[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL AND THE DEPARTMENT OF BIOCHEMISTRY, NEW YORK UNIVERSITY COLLEGE OF MEDICINE]

Nucleoside Polyphosphates. VII.[1] The Use of Phosphoramidic Acids in the Synthesis of Nucleoside-5′ Pyrophosphates

BY ROBERT WARNER CHAMBERS[2] AND H. G. KHORANA[3]

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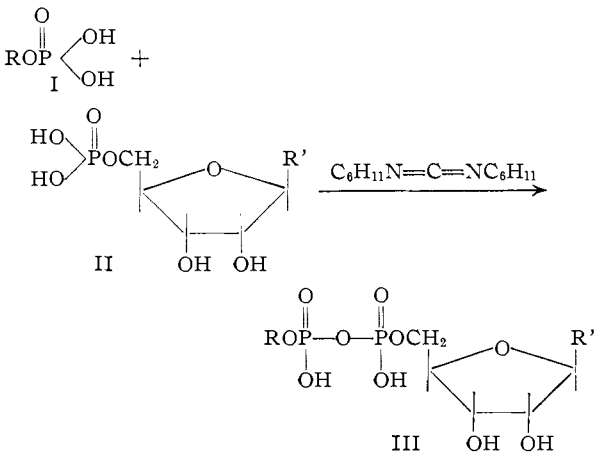
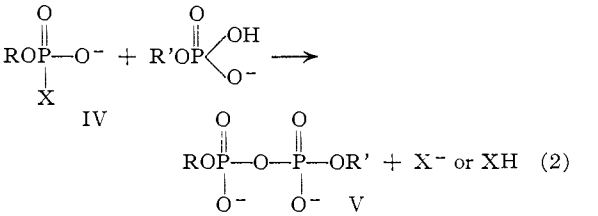
The use of phosphoramidic acids in the chemical synthesis of pyrophosphate bonds has been investigated. The reaction of adenosine-5′ phosphate with an excess of phosphoramidic acid gave a mixture of adenosine-5′ di-, tri- and higher phosphates. In experiments directed toward the specific synthesis of nucleoside-5′ diphosphates and nucleotide coenzymes, adenosine-5′ phosphoramidate was synthesized by the phosphorylation of 2′,3′-di-*O*-acetyladenosine with monophenyl phosphorodichloridate followed by treatment of the resulting nucleoside-5′ monophenylphosphorochloridate with an excess of ammonia and subsequent alkaline removal of the acetyl and phenyl groups. A satisfactory synthesis of adenosine-5′ diphosphate by the reaction of adenosine-5′ phosphoramidate with an excess of phosphoric acid is described.

Of the methods developed for the synthesis of nucleoside pyrophosphates[4-6] and related compounds, that involving the use of carbodiimides[6] has proved to be the most generally useful. In this method, no protecting groups are required and the desired products (general formula III) may be obtained directly after a one-step condensation reaction (eq. 1). However, the lack of specificity in effecting condensation between two dissimilar components is a serious drawback in the method and often leads to complex mixtures of desired and side products. The formation of the desired products (III) can in many cases be promoted by using an excess of one of the components, and utilizing this principle satisfactory syntheses of ribo- and deoxyribonucleoside-5′ triphosphates[1] and certain nucleotide coenzymes[7,8] have been realized. Still, the problems of the isolation of the desired products from large amounts of side products (*e.g.*, inorganic polyphosphates in nucleoside pyrophosphate syntheses) are unavoidable.



R = H or sugar residue, etc.; R′ = purine or pyrimidine

We have therefore devoted attention to devising an alternative method which, while retaining the desirable features of the procedure discussed above, would bring about the specific synthesis of unsymmetrical pyrophosphates. The underlying aim in this research has been to convert one of the components (*e.g.*, I) to a derivative of the general type IV,[9] which now contains a highly reactive P → X linkage, with the phosphorus being rendered electrophilic, and then to bring about pyrophosphate V synthesis, in a second step, through anionic attack (*phosphorolysis*) on IV by phosphoric acid or another phosphomonoester (eq. 2). Necessary attributes of an intermediate such as IV were therefore considered to be that it should be stable under a given set of conditions, so as to enable its isolation and storage, and yet it should be reactive enough to form a pyrophosphate bond under relatively mild conditions.



