl\cdot\frac{1}{4}H_2O$: C, 39.23; H, 4.11; N, 22.87; Cl, 11.58%.

This product consumed one equivalent mole of periodic acid per mole of the nucleoside as formula IVb upon oxidation by the Fleury-Lange method.[12] When refluxed in 0.1 N hydrochloric acid and 0.1 N sodium hydroxide solutions, this product gave guanine and a small amount of guanosine respectively. The treatment of this solid with acetone according to the method of Mori *et al.*[13] afforded 5′-chloro-5′-deoxy-2′,3′-*O*-isopropylideneguanosine (IIIb).

The Isolation of 5′-Deoxy-2′,3′-*O*-isopropylidene-3,5′-cycloguanosine (Vb). To a mixture of 27.6 m*l* of phosphoryl chloride and 10.9 m*l* of pyrophosphoryl tetrachloride, 10 g of Ib were added, after which the mixture was stirred for 7 hr at 40°C. Ether was added to the reaction mixture, and the precipitated product was collected on a filter and dissolved in 300 m*l* of ice water. The solution was adjusted to pH 5, and passed through a Dowex 1-X2 (Cl^- form) (100—200 mesh) column (200 m*l* bed volume), and the column was washed with water. The ultraviolet absorbing effluent was then collected, adjusted to pH 7, and concentrated under reduced pressure until the product began to precipitate, after which it was kept at room temperature. The precipitate was collected on a filter, washed with 10% ethyl alcohol, and dried to give 5.2 g of white crystals. Recrystallization from water afforded 4.25 g of white needles, mp 250—255°C (decomp., with a gradual coloring from 240°C).

Found: C, 50.70; H, 5.02; N, 22.55%. Calcd for $C_{13}H_{15}O_4N_5$: C, 51.14; H, 4.95; N, 22.94%.

When a part of the crystals were refluxed in 1N hydrochloric acid for 1 hr and the solution was developed on paper chromatography, a new spot was found at the R_f value of 0.09 by Solv. 1 and at 0.08 by Solv. 2. This spot was identified as 3,5′-(5′-deoxy-D-ribofuranosyl)-guanine by comparing its ultraviolet absorption maxima with the values of the literature.[6] When refluxed in a 1 N sodium hydroxide solution, Vb gave a new spot which showed R_f values of 0.68 (Solv. 1) and 0.58 (Solv. 2). This product was similarly identified as 5′-deoxy-2′,3′-*O*-isopropylidene-3,5′-cycloxanthosine.

The Preparation of 5′-Deoxy-2′,3′-*O*-isopropylidene-3,5′-cycloguanosine (Vb). A solution containing 3.4 g of Ib and 1.25 g of methanesulfonyl chloride in 50 m*l* of anhydrous pyridine was heated for 2 hr at 80°C. The resulting solution was then poured into 200 m*l* of water and evaporated to dryness under reduced pressure. After the residue had been dissolved in water, the pH of the solution was adjusted to 7 and ethyl alcohol was added to the solution. The precipitate was collected on a filter and recrystallized three times from water to give 1.3 g (40%) of white needles.

The crystal was indistinguishable spectrally (infrared and ultraviolet) from a sample of the Vb isolated in the preceding experiment.

The Preparation of 5′-Deoxy-3,5′-cycloguanosine (VIb). A solution of 2.0 g of Vb in 50 m*l* of water was adjusted to pH 1.5 with hydrochloric acid and then heated for 6 hr at 70°C. The resulting solution was neutralized with concentrated ammonium hydroxide, concentrated, and cooled to give a gelationous product. This product was changed to a powdery white precipitate with slight warming in ethyl alcohol. The precipitate was filtered, quickly dried, and reprecipitated from water-ethyl alcohol to give 0.2 g of a product. Although an attempt at elemental analysis failed due to its hygroscopic nature, it was confirmed by the ultraviolet spectra and R_f values that the product was G-2 itself.

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12) P. F. Fleury and J. Lange, *J. Pharm. Chim.*, **17**, 107, 196 (1933).

13) H. Mori et al., Presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Sapporo, July 21, 1964, p. 269; Ajinomoto Co., U. S. Pat. 3201388 (1965).