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Studies of Phosphorylation. IV. The Phosphorylation of Nucleosides with Phosphorus Trihalide.[1]

Masaharu Yoshikawa, Mitsuru Sakuraba and Koji Kusashio
Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki

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The treatment of a 2′,3′-*O*-isopropylidene nucleoside with phosphorus trichloride in the presence of trimethyl phosphate and subsequent hydrolysis gave a nucleoside 5′-phosphite in an excellent yield. Triaryl phosphates, trialkylphosphine oxides, and trialkyl phosphites were also found to be potential accelerators of the reaction. Their effects were comparable to that of trimethyl phosphate. The similar treatment of a 5′-*O*-acetyl nucleoside gave mixed nucleoside 2′- and 3′-phosphites in an approximate mole ratio of 4 : 6. The reaction intermediate, nucleoside phosphorodichloridite, was subjected to partial hydrolysis, giving the corresponding monochloridite. The product was readily oxidized to give the 5′-nucleotide. Chlorine was generally suitable as an oxidizing agent, but iodine gave better results for guanosine derivatives than did chlorine or bromine. The treatment of an unprotected nucleoside with phosphorus oxychloride in a trialkyl phosphate, prior to the reaction with phosphorus trichloride, could be used for the synthesis of a nucleoside 2′(or 3′),5′-diphosphate in a good yield.

Two methods have been reported for the preparation of nucleoside 5′-phosphates from 2,′3′-*O*-substituted nucleosides *via* the corresponding 5′-phosphites: phosphorylation with *O*-benzylphosphorous *O*,*O*-diphenylphosphoric anhydride, followed by oxidation with *N*-chlorosuccinimide,[2] and condensation with phosphorous acid by di-*p*-tolylcarbodiimide or dicyclohexylcarbodiimide, followed by oxidation with potassium permanganate.[3,4] The latter method has also been used in the preparation of nucleoside 2′(or 3′)-phosphites and- phosphates from the corresponding 5′-*O*-substituted nucleosides.

Holý *et al.* have reported that the treatment of adenosine with triethyl phosphite in dimethylformamide containing hydrochloric acid, followed by alkaline hydrolysis, gives a mixture of adenosine 2′- and 3′-phosphites in a 60% yield. A similar reaction, but carried out with triphenyl phosphite in place of triethyl phosphite, yielded a mixture of 2′(or 3′)-phosphite and 2′(or 3′),5′-diphosphite.[5]

The phosphorylation of a 5′-*O*-substituted nucleoside to give the corresponding 2′(or 3′)-phosphate has been carried out successfully by the condensation of the nucleoside with 2-cyanoethyl phosphate by dicyclohexylcarbodiimide,[6] but when dibenzyl phosphorochloridate was used, the reaction was not achieved because of the inertness of the chloridate to the secondary hydroxyl groups of nucleoside sugar.[7] The present authors attempted to prepare guanosine 2′(or 3′)-phosphate from 5′-*O*-acetylguanosine and pyrophosphoryl tetrachloride in triethyl phosphate, but the yield was 50% at best. A better method, using P^1,P^1-diphenyl P^2-morpholinopyrophosphorochloridate, has been described by Ikehara and Tazawa.[8]

Recently, Honjo *et al.*[9] have reported that the reaction of 2′,3′-*O*-isopropylideneinosine with phosphorus trichloride in acetone (or methyl ethyl ketone) in an open vessel and subsequent hydrolysis gave inosine 5′-phosphate as the major product, although similar treatment in a closed vessel gave mainly inosine 5′-phosphite. The route *via* nucleoside phosphorodichloridite was considered to be a more plausible mechanism than the phosphorylation with phosphorus oxychloride formed by the air oxidation of phosphorus trichloride.

Secondary alkyl phosphorodichloridites have been prepared by the treatment of secondary alcohols

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[1] M. Yoshikawa, T. Kato and T. Takenishi, This Bulletin, **42**, 3505 (1969).

[2] N. S. Corby, G. W. Kenner and A. R. Todd, *J. Chem. Soc.*, **1952**, 3669.