Phosphoramidic acid and its esters (IV, X = NH_2) appeared to meet the above criteria. Thus, the high energy character of the P → N bond in such compounds is well-known[10] and the ability of biological systems to utilize the phosphoramidates such as creatine phosphate for the synthesis of pyrophosphate bonds was demonstrated many years ago by Lohmann.[11] In addition, many of the phosphoramidates such as phosphoramidic acid are stable under neutral or alkaline conditions, but extremely reactive in acid.[12] The experiments

(1) Paper VI, M. Smith and H. G. Khorana, THIS JOURNAL, **80**, 1141 (1958).

(2) A part of this work was carried out during the tenure of a Life Insurance Medical Research Post-doctoral Fellowship held by R. W. C. (1954–1956). The work at New York University was aided by grants from the National Cancer Institute (Grant C-2784) of the National Institutes of Health, U. S. Public Health Service and the Rockefeller Foundation.

(3) Financial support from the National Research Council of Canada, Ottawa, is acknowledged.

(4) J. Baddiley and A. R. Todd, *J. Chem. Soc.*, 648 (1947).

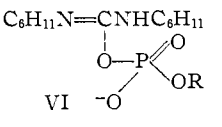
(5) B. H. Chase, G. W. Kenner, A. R. Todd and R. F. Webb, *ibid.*, 1371 (1956).

(6) H. G. Khorana, THIS JOURNAL, **76**, 3517 (1954).

(7) E. P. Kennedy, *J. Biol. Chem.*, **222**, 185 (1956).

(8) N. A. Hughes, G. W. Kenner and A. R. Todd, *J. Chem. Soc.*, 3733 (1957).

(9) In the synthesis of pyrophosphates using dicyclohexylcarbodiimide, an adduct, VI or its protonated form, must be an intermediate, H. G. Khorana, *Chem. Revs.*, **53**, 145 (1953). Such an intermediate, if isolated, could be used in the specific synthesis of unsymmetrical pyrophosphates. Attempts to prepare compounds of the type VI have, however, been unsuccessful so far.



(10) For a review, see P. Oesper in "Phosphorus Metabolism," Vol. I, The John Hopkins Press, Baltimore, Md., 1951, p. 523.

(11) K. Lohmann, *Biochem. Z.*, **271**, 264 (1934).

(12) See *e.g.*, O. Meyerhof and K. Lohmann, *ibid.*, **196**, 22 (1928).

now described have demonstrated the usefulness of these derivatives in the specific chemical synthesis of nucleoside-5′ pyrophosphates and related compounds.

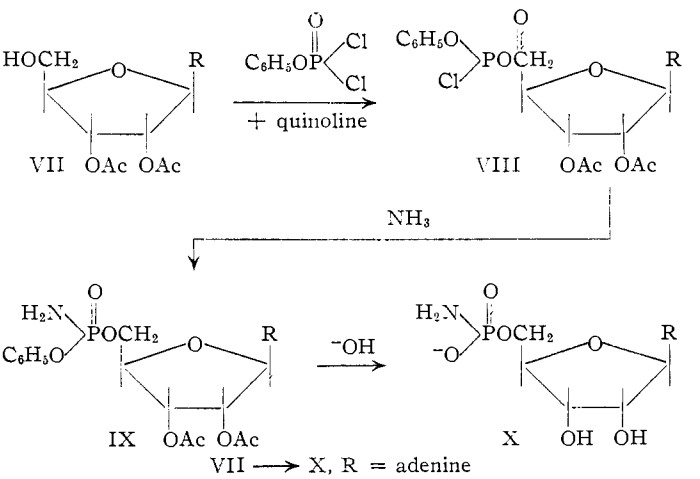
Phosphoramidic acid, a readily available substance,[13] was selected for our initial experiments. Its monotriethylammonium salt was allowed to react with 85% phosphoric acid in dimethylformamide. Paper chromatography indicated the formation of pyrophosphoric acid as well as of some higher polyphosphates, the latter probably arising from the anionic attack of the initially formed pyrophosphoric acid on unreacted phosphoramidic acid.

