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PHOSPHORYLATION OF RIBONUCLEOSIDES WITH PHOSPHORUS TRICHLORIDE

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The reaction between an alcohol and a large excess of PCl_3 generally gives a dichlorophosphite, which on hydrolysis affords a phosphite. Thus, treatment of an I_p* ribonucleoside with PCl_3 should give, after hydrolysis and de-blocking the protective group, a ribonucleoside-5'-phosphite.

Contrary to these expectations, however, when I_p inosine was allowed to react with PCl_3 in acetone in an open vessel and the reaction mixture was poured into ice water, the main product, after removal of the protective group, was 5'-IMP (yield 91%) (Table I, No. 2). The structure of this 5'-IMP was confirmed by its ultraviolet absorption spectrum, its paper electrophoretic** and chromatographic*** behavior, and by the liberation of phosphoric acid with bull semen 5'-nucleotidase. Levene and Tipson (1) had reported that I_p inosine was phosphorylated with $POCl_3$ in the presence of pyridine in low yields. The present phosphorylation has been achieved with PCl_3 in the absence of base. In general, purine nucleosides are not very

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* I_p, 2',3'-O-isopropylidene; 5'-IMP, inosine-5'-monophosphate; 5'-GMP, guanosine-5'-monophosphate.

** 0.05 M phosphate buffer (pH 7.5), 22 V/cm.

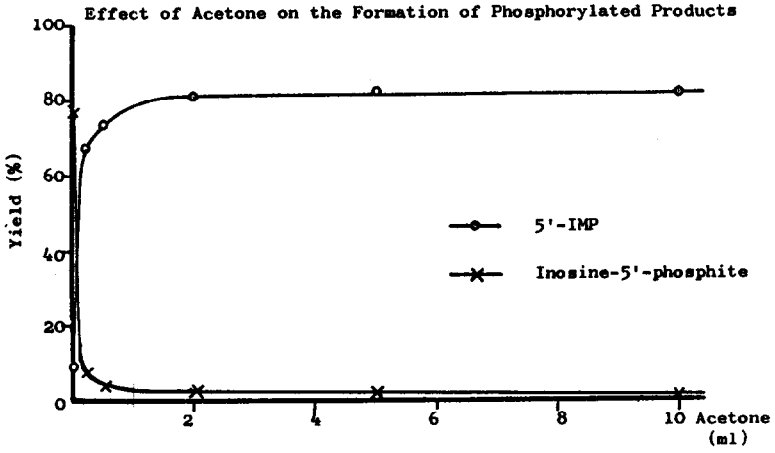
*** n-PrOH : 28% aq.NH_3 : H_2O (10 : 6 : 1), ascending method.

stable in acid solution. It was somewhat of interest to note that the glycosyl cleavage had not occurred under the reaction conditions where HCl was inevitably produced.

In addition to 5'-IMP, a minor product was produced which gave a positive phosphorus color reaction (2, 3) and showed an ultraviolet absorption spectrum identical with that for inosine. This substance was identified as inosine-5'-phosphite, because enzymic hydrolysis with bull semen 5'-nucleotidase or snake venom 5'-nucleotidase (Trimeresurus flavoviridis Hallowell) yielded inosine and phosphorous acid, and oxidation with dilute alkaline $KMnO_4$ solution (4) gave 5'-IMP. It should be mentioned that inosine-5'-phosphite was resistant to human prostatic phosphatase, Escherichia coli phosphatase or calf mucosa phosphatase.

No improvement in the yield of 5'-IMP was attained when the amount of acetone employed exceeded a certain limit (Fig. 1). In the absence of

FIG. 1



Isopropylidene inosine 20 mg, PCl_3 0.5 ml,
Reaction time 1 hr, Reaction temp. 0-5°

acetone, however, the yield of 5'-IMP was markedly supressed, while that of inosine-5'-phosphite increased. When methyl ethyl ketone was used in place of acetone, a comparable yield of 5'-IMP was obtained. However, the use of benzaldehyde gave poor yields of both and the use of other solvents gave very poor yields of 5'-IMP (Table I).

TABLE I

Effect of Solvents on Phosphorylation with PCl_3

Isopropylidene inosine 20 mg, Reaction temp. 0-5°, Reaction time 2 hr

No.	Solvent (ml)		PCl_3 (ml)	Yield of 5'-IMP (%)	Yield of Inosine-5'-phosphite (%)
1	None		0.8	10	77
2	Me_2CO	2.0	0.4	91	5
3	MeCOEt	2.0	0.4	83	7
4	C_6H_5CHO	2.0	0.4	22	24
5	$n\text{-}C_3H_7CHO$	1.0	0.3	0	20
6	Tetrahydrofuran	2.0	0.4	14	73
7	m-Cresol	2.0	0.4	trace	70
8	$HCONMe_2$	2.0	0.4	0	0
9	AcOEt	2.0	0.4	6	83
10	$MeNO_2$	2.0	0.4	5	76
11	$CH_2(CO_2Et)_2$	2.0	0.3	0	96

When the phosphorylation of I_p inosine with PCl_3 in acetone was carried out in a closed vessel, the yield of 5'-IMP was markedly lowered (20%). In order to examine the effect of the moisture, the reaction was carried out in an atmosphere of dry air. The yield of 5'-IMP (86%) was almost the same as that in the case with an open vessel. However, when the reaction was carried out in an atmosphere of nitrogen, a very poor yield of 5'-IMP (10%) was obtained. These facts indicate that the acetone

alone does not act as an oxidizing agent, and that the presence of oxygen in the reaction system is essential for the phosphorylation.