[3] J. A. Schofield and A. R. Todd, *ibid.* **1961**, 2316.

[4] A. Holý, J. Smrt and F. Šorm, *Collect. Czech. Chem. Commun.*, **30**, 1635 (1965).

[5] A. Holý, *Chem. Ind.* (London), **1965**, 721.

[6] P. I. Gilham and G. M. Tenner, *ibid.*, **1959**, 542; G. M. Tenner, *J. Amer. Chem. Soc.*, **83**, 159 (1961); H. J. Thomas, K. Hewson and J. A. Montgomery, *J. Org. Chem.*, **27**, 192 (1962).

[7] D. H. Hayes, A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, **1955**, 808.

[8] M. Ikehara and I. Tazawa, *J. Org. Chem.*, **31**, 819 (1966).

[9] M. Honjo, R. Marumoto, K. Kobayashi and Y. Yoshida, *Tetrahedron Lett.*, **32**, 3851 (1966).

with phosphorus trichloride under mild warming conditions.10) This method, however, could not be directly adapted to the synthesis of nucleotides because of the highly heat-sensitive nature of nucleoside phosphorodichloridites in an acidic condition.

We have attempted to find some potentially active promotors for the reaction of a nucleoside with phosphorus trichloride at a low temperature. The method involving the reaction of 2′,3′-*O*-isopropylidene and 5′-*O*-acetyl nucleosides with a phosphorus trihalide in the presence of an appropriate organo-phosphorus compound gave nucleoside 5′- and 2′(or 3′)-phosphites respectively. The oxidation of a nucleoside phosphorochloridite with a halogen offered a convenient method for the synthesis of a nucleoside 5′- or 2′(or 3′)-phosphate. The reaction of phosphorus trichloride with a nucleoside 5′-phosphorodichloridate proceeded readily. The subsequent hydrolysis of the product after oxidation gave a nucleoside 2′(or 3′),5′-diphosphate in a good yield. This paper will deal with the synthesis of the nucleoside phosphite and phosphate using phosphorus trihalide.

Results and Discussion

Phosphorylation of 2′,3′-*O*-Isopropylidene Nucleoside. Trialkyl phosphate has shown an excellent effect in the phosphorylation of a wide field of nucleosides with phosphorus oxychloride, as has been reported in a preceding paper.1) The addition of a trialkyl phosphate to the reaction mixture of 2′,3′-*O*-isopropylideneinosine with phosphorus trichloride caused an even more remarkable promotion and resulted in a high yield of inosine 5′-phosphite after hydrolysis, in contrast with the low yield (14%) in the reaction with phosphorus trichloride alone, as is shown in Table 1. The effect was greater with a lower alkyl phosphate. The addition of a triaryl phosphate, such as triphenyl or diphenyl tolyl phosphate, in place of a trialkyl phosphate was also useful in promoting the reaction.

An increase in the amount of a trialkyl phosphate resulted in an acceleration of the reaction. Hence, the reaction must be discontinued quickly because of the rapid decomposition of the produced 5′-phosphorodichloridite. On the phosphorylation of 2′,3′-*O*-isopropylideneinosine with two mole-equivalents of phosphorus trichloride in trimethyl phosphate, only a quarter of an hour at 0°C was enough to give inosine 5′-phosphite in a 93% yield after hydrolysis; if the reaction period was prolonged, the yield was about 60%.

The oxidation of a nucleoside phosphite was carried out successfully by a halogen and yielded a nucleoside 5′-phosphate in a good yield. After the phosphorylation mixture had been poured into ether, the 2′,3′-*O*-isopropylideneinosine 5′-phosphorodichloridite (II) thus precipitated was centrifuged and dissolved again in fresh trimethyl phosphate.