In order to demonstrate that a similar reaction occurred with phosphomonoesters such as mononucleotides, the reaction between AMP[14] and an excess of phosphoramidic acid at room temperature was investigated. The products after three days were found, by ion exchange analysis, to be: AMP, 34%; ADP, 27%; ATP, 21%; higher nucleoside polyphosphates, 17%.

The above results were encouraging in that they established the feasibility of using phosphoramidates for the formation of unsymmetrical nucleoside pyrophosphates under mild conditions. However, this particular approach using nucleotide anions offered no practical advantage over the carbodiimide method[6] since the specific formation of any one of the nucleoside polyphosphates could hardly be expected. The alternative approach, namely, the activation of nucleotides by conversion to amidate derivatives, appeared to be much more attractive. For example, in the synthesis of nucleoside diphosphates, only the anions of phosphoric acid would be required and the formation of inorganic polyphosphates would be avoided. Further, the only possible side reaction would be the condensation of the nucleoside phosphoramidate with itself to form a symmetrical dinucleoside pyrophosphate[15] and it seemed likely that under suitable conditions this reaction could be minimized.

Since the nucleoside-5′ phosphoramidates were unknown, an unequivocal synthesis utilizing appropriately blocked intermediates was undertaken. The protective groups used were such that they could be removed by alkaline treatment, conditions under which the nucleoside-5′ phosphoramidates themselves were expected to be stable. Accordingly, 2′,3′-di-*O*-acetyladenosine[16] (VII) was phosphorylated with phenylphosphorodichloridate[17] in the presence of one mole of quinoline and the intermediate, presumably 2′,3′-di-*O*-acetyladenosine-5′ phenylphosphorochloridate (VIII), was treated directly with dry ammonia in dioxane. The product of this reaction (presumably IX) was treated with lithium hydroxide at room temperature to remove the acetyl and phenyl groups, and adenosine-5′ phosphoramidate (X) finally was isolated as its chromatographically pure cyclohexylammonium salt in 25% yield based on diacetyladenosine.

The reaction between adenosine-5′ phosphoramidate and 85% phosphoric acid was then studied under a variety of conditions. The most satisfactory procedure devised so far employs *o*-chlorophenol as the solvent and an excess (5 equiv.) of phosphoric acid. When this reaction is carried out at 0° for about three hours, ADP can be isolated on a preparative scale in about 50% yield based on the amidate. It should be emphasized that in this synthesis no side reactions were observed other than some hydrolysis of AMP-NH_2 to AMP, caused undoubtedly by the presence of water in the phosphoric acid used. Thus, AMP, ADP and unreacted phosphoric acid were the only products present and because of the simplicity of this mixture, ADP could be isolated readily without any significant losses.



The main obstacle in the practical application of the present approach to the synthesis of unsymmetrical pyrophosphates obviously lay in the relative inaccessibility of the nucleoside-5′ phosphoramidates. However, the work reported separately[18] has provided a simple one-step synthesis of these intermediates in high yield. The extension of the present work to the synthesis of nucleotide coenzymes is reported in the following paper.[15]

An abbreviated account of this work appeared in 1956[19] and subsequently Clark, *et al.*,[20] described the use of monobenzylphosphoramidate for the synthesis of ADP and ATP.

Experimental

Methods.—Paper chromatography was performed using descending technique and the following solvent systems: (1) isopropyl alcohol (75 ml.)–water (25 ml.)–trichloroacetic acid (5 g.)–ammonia (0.25 ml., sp. gr., 0.9)[21] (solvent A); (2) isopropyl alcohol–ammonia–water, 7–1–2 (v./v.)[22]

(13) R. Klement and K. H. Becht, *Z. anorg. Chem.*, **254**, 217 (1947).

(14) Abbreviations used are: AMP, adenosine-5′ phosphate; ADP, adenosine-5′ pyrophosphate; ATP, adenosine-5′ triphosphate.

(15) For a fuller discussion of the formation of this side product see the accompanying paper, J. G. Moffatt and H. G. Khorana, THIS JOURNAL, **80**, 3756 (1958).

