UDC 547.964 : 547.857.7'456'118.5

36. Kuniyoshi Tanaka, Mikio Honjo, Yasushi Sanno, and Hiroki Moriyama: A New Synthetic Method for Adenosine 5'-Triphosphate and Other Nucleoside 5'-Triphosphates.*1

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There have so far been reported various methods[1~7] for the chemical synthesis of ATP.*3 Among them, the method of Khorana, *et al.*,[6,7] which consists of allowing *o*-phosphoric acid and DCC to act on AMP, seemed most advantageous, but duplication of the method showed that it requires a large amount of DCC and produces fairly large amounts of ADP and DAPP as by-products. Therefore, as a new method for the preparation of ATP, attempt was made for condensation of AMP-NH_2,[8] which is an important intermediate in the synthesis of various nucleotide coenzymes and ADP, with pyrophosphoric acid under suitable conditions, and the method was found to be usable.

In the first place, a solution of the 1,3-dicyclohexylguanidinium salt of AMP-NH_2 (I) *o*-chlorophenol was allowed to react with an aqueous solution of pyrophosphoric acid,

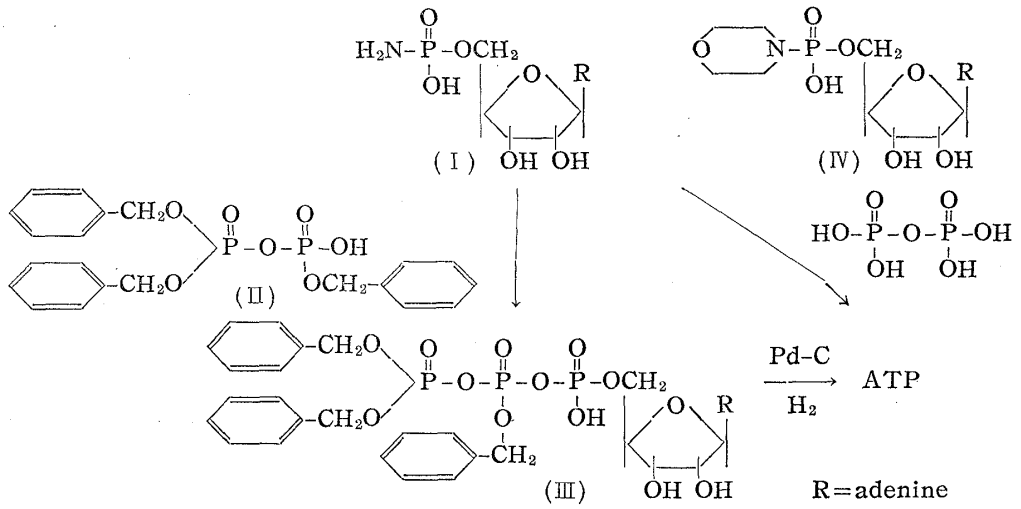

Chart 1.

*1 Published briefly as a Communication to the Editor in this Bulletin, 8, 750 (1960). While this full paper was prepared for publication, a similar method was reported by J. G. Moffatt and H. G. Khorana (J. Am. Chem. Soc., 83, 649 (1961)).

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*3 Abbreviations used : AMP, adenosine 5'-phosphate; AMP-NH_2, adenosine 5'-phosphoramidate; AMP-$\overset{\frown}{\underset{\smile}{N\ O}}$, adenosine 5'-phosphoromorpholidate; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; CMP-NH_2, cytidine 5'-phosphoramidate; CTP, cytidine 5'-triphosphate; DAPP, P^1,P^2-diadenosine 5'-pyrophosphate; DCC, dicyclohexylcarbodiimide; UMP, uridine 5'-phosphate; UMP-NH_2, uridine 5'-phosphoramidate; UDP, uridine 5'-diphosphate; UTP, uridine 5'-triphosphate; dAMP, deoxyadenosine 5'-phosphate; dAMP-NH_2, deoxyadenosine 5'-phosphoramidate; dATP, deoxyadenosine 5'-triphosphate.