The direct treatment of the solution with chlorine resulted in a serious decomposition of the nucleoside

TABLE 1. PHOSPHORYLATION OF 2′,3′-*O*-ISOPROPYLIDENE NUCLEOSIDES WITH PHOSPHORUS TRIHALIDE*

No.	Isopropylidene nucleoside	Organo-phosphorus compound, mmol	PX_3 mmol	Time hr	Yield of 5′-phosphite mol%
1 (2)**	Inosine	none	PCl_3: 100 (PBr_3: 100)	6 (4)	13 (2)
3 (4)	Inosine	$(CH_3O)_3PO$: 10	PCl_3: 100 (PBr_3: 100)	1 (4)	90 (54)
5 (6)	Inosine	$(C_2H_5O)_3PO$: 10	PCl_3: 100 (PBr_3: 100)	1 (3)	84 (80)
7 (8)	Inosine	$(n\text{-}C_4H_9O)_3PO$: 10	PCl_3: 100 (PBr_3: 100)	2 (6)	74 (72)
9	Inosine	$(C_6H_5O)_3PO$: 10	PCl_3: 100	2	72
10	Inosine	$(C_6H_5O)_2(p\text{-}CH_3C_6H_4O)PO$: 10	PCl_3: 100	3	72
11	Inosine	$(n\text{-}C_8H_{17})_3PO$: 10	PCl_3: 100	6	77
12	Inosine	$(CH_3O)_3P$: 10	PCl_3: 100	1	89
13	Inosine	$(C_2H_5O)_3P$: 10	PCl_3: 100	3	88
14	Inosine	$(i\text{-}C_3H_7O)_3P$: 10	PCl_3: 100	7	68
15	Inosine	$(n\text{-}C_4H_9O)_3P$: 10	PCl_3: 100	7	34
16 (17)	Inosine	$(CH_3O)_3PO$: 100	PCl_3: 10 (PBr_3: 10)	0.25 (2)	93 (80)
18 (19)	Adenosine	$(CH_3O)_3PO$: 10	PCl_3: 100 (PBr_3: 100)	1 (2)	87 (73)
20	Adenosine	$(CH_3O)_3PO$: 100	PCl_3: 15	0.5	78
21 (22)	Guanosine	$(CH_3O)_3PO$: 100	PCl_3: 15 (PBr_3: 15)	2 (2)	87 (83)
23	Uridine	$(CH_3O)_3PO$: 100	PCl_3: 10	3	98
24	Cytidine	$(CH_3O)_3PO$: 10	PCl_3: 100	6	75

* 5 mmol of 2′,3′-*O*-isopropylidene nucleoside was treated at 0°C.

** The results of phosphorylation with phosphorus tribromide comparable to phosphorus trichloride are shown in parentheses.

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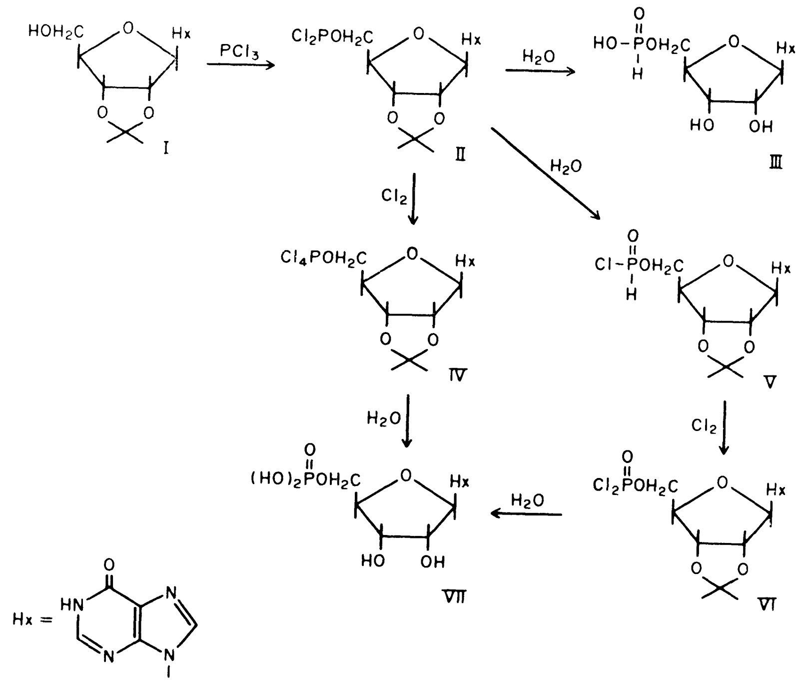
10) G. M. Kosolapoff, "Organo-phosphorus Compounds," John Wiley & Sons, New York (1950), p. 180.