(16) H. Bredereck, E. Berger and J. Ehrenberg, *Ber.*, **73**, 269 (1940).

(17) E. Baer and H. C. Stancer, THIS JOURNAL, **75**, 4510 (1953).

(18) R. W. Chambers and J. G. Moffatt, *ibid.*, **80**, 3752 (1958).

(19) R. W. Chambers and H. G. Khorana, *Chemistry & Industry*, 1022 (1956).

(20) V. M. Clark, G. W. Kirby and A. R. Todd, *J. Chem. Soc.*, 1497 (1957).

(21) J. P. Ebel, *Bull. soc. chim. (France)*, 991 (1953).

(22) R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952); D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 2040 (1953).

(solvent B); (3) 1% ammonium sulfate–isopropyl alcohol, 1–2 (v./v.)[23] (solvent C).

Paper electrophoresis was carried out as previously described.[24]

Preparation of Phosphoramidic Acid. Method A.—Dibenzyl phosphoramidate was prepared by the method of Atherton, *et al.*[25] It (1.385 g., 5 mmoles) was dissolved in 10 ml. of anhydrous methanol and hydrogenated in the presence of 0.71 ml. (5.1 mmole) of triethylamine and 100 mg. of palladium–charcoal (5% palladium on acid-washed Norite A). After the theoretical uptake of hydrogen (40 minutes), the catalyst was removed by filtration and washed with methanol. The filtrate and washings were concentrated to a white powder and dried over phosphorus pentoxide *in vacuo* at room temperature; 834 mg. (84% for monotriethylammonium salt).

Method B.—The method of Klement and Becht[13] was modified somewhat: Diphenyl phosphorochloridate[26] (168 ml.) was added dropwise (about 2 hr.) to a rapidly stirred ice-cold solution of ethanol (300 ml. of absolute) previously saturated at 0° with ammonia gas. After the addition was complete the reaction mixture had become slightly acidic and it was immediately resaturated with ammonia. Addition of an excess of ice-cold water gave the crystalline diphenyl phosphoramidate which was collected by filtration and then dried over phosphorus pentoxide *in vacuo* at room temperature; yield 176 g. (89%), m.p. 141–148°. Recrystallization from 95% ethanol (700 ml.) gave the amidate (158 g.) with m.p. 148.7–149.3°, reported[13] m.p. 148°.

The diphenyl phosphoramidate was converted to potassium monohydrogen phosphoramidate as described by Klement and Becht.[13] This material did not give a test for inorganic phosphate with silver nitrate nor any test for ammonia with Nessler reagent. After heating a small sample for a few minutes in 0.1 *N* hydrochloric acid, the Nessler test was positive. Titration with standard acid gave pK_a' 8.16, reported[27] 8.2; neut. equiv. calcd. for $KHPO_3NH_2$ 135, found 134.

Formation of Pyrophosphoric Acid from Phosphoramidic Acid.—A small sample of the monotriethylammonium phosphoramidate was suspended in a little freshly distilled dimethylformamide and dry hydrogen chloride gas was passed into the mixture for about 1 min. Most of the solid dissolved. After 24 hr. at room temperature, an aliquot was removed from the reaction mixture and examined by paper chromatography in solvent A. Spots corresponding to ortho- and pyrophosphoric acid were detected as well as some other unidentified slower travelling phosphorus containing material.

In a similar experiment, 1 drop of 85% orthophosphoric acid was added instead of the hydrogen chloride. The chromatographic results were similar to those described above.