The mechanism of the reaction appears to be either that the PCl_3 is first oxidized to a pentavalent phosphorus compound (e.g. $POCl_3$) which then acts as a phosphorylating agent, or that the reaction between a nucleoside and PCl_3 takes place to give a dichlorophosphite which is then oxidized to the dichlorophosphate. The latter mechanism is more plausible by the following experiments:

1) The reaction mixture was examined throughout the course of the reaction period. Inosine was subjected to acetonization with acetone, PCl_3 and water, followed by phosphorylation with acetone and PCl_3 (crystalline I_p inosine is hardly soluble in a mixture of acetone and PCl_3). The results revealed that I_p inosine-5'-dichlorophosphite**** (I) was produced in about 50% yield shortly after the reaction had set in (Fig. 2). With lapse of time, the amount of this substance decreased. After 10 minutes from the start of the reaction only a few per cent of I remained. On the other hand, I_p inosine-5'-dichlorophosphate (II) gradually increased, and after 10 minutes its yield reached about 80%. The total yield of I and II did not change with further lapse of time. These facts clearly indicate that I_p inosine is first esterified with PCl_3 to give I which is then oxidized to II.

2) When I_p inosine (20 mg) was allowed to react with PCl_3 (0.3 ml) in ethyl acetate (2 ml) 0-5° for 1 hr and the organic solvent and PCl_3 were distilled off at low temperature under reduced pressure, crude compound I was obtained in a powdery form (yield 90%). This was further allowed to

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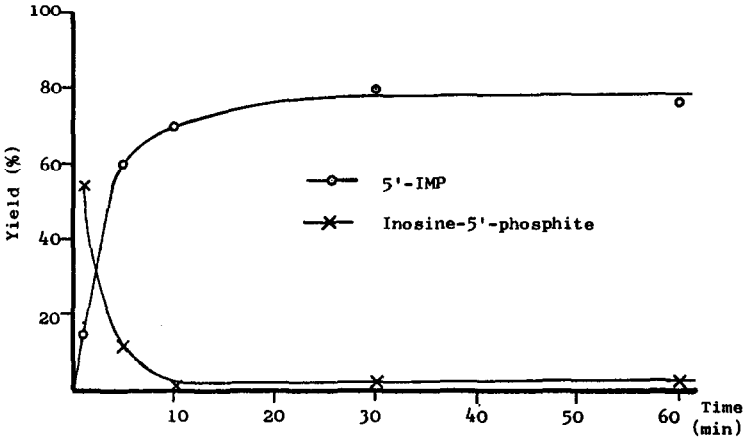
**** The compound has not been isolated, however, upon hydrolysis it gave inosine-5'-phosphite.

FIG. 2

Phosphorylation of Inosine with PCl_3



Acetonization (1 hr): Inosine 20 mg, Acetone 0.5 ml, PCl_3 0.03 ml, H_2O 0.01 ml

Phosphorylation (0-5°): Acetone 2 ml, PCl_3 0.5 ml

react in acetone and PCl_3 in an open vessel to obtain 5'-IMP in a good yield (62%). No oxidation took place in the absence of either acetone or PCl_3. The observation, therefore, confirms that the phosphate is produced by the oxidation of I, which requires the co-existence of acetone, PCl_3 and oxygen. One possibility for the mechanism of the present phosphorylation might be as follows: PCl_3 and acetone form an addition product, which in turn reacts with atmospheric oxygen to yield another intermediate capable of oxidizing phosphite to phosphate.

The phosphorylation of other ribonucleosides by the similar procedure was investigated. I_p adenosine, I_p cytidine and I_p uridine afforded the corresponding 5'-nucleotides in 76-91% yields. The reaction product from I_p guanosine, however, was not identical with 5'-GMP (paper electrophoretic behavior)**. It was assumed from the ultraviolet absorption spectrum of

the product that, in addition to phosphorylation of the 5' position, the aglycon had been altered in an unidentified manner.

Studies of the phosphorylation of deoxyribonucleosides and other alcohols are now in progress.

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REFERENCES

1. P. A. Levene and R. S. Tipson, J. Biol. Chem. 111, 313 (1935).
2. C. S. Hanes and F. A. Isherwood, Nature 164, 1107 (1949).
3. H. E. Wade and D. M. Morgan, Nature 171, 529 (1953).
4. J. A. Schofield and Sir A. Todd, J. Chem. Soc. 2316 (1961).