because of the probable instability of the tetrachlorophosphorane (IV) produced during the reaction. However, inosine 5′-phosphate (VII) was obtained in a 73% over-all yield from the starting nucleoside (I) by the careful partial hydrolysis of the phosphorodichloridite (II) by adding about an equimolar amount of water per mole of the intermediate II and by the subsequent oxidation of V with chlorine. Other halogens, such as bromine and iodine, could also be used for oxidation in the same way, but a number of usual oxidizing agents could not be used for this purpose.*[1]

The use of phosphorus tribromide in place of phosphorus trichloride resulted in a lower yield of a nucleoside 5′-phosphite. Especially, the treatment of 2′,3′-*O*-isopropylideneinosine with phosphorus tribromide alone gave only a trace of inosine 5′-phosphite.

The addition of a trialkylphosphine oxide or trialkyl phosphite to this reaction gave good results in spite of the ineffectiveness of both on phosphorylation with phosphorus oxychloride. However, the latter's effectiveness as an additive decreased sharply according to the increase in the carbon numbers of the alkyl group.

This method is generally applicable to the synthesis of other 5′-nucleotides; the results are shown, along with the results of the investigation of 2′,3′-*O*-isopropylideneinosine, in Table 1.

Phosphorylation of 5′-*O*-Acetyl Nucleoside. The reaction of 5′-*O*-acetyladenosine with a large excess of phosphorus trichloride did not give adenosine 2′(or 3′)-phosphite. The addition of a small amount of triethyl phosphate to the reaction mixture also resulted in only a small percentage of the phosphorylation product, unlike the case of a 2′,3′-*O*-isopropylidene nucleoside. However, adenosine 2′(or 3′)-phosphite was produced in a quantitative yield by the treatment of 5′-*O*-acetyladenosine with two mole-equivalents of phosphorus trichloride in triethyl phosphate and by subsequent hydrolysis. The phosphorylation was completed after a longer period than that needed for 2′,3′-*O*-isopropylideneadenosine. The mole ratio of the adenosine 2′- and 3′-phosphites was 36 : 64, which was very close to the result reported by Holý.[5] Since other 5′-*O*-acetyl ribonucleosides also produced mixtures of the corresponding 2′- and 3′-phosphites in similar mole ratios, as is shown in Table 2, it was clear that the 2′-hydroxyl group was less reactive than the 3′-hydroxyl group.

An oxidation giving a mixture of adenosine 2′- and 3′-phosphates in a 84% yield was carried out by a similar technique to that used for inosine 5′-phosphate. In the course of an investigation of the oxidation of a nucleoside 2′(or 3′)-phosphorodichloridite, it was found that the ease of oxidation depended upon the kind of halogen used and was generally in the order: chlorine > bromine > iodine. However, the oxidation of guanosine 2′(or 3′)-phosphorodichloridite with chlorine gave only a trace of the corresponding phosphate because of the destruction of the heterocyclic nucleus, which was detected by the loss of the ultraviolet absorption. In this case, the highest yield was

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*[1] The investigation of the oxidation was carried out with the cooperation of Mr. Takashi Yamashita.