Reaction of Adenosine-5′ Dihydrogen Phosphate and Phosphoramidic Acid. Experiment I.—Adenosine-5′ dihydrogen phosphate (70 mg., 0.2 mmole) and phosphoramidic acid (dihydrogen form prepared according to Klement and Becht,[13] 460 mg., 4 mmoles) were dissolved in 7 ml. of freshly distilled formamide containing 4 mmoles of hydrogen chloride. Monopotassium phosphoramidate (about 50 mg. each) was added after 30 and 60 hr. After 72 hr., the nucleotides were precipitated with acetone, removed by centrifugation and washed with acetone followed by ether. The product was dried briefly *in vacuo* at room temperature and then dissolved in water. An aliquot containing about 150 optical density units (at 260 mμ) was removed and adjusted to *p*H 8.5 with ammonium hydroxide. The mixture was analyzed by ion exchange chromatography on a 3 cm. long × 1 cm. diameter column of Dowex-2 resin (chloride form) using a linear gradient elution technique similar to that described by Paar.[28] The mixing vessel contained 500 ml. of 0.003 *N* HCl and the reservoir an equal volume of 0.003 *N* HCl + 0.1 *M* NaCl. The peak fractions, as determined by their optical density at 260 mμ, were pooled. The percentages of the various products on the basis of total optical density recovered were: AMP, 34; ADP, 27; ATP, 21; and higher polyphosphates 18%.

Experiment II.—Triethylammonium phosphoramidic acid was prepared by passing an aqueous solution of monopotassium hydrogen phosphoramidate through a column of Dowex-50–triethylammonium resin and evaporating the total effluent and washings under reduced pressure. Crystallization from aqueous acetone gave the product as needles (hygroscopic). A solution of this material (19.8 mg., 0.1 mmole) and AMP (17.5 mg., 0.05 mmole) in freshly distilled formamide (3 ml.) was kept at room temperature. Aliquots were removed at intervals, examined by paper electrophoresis at *p*H 4.4 and the products analyzed after elution from paper by their optical density at 260 mμ; ADP was the only new product and its yield was 13% after 2.5 hours, 24% after 16 hours and remained constant thereafter up to 72 hours.

Preparation of Adenosine-5′ Phosphoramidate.—2′,3′-Di-*O*-acetyladenosine (351 mg., 1 mmole) was dissolved in 3 ml. of anhydrous dioxane and 0.118 ml. (1 mmole) of purified quinoline[29] was added. This solution was added dropwise over a 30-min. period to a stirred solution of phenylphosphorodichloridate[17] (231 mg., 1.1 mmoles) in dioxane (2 ml.). The precipitate which formed after about 5 minutes redissolved to give a clear solution after about 1.5 hr. After 2 hr. at room temperature the solution became cloudy and after 4 hr. an oil had separated. The entire reaction mixture was cooled in an ice-bath and dry ammonia gas[30] was passed into the solution for 5 minutes. The ammoniacal solution was allowed to stand at room temperature for 20 min. and then the fine white precipitate of ammonium chloride was removed by centrifugation. The solid was washed thoroughly with dioxane and the combined supernatant and washings were concentrated to a thick sirup. Hydrolysis of a trace of this oil in a mixture of acetone and 1 *N* lithium hydroxide followed by chromatography in solvent B and C revealed a spot corresponding to adenosine and another spot having the expected mobility of the amidate X. The total oil was triturated with dry ether to give a powder which was collected by centrifugation and washed 3 times with dry ether. The yield was 480 mg., theoretical for 2′,3′-di-*O*-acetyladenosine-5′ phenylphosphoramidate is 506 mg.

A portion (226 mg.) of the crude product was dissolved in 1 ml. of dioxane and 1.5 ml. of 1 *N* lithium hydroxide was added. After 1 hr. at room temperature, the *p*H was adjusted to 6.8 with glacial acetic acid and the solution evaporated under reduced pressure. The residue was dissolved in water and the *p*H of the solution readjusted to 6.8. Phenol was removed by five extractions with chloroform and the aqueous layer evaporated under reduced pressure. Last traces of water were removed by re-evaporation after the addition of absolute ethanol and the residual white powder was triturated with methanol 3 times to remove the lithium acetate. Finally, the product was washed with acetone and then ether; 72 mg. Paper chromatography in solvent B indicated that the product consisted mainly of the amidate X although a small amount of adenosine also was detected. This powder was dissolved in 0.2 ml. of water and brought to turbidity with acetone. A small amount of solid was removed by centrifugation and the product was precipitated by addition of more acetone. Centrifugation gave 50 mg. of the lithium salt of X, which now contained only a trace of impurity. It was converted to the cyclohexylammonium salt as follows: the solid was dissolved in 5 ml. of water and passed slowly through a Dowex-50–cyclohexylammonium ion exchange column (2.5 cm. long × 1 cm. diameter). The column was washed with 5 ml. of water and the effluent was concentrated to a sirup under reduced pressure. Re-evaporation after addition of ethyl alcohol gave a white powder; 50 mg. (78% recovery based on the lithium salt). This material was purified for analysis by dissolving it in 1 ml. of methanol and removing a small amount of insoluble material by centrifugation. Addition of anhydrous ether gave an amorphous solid (39 mg.) which was chromatographically homogeneous in solvent B, R_f 0.24.

