Phosphorylation by Oxidation-Reduction Condensation.[1] Preparation of Active Phosphorylating Reagents

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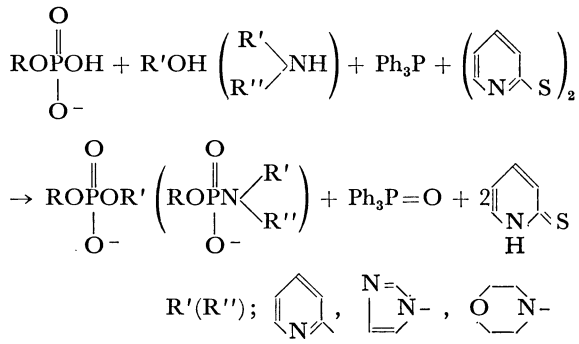
Active phosphorylating reagents such as imidazolylphosphonate,[2,3] 2-pyridyl ester of phosphoric acid,[4,5] and phosphoramidate,[6] especially phosphoromorpholidate,[7] are considered to be important intermediates in nucleotide synthesis. In the present experiment, the preparation of these active phosphorylating reagents such as imidazolylphosphonate, 2-pyridyl ester of phosphoric acid and phosphoromorpholidate by the oxidation-reduction condensation reaction[1] was studied.

First, the preparation of imidazolylphosphonate was attempted. In a typical reaction, triphenylphosphine (2 mmol) was added rapidly into a mixture of *p*-chlorophenyl dihydrogen phosphate (1 mmol), imidazole (5 mmol), triethylamine (1 mmol) and 2,2'-dipyridyl disulfide (2 mmol) in 5 m*l* of THF at room temperature. After stirring for 20 min, acetone solution of sodium iodide (1.5 mmol) was added to the reaction mixture and cooled down to 0°C. After being kept standing for 1 hr at 0°C, the precipitated white needle crystals were filtered off and washed with cold acetone. After removal of the solvent *in vacuo*, the sodium salt of *p*-chlorophenyl imidazolylphosphonate was obtained in a quantitative yield. UV: λ_{max} 270 mμ.

Found: C, 38.50; H, 2.51; N, 10.00%. Calcd for $C_9H_7O_3N_2PClNa$: C, 38.53; H, 2.52; N, 9.99%.

In the case of adenosine 5'-monophosphate, DMF was used in place of THF in the above experiment, and the corresponding adenosine 5'-imidazolylphosphonate was obtained in a quantitative yield without producing symmetrical pyrophosphate (AppA). *p*-Chlorophenyl-2-pyridyl phosphate was isolated as its cyclohexylammonium salt from *p*-chlorophenyl dihydrogen phosphate and 2-hydroxypyridine in high yield according to the following procedure. *p*-Chlorophenyl dihydrogen phosphate (1 mmol) was allowed to react with 2-hydroxypyridine (5 mmol), triphenylphosphine (2 mmol) and 2,2'-dipyridyl disulfide (2 mmol) in THF at room temperature for 3 hr. Cyclohexylamine (1.5 mmol) was then added to the reaction



mixture cooled in an ice-bath and kept standing for 2 hr. The precipitated crystals were filtered off, washed with cold THF and dried *in vacuo*. The corresponding cyclohexylammonium salt of *p*-chlorophenyl-2-pyridyl phosphate, mp 164°C, was obtained in 80% yield. UV: λ_{max} 262 mμ.[8] Found: C, 52.95; H, 5.85; N, 7.40%. Calcd for $C_{17}H_{22}O_4N_2ClP$: C, 53.10; H, 5.76; N, 7.29%.

Next, it was found that adenosine 5'-phosphoromorpholidate, an important intermediate in the synthesis of pyrophosphates such as FAD (flavin Adenine dinucleotide), was obtained in a quantitative yield by this method. Triphenylphosphine (2 mmol) was added into the suspended DMF solution of adenosine monophosphate (1 mmol), morpholine (5 mmol), triethylamine (1 mmol) and 2,2'-dipyridyl disulfide (2 mmol) at room temperature. After the mixture was stirred for 1 hr, the suspended solution turned clear. By this time, paper chromatography and paper electrophoresis showed the presence of only one spot corresponding to adenosine 5'-phosphoromorpholidate. Determination by UV absorption after separation with paper chromatography showed that the adenosine 5'-phosphoromorpholidate was obtained in a quantitative yield. $\lambda_{max}^{pH\,7}$ 260 mμ (ε 1.59×10^4). R_f 0.41, isopropanol - concd. ammonium hydroxide - water (7 : 1 : 2).

In conclusion, it is noted that the present oxidation-reduction condensation produces active phosphorylating reagents such as imidazolylphosphonate, 2-pyridyl ester of phosphoric acid and phosphoromorpholidate in high yields in a short reaction time by simply mixing triphenylphosphine, 2,2'-dipyridyl disulfide and phosphate with imidazole, 2-hydroxypyridine or morpholine, respectively.

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