TABLE 2. PHOSPHORYLATION OF 5′-*O*-ACETYL NUCLEOSIDES WITH PHOSPHORUS TRIHALIDE

No.	Acetyl nucleoside mmol	Trialkyl phosphate mmol	PX_3 mmol	Time hr	Yield of 2′(3′)-phosphite mol%	Mole ratio	
						2′	3′
25	Adenosine: 5	$(C_2H_5O)_3PO$: 5	PCl_3 : 100	6	2	—	—
26	Adenosine: 5	$(C_2H_5O)_3PO$: 20	PCl_3 : 100	6	21	—	—
27	Adenosine: 5	$(C_2H_5O)_3PO$: 125	PCl_3 : 15	1	94	36	64
28	Adenosine: 5	$(CH_3O)_3PO$: 100	PCl_3 : 10	1	97	39	61
29	Adenosine: 5	$(CH_3O)_3PO$: 100	PBr_3 : 10	1	91	35	65
30	Inosine: 5	$(C_2H_5O)_3PO$: 125	PCl_3 : 15	1	97	39	61
31	Inosine: 5	$(CH_3O)_3PO$: 100	PBr_3 : 10	1	88	33	67
32	Guanosine: 5	$(C_2H_5O)_3PO$: 125	PCl_3 : 15	1	95	40	60
33	Guanosine: 5	$(CH_3O)_3PO$: 100	PBr_3 : 10	1	97	47	53

Temperature: 0°C

TABLE 3. SYNTHESIS OF NUCLEOSIDE 2′,5′- AND 3′,5′-DIPHOSPHATES*

No.	Nucleoside	Time** hr	Halogen	Yield of diphosphate mol%	Mole ratio	
					2′,5′	3′,5′
34	Adenosine	2	Cl_2	92	43	57
35	Inosine	4	Cl_2	78	48	52
36	Guanosine	9	I_2	64	45	55
37	Cytidine	1	Br_2	78	46	54
38	Uridine	12	I_2	80	46	54

* 0.04 mol of nucleoside was treated successively at 0°C by 0.08 mol of phosphorus oxychloride, 0.08 mol of phosphorus trihalide for 1 hr, 0.48 mol of water and 0.12 mol of halogen for 2 hr in 100 m*l* of trimethyl phosphate.

** This represents the time of the first phosphorylation with phosphorus oxychloride.

achieved by less reactive iodine.

Synthesis of Nucleoside 2′(or 3′),5′-diphosphate. It is known that the 2′- and 3′-hydroxyl groups were inactive compared with the 5′-hydroxyl group. On the other hand, a nucleoside 2′(or 3′)-phosphorodichloridite in a reaction mixture was relatively stable even over a prolonged reaction time, despite the sharp decomposition of the 5′-phosphorodichloridite. Thus, the differences in reaction rate between sugar 2′(or 3′)- and 5′-hydroxyl groups, and in stability between the corresponding phosphorodichloridites, interfered with the extension of this procedure to the synthesis of a nucleoside 2′(or 3′),5′-diphosphate directly from an unprotected nucleoside.

A combination of phosphorus oxychloride and phosphorus trichloride was utilized for the synthesis. An attempt at the phosphorylation of an unprotected nucleoside in one step with a mixed solution of the combining phosphorylating agents in a trialkyl phosphate resulted in failure. This is considered to be due to the markedly rapid reaction of the unprotected nucleoside with phosphorus trichloride in comparison with that with phosphoryl chloride and to the different properties, described above. However, the treatment of an unprotected nucleoside with phosphorus trichloride, after phosphorylation with phosphorus oxychloride sufficient to give a nucleoside 5′-phosphorodichloridate, and subsequent oxidation gave an excellent yield of the corresponding diphosphate. All of these procedures were carried out in one solution without the isolation of the intermediate.

Experimental

Detection and Analysis of Nucleotides. Paper chromatographies were developed by the ascending technique on Toyo Roshi No. 51 paper with Solvent 1: *n*-propyl alcohol - concentrated ammonium hydroxide - water (20 : 20 : 3), and Solvent 2: isopropyl alcohol - saturated ammonium sulfate - water (2 : 79 : 19). The nucleotides were detected by ultraviolet light and by spraying on a Hanes-Isherwood reagent, as has previously been descrived.[11] The yields were estimated by means of the ultraviolet absorption intensities of the solutions eluted from the spots with 0.1 N hydrochloric acid.