(23) N. Anand, V. M. Clark, R. H. Hall and A. R. Todd, *J. Chem. Soc.*, 3665 (1950).

(24) R. W. Chambers, J. G. Moffatt and H. G. Khorana, THIS JOURNAL, **79**, 3747 (1957).

(25) F. R. Atherton, H. T. Openshaw and A. R. Todd, *J. Chem. Soc.*, 382 (1945).

(26) E. Baer, *Biochem. Preps.*, **1**, 50 (1949).

(27) O. Meyerhof and K. Lohmann, *Biochem. Z.*, **196**, 49 (1928).

(28) C. W. Paar, *Biochem. J.*, **56**, xxvii (1954).

(29) Commercial synthetic quinoline was fractionated through a 24-inch Podbielniak column.

(30) Ammonia gas was dried by passing it through a barium oxide tube.

Anal. Calcd. for $C_{16}H_{28}N_7O_6P$ (445.40): P, 6.95; adenine (Ad)/P, 1.00. Found: P, 6.96; Ad/P, 1.00 (adenine was determined spectrophotometrically).

Preparation of Adenosine-5' Diphosphate.—1,3-Dicyclohexylguanidinium adenosine-5' phosphoramidate was prepared from 1.05 g. of adenosine-5' dihydrogen phosphate (monohydrate) using the conditions described by Chambers and Moffatt[18]; the yield was 1.38 g. (89% based on ultraviolet measurements). This material was chromatographically identical with the material prepared by the method described above.

The amidate was dissolved in 5 ml. of twice-distilled *o*-chlorophenol contained in a 15-ml. pear-shaped flask and the solution cooled in an ice-bath. Orthophosphoric acid (2.7 ml. of 85%) was added and the two-phase mixture stirred vigorously. After 3.5 hours 10 ml. of chloroform was added and the oil which was deposited was triturated with acetone to give a gum, which was dissolved in 20 ml. of 1 *N* ammonium hydroxide and made up to 25 ml. with water. The *p*H was adjusted to 8 with ammonia and the solution (TOD[31] 33,000) applied to the top of a 7 cm. long × 4 cm. diameter column of Dowex-1 (chloride form) resin at the rate of about 2 ml./min.; AMP and inorganic phosphate were eluted with 0.003 *N* hydrochloric acid + 0.03 *M* lithium chloride at a flow rate of about 8 ml./min. The total effluent was 3 liters and contained 36% of the total optical density applied to the column; ADP was then eluted with 0.003 *N* hydrochloric acid +0.05 *M* lithium chloride (3.25 liters, TOD[31] 21,000).

The ADP fraction was neutralized with lithium hydroxide and concentrated to 25 ml. under reduced pressure (bath temperature below 37°) and the gelatinous mixture was transferred to a 40-ml. centrifuge tube with the aid of three 2-ml. portions of water. The suspension was centrifuged and the solid was washed with 5, 2 and then 1 ml. of water. Barium acetate (3 ml. of 2 *M*) was added to the combined supernatant and washings (TOD, 19,900) and the mixture kept at 0° overnight in a stoppered tube. The precipitate was then collected by centrifugation and washed (2 × 5 ml.) with 50% ethanol and then once each with 5 ml. of 95% ethanol, acetone and ether. The solid was dried at room temperature over phosphorus pentoxide; 690 mg. (TOD 12,500). An equal volume of 95% ethanol was added to the mother liquor (TOD 7,030) from the first precipitation and the precipitate collected in the usual manner except for two additional cold water washes (1 ml. and then 0.5 ml.) at the beginning. The dry solid weighed 347 mg. (TOD 6,280). The total recovery was 18,780 O.D. units (89%); yield 49% based on AMP. The first crop was electrophoretically homogeneous while the second crop contained a trace of AMP.

The main crop was dissolved in 4 ml. of ice-cold water by addition of 1 ml. of cold 2 *N* hydrochloric acid. A small amount of insoluble solid was removed by centrifugation in the cold and washed with 1 ml. of cold water containing 5 drops of 2 *N* hydrochloric acid. The *p*H of the combined supernatant and wash was 1.6. Cold 95% ethanol (5 ml.) was added and the precipitate was collected in the usual manner; 413 mg. (TOD 10,800, 86% recovery). An additional 25 mg. of material (TOD 609) was recovered from the supernatant by addition of 5 ml. of 95% ethanol. The total recovery was 438 mg., TOD 11,409 (91%). Electrophoresis of these two solids indicated the presence of trace amounts of AMP and material having the same mobility as ATP. The material was very hygroscopic and unstable. On standing overnight at room temperature significant conversion to AMP and ATP (identified by electrophoresis) occurred.

Anal. Calcd. for $BaHADP{\cdot}2H_2O$: mol. wt., 600; $Ba^1/_2H_2ADP{\cdot}6H_2O$, mol. wt., 604; Ad:labile P:total P, 1.0:1.0:2.0. Found: equiv. wt., 597 (ultraviolet measurements); Ad:labile P:total P, 1.0:1.0:1.9.

This barium salt was converted to the sodium salt by dissolving 100 mg. in 5 ml. of water and passing the solution through a 0.8 × 4 cm. long column of Dowex-50 (Na^+) and washing the column with water until the optical density dropped to 1.5. The effluent was lyophilized to a fluffy, white, extremely hygroscopic powder, 69 mg.

Anal. Calcd. for $Na_2HADP.3H_2O$: mol. wt., 525; Ad:labile P: total P, 1.0:1.0:2.0. Found: equiv. wt., 530; Ad:labile P: total P, 1.0:0.95:2.0.

These data do not rule out $NaH_2ADP3{\cdot}4H_2O$ as the correct formula. Electrophoresis indicated a trace of ATP and a small amount of AMP (less than 10%). Biological assay using phosphoenol pyruvate kinase and lactic dehydrogenase and measuring the disappearance of reduced diphosphopyridine nucleotide indicated 107% ADP.[32] The high value is attributed to the presence of AMP in the ADP and myokinase in the enzyme system. This synthetic ADP rapidly formed AMP polymer with polynucleotide phosphorylase.[32,33]

(31) This refers to total optical density units as measured at 260 mμ at *p*H 2. FOOTNOTE ADDED IN PROOF.—An alternative more satisfactory procedure for isolation of ADP was developed: The ADP fraction is neutralized with lithium hydroxide, concentrated to a small volume and dried to a powder *in vacuo* over phosphorus pentoxide. The dry solid is triturated with absolute methanol, filtered and washed with methanol until the filtrate is chloride negative. The recovery of lithium ADP is almost quantitative and the product is electrophoretically pure.

(32) This assay was kindly performed by Dr. Sana Mii.

(33) M. Grunberg-Manago and S. Ochoa, *Biochem. Biophys. Acta*, **20**, 269 (1956).

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The Synthesis of Adenosine-5' and Uridine-5' Phosphoramidates

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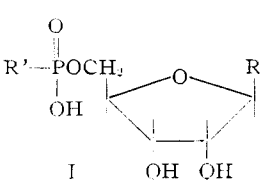
A simple, one-step synthesis of adenosine-5' and uridine-5' phosphoramidates utilizing a novel reaction between the appropriate 5'-nucleotide, ammonia and dicyclohexylcarbodiimide is described. 1,3-Dicyclohexylguanidine was formed simultaneously and hence the amidates were isolated as the crystalline salts of this base. Preparations of adenosine-5' methyl phosphate and a compound tentatively identified as adenosine-5' phosphoroimidazole are reported and some properties of these compounds are discussed.

The nucleoside phosphoramidates (I, R = purine or pyrimidine, R' = NH_2) are endowed with certain properties which make them ideally suited for the synthesis of unsymmetrical nucleoside pyro-



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