Nucleoside 2′(or 3′)-phosphites, 2′(or 3′)-phosphates, and 2′(or 3′),5′-diphosphates were chromatographed on thin layers of MN-Cellulose powder 300 (0.25 mm

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[11] M. Yoshikawa and T. Kato, This Bulletin, **40**, 2849 (1967).

thick) (Macherey, Nagel & Co., Germany) on a glass plate (20×20 cm) using the ascending technique with Solvent 3: isopropyl alcohol - saturated ammonium sulfate - 0.5 M sodium acetate (2 : 79 : 19). For nucleoside diphosphates, the faster-running compounds were assumed to be 2′,5′-diphosphates, judging from the behavior of inosine 2′,5′-diphosphate.

The R_f values are summarized in Table 4.

Table 4. R_f Values of nucleotides

Nucleotide	R_f Value of chromatogram			
	Paper		Thin-layer	
	Solv. 1	Solv. 2	Solv. 3	
			2′	3′
Adenosine 2′(3′)-phosphate	0.30	0.29	0.35	0.24
Adenosine 2′(3′)-phosphite	0.55		0.23	0.13
Adenosine 5′-phosphate	0.30	0.32		
Adenosine 5′-phosphite	0.60			
Adenosine 2′(3′),5′-diphosphate	0.15	0.37	0.37	0.32
Inosine 2′(3′)-phosphate	0.34	0.57	0.61	0.52
Inosine 2′(3′)-phosphite	0.54		0.50	0.41
Inosine 5′-phosphate	0.30	0.67		
Inosine 5′-phosphite	0.57			
Inosine 2′(3′),5′-diphosphate	0.16	0.76	0.81	0.76
Guanosine 2′(3′)-phosphate	0.23	0.43	0.50	0.39
Guanosine 2′(3′)-phosphite	0.46		0.36	0.25
Guanosine 5′-phosphate	0.21	0.47		
Guanosine 5′-phosphite	0.50			
Guanosine 2′(3′),5′-diphosphate	0.09	0.55	0.76	0.65
Cytidine 5′-phosphate	0.32	0.73		
Cytidine 5′-phosphite	0.55			
Cytidine 2′(3′),5′-diphosphate	0.23	0.81	0.88	0.81
Uridine 5′-phosphate	0.31	0.69		
Uridine 5′-phosphite	0.61			
Uridine 2′(3′),5′-diphosphate	0.20	0.77	0.87	0.81

Phosphorylation of 2′,3′-*O*-Isopropylideneinosine with Phosphorus Trichloride (as a Typical Example). To a cold mixture of phosphorus trichloride (8.7 m*l*, 100 mmol) and trimethyl phosphate (1.17 m*l*, 10 mmol), 1.54 g (5 mmol) of 2′,3′-*O*-isopropylideneinosine were added; the mixture was stirred for 1 hr at 0°C and then poured into 50 m*l* of ether. The precipitate was separated by a centrifuge and dissolved in about 30 m*l* of ice water, and the aqueous solution was hydrolyzed at 70°C after being adjusted to pH 1.5 with 6 N sodium hydroxide. Paper chromatography showed the presence of inosine 5′-phosphite in a 90% yield, as is shown in No. 3 of Table 1.

The effects of the other additives used in the same manner instead of trimethyl phosphate are summarized in Table 1, along with those of some other 2′,3′-*O*-isopropylidene nucleosides.

The solution was neutralized, absorbed on a Dowex 1×8 (HCOO⁻) (50—100 mesh) column, and eluted with 0.02 N formic acid. The fraction of inosine 5′-phosphite was neutralized with 6 N ammonium hydroxide and concentrated to about 20 m*l*. Ammonium inosine 5′-phosphite (1.23 g) was separated by the addition of 60 m*l* of ethyl alcohol and purified by reprecipitation from the aqueous solution with ethyl alcohol.

Found: C, 32.96; H, 5.00; N, 19.09; P, 8.77%. Calcd for $C_{10}H_{16}O_7N_5P \cdot H_2O$: C, 32.70; H, 4.94; N, 19.08; P, 8.43%.

The product was converted into inosine 5′-phosphate in a 39% yield by permanganate oxidation according to the method described by Schofield and Todd.3)

Synthesis of Inosine 5′-Phosphate. To a solution of 0.87 m*l* (10 mmol) of phosphorus trichloride in 11.7 m*l* (100 mmol) of trimethyl phosphate, 1.55 g (5 mmol) of 2′,3′-*O*-isopropylideneinosine were added on cooling; the mixture was then stirred at 0°C for 15 min. The reaction mixture was poured into about 100 m*l* of ether. The precipitate was separated by a centrifuge and dissolved in 10 m*l* of trimethyl phosphate. The solution was partially hydrolyzed with 0.09 m*l* (5 mmol) of water on cooling, and then 1.68 *l* (7.5 mmol) of chlorine was introduced into the sealed vessel of the solution. After having been vigorously stirred for 45 min at 0°C, the solution was treated with 100 m*l* of ether and the precipitate was hydrolyzed at pH 1.5 in a manner similar to that described above. Inosine 5′-phosphate was obtained in an over-all yield of 73%, as estimated by paper chromatographical analysis.

Phosphorylation of 5′-*O*-Acetyladenosine with Phosphorus Trichloride (as a Typical Example). To a solution of phosphorus trichloride (1.3 m*l*, 15 mmol), in 20 m*l* (125 mmol) of triethyl phosphate, 1.55 g (5 mmol) of 5′-*O*-acetyladenosine were added, and the mixture was stirred at 0°C for 1 hr. After the treatment of the reaction mixture with ether, the precipitate was dissolved in concentrated ammonium hydroxide and the solution was allowed to stand overnight at room temperature. This solution was found by chromatographical analysis to contain adenosine 2′- and 3′-phosphites in the mole ratio of 36 : 64 and in a total yield of 94%.

The results of the phosphorylation of several 5′-*O*-acetyl nucleosides with phosphorus trihalide are summarized in Table 2.

The alkaline solution was concentrated to about 10 m*l* and then adjusted to pH 1 with concentrated hydrochloric acid. After the acidic solution had been kept overnight in a refrigerator, the precipitate was filtered and dried. The mixture of adenosine 2′- and 3′-phosphites (1.14 g) thus obtained was reprecipitated from diluted alkali - hydrochloric acid.

Found: C, 35.58; H, 4.33; N, 21.21; P, 9.41%. Calcd for $C_{10}H_{14}O_6N_5P$: C, 36.26; H, 4.26; N, 21.15; P, 9.35%.

Synthesis of Adenosine 2′- and 3′-Phosphates. After working up in the same way with the method described in the previous experiment, the solution of the phosphorylated mixture was hydrolyzed carefully with 0.8 m*l* (45 mmol) of water: 0.5 *l* (22.5 mmol) of chlorine gas was then gradually passed through the solution at 0°C with stirring. After stirring had been continued for 3 hr, the reaction mixture was poured into a cold diluted ammonium hydroxide; analysis by paper chromatography showed adenosine monophosphate to be produced in an over-all yield of 84%. The solution was then concentrated to about 10 m*l* under reduced pressure, acidified to pH 1 with concentrated hydrochloric acid, and kept in a refrigerator. A white powder of adenosine monophosphate (1.08 g) was obtained by filtration; it was identical in paper-chromatographical behavior in solvents 1 and 2 with authentic adensosine 3′-phosphate. However, on thin-layer chromatography in Solvent 3, it was recognized to be a mixture of adenosine 2′- and 3′-phosphates in a mole ratio of 50 : 50.

Found: C, 33.83; H, 4.22; N, 20.00; P, 8.57%. Calcd for $C_{10}H_{14}O_7N_5P \cdot \frac{1}{2}H_2O$: C, 33.72; H, 4.42; N, 19.66; P, 8.48%.

Synthesis of the Disodium Salt of Guanosine 2′- and 3′-Phosphates. 5′-*O*-Acetylguanosine (1.63 g, 5 mmol) was phosphorylated much as has been described above, hydrolyzed partially, and oxidized for 9 hr at 5—10°C by 5.7 g (22.5 mmol) of iodine in place of chlorine gas. The resulting solution was poured into 100 m*l* of ether, and then the precipitate was dissolved in diluted ammonium hydroxide after centrifuging. Paper chromatography showed the presence of guanosine monophosphate in an 85% yield. The alkaline solution was then evaporated under reduced pressure, and the aqueous solution of the residue was adsorbed on a Dowex 1×2 (Cl^-) (100—200 mesh) column. The column was washed with water and eluted with 0.1 M sodium chloride in 0.003 N hydrochloric acid. The eluate was concentrated and adsorbed again on active carbon. The active carbon was washed with water and eluted with 0.5 N ammonium hydroxide. To the eluate, 6 N sodium hydroxide was added in small portions, with care taken to maintain the pH under 9 until no more ammonia was eliminated by concentration. The solution was then concentrated to about 10 m*l*, and 30 m*l* of ethyl alcohol was gradually added to it. The mixture of the disodium salt of guanosine 2′- and 3′-phosphates (1.19 g) thus obtained was recrystallized from water - ethyl alcohol and identified in a manner similar to that used for adenosine 2′- and 3′-phosphates.

Found: C, 28.74; H, 3.24; N, 16.55; P, 7.34%. Calcd for $C_{10}H_{12}O_8N_5PNa_2 \cdot H_2O$: C, 28.26; H, 3.32; N, 16.47; P, 7.28%.

Synthesis of the Dibarium Salt of Inosine 2′,5′- and 3′,5′-diphosphates (as a Typical Example). After the treatment of inosine (10.7 g, 0.04 mol) with phosphorus oxychloride (7.4 m*l*, 0.08 mol) in 10 m*l* of trimethyl phosphate for 4 hr at 0°C, phosphorus trichloride (7.0 m*l*, 0.08 mol) was added to the solution and the mixture was stirred for 1 hr. Water (8.6 m*l*, 0.48 mol) was then carefully added to the solution, and chlorine gas (2.7 *l*, 0.12 mol) was passed through with stirring. After 2 hr of oxidation at 0°C, the reaction mixture was poured into about 1 *l* of ether; the precipitate was hydrolyzed with 0.5 *l* of ice water and neutralized with 6 N sodium hydroxide. Paper chromatography revealed the presence of inosine diphosphates in a 78% yield.

In a similar manner, nucleoside diphosphates are obtained in high yields. The results are summarized in Table 3. The neutralized solution was passed through a column of a Dowex 1×2 (Cl^-) (50—100 mesh). The column was washed with water and eluted with 0.015 M sodium chloride in 0.003 N hydrochloric acid, and subsequently with 0.2 M sodium chloride in 0.003 N hydrochloric acid. The latter eluate was neutralized with 6 N sodium hydroxide, concentrated to about 100 m*l* under reduced pressure, and filtered to remove the sodium chloride. A saturated solution of 22 g of barium acetate was then added to the filtrate, and the precipitate was collected and dried. The mixture of dibarium inosine 2′,5′- and 3′,5′-diphosphates (15.4 g) thus obtained was dissolved again in 6 N hydrochloric acid, filtered, neutralized with 6 N sodium hydroxide, and reprecipitated by adding a barium acetate solution.

Found: C, 15.87; H, 2.24; N, 7.01; P, 8.24%. Calcd for $C_{10}H_{10}O_{11}N_4P_2Ba_2 \cdot 3H_2O$: C, 15.95; H, 2.14; N, 7.44; P, 8.23%.

Thin-layer chromatography showed the presence of two components with R_f values of 0.81 and 0.76 (mole ratio, 48 : 52). The faster-running component was identical with the inosine 2′,5′-diphosphate which was separated on the column chromatography of a Dowex 1×2 (Cl^-) according to the method reported by Mitsugi *et al.*[12] The slower-moving component was similarly identified as inosine 3′,5′-diphosphate.

The authors are indebted to Dr. Tetsuya Kato for his continuing guidance.

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12) K. Mitsugi, E. Nakazawa, S. Okumura, M. Takahashi and H. Yamada, *Agr. Biol. Chem.* (Tokyo), **29**, 1051 (1965).