1) J. Baddiley, A. M. Michelson, A. R. Todd : J. Chem. Soc., **1949**, 582.
2) A. M. Michelson, A. R. Todd : *Ibid.*, **1949**, 2487.
3) B. H. Chase, G. W. Kenner : *Ibid.*, **1956**, 1371.
4) V. M. Klark, G. W. Kirby, A. R. Todd : *Ibid.*, **1957**, 1497.
5) H. G. Khorana : J. Am. Chem. Soc., **76**, 3517 (1954).
6) M. Smith, H. G. Khorana : *Ibid.*, **80**, 1141 (1958).
7) R. W. Chambers, H. G. Khorana : *Ibid.*, **80**, 3749 (1958).
8) R. W. Chambers, J. G. Moffatt : J. Am. Chem. Soc., **80**, 3752 (1958).

with stirring and cooling with ice-water, but paper partition chromatography*[4] of the reaction mixture scarcely showed the formation of ATP. The cause of the failure seemed to be that the pyrophosphoric acid used was impure and the solution of the reactants did not become homogeneous because pyrophosphoric acid is sparingly soluble in *o*-chlorophenol. Therefore, tribenzyl pyrophosphate[9] (II) was selected as the derivative of pyrophosphoric acid soluble in *o*-chlorophenol and its solution in *o*-chlorophenol was mixed with a solution of the dicyclohexylguanidinium salt of (I) in the same solvent, with stirring and cooling. The mixture was left standing at 5° for 2 days. The reaction product (III) was heated at 60° for 15 minutes to effect partial debenzylation and, after addition of water, shaken with ether to eliminate *o*-chlorophenol. The aqueous solution was subjected to reductive debenzylation on palladium-charcoal, the resulting nucleotides were adsorbed on charcoal, eluted with 50% ethanol containing ammonia, the eluate was concentrated *in vacuo*, and submitted to paper electrophoresis,*[5] detecting the ultraviolet absorbing spot of ATP besides those of AMP and ADP. The concentrated eluate, when chromatographed[10] on Amberlite CG-400 (Cl-form), exhibited the absorption of ATP at 260 mμ, which was 12.2% of total optical density in molar ratio. Since Khorana, *et al.*[11] reported that the activity of AMP-N⌒O (IV) was stronger than that of (I) in their synthesis of coenzyme A, the 4-morpholine N,N′-dicyclohexylcarboxamidinium salt of (IV) was condensed with (II) in *o*-chlorophenol and the formation of ATP was confirmed by paper electrophoresis after treating the reaction product (III) as above, but the yield was not improved. As other derivatives of pyrophosphoric acid, organic amine salts of the acid, which might be soluble in the solvent used, was considered and triethylammonium pyrophosphate (V) was prepared from tetrasodium pyrophosphate through barium pyrophosphate. (V) was also prepared by mixing an aqueous solution of tetrasodium pyrophosphate, which had been treated with Amberlite IR-120 (H-form), with a little more than 2 equivalent amount of triethylamine and evaporating the mixture to dryness in a reduced pressure. The product gave in paper electrophoresis a single colored spot[12] at the site corresponding to pyrophosphate and its analytical and pyrophosphate values[13] were in accord with those of bistriethylammonium pyrophosphate (V). When the reaction was effected with 4 equivalent amount of triethylamine, part of the triethylamine escaped during the evaporation of the reaction mixture and neither tris- nor tetrakis (triethylammonium pyrophosphate but (V) was isolated.

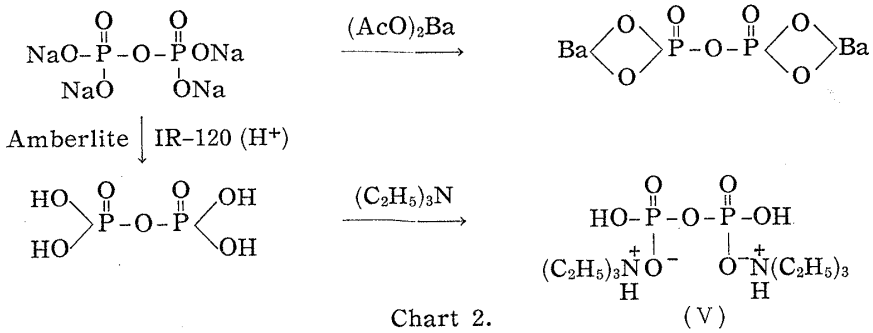

Chart 2. (V)

*[4] Solvent system : Isobutyric acid-water-acetic acid (100:50:1) of F. Turba, H. Pelzer, H. Schuster: Z. physiol. Chem., **296**, 97 (1954).

*[5] This is a modification of the method published by F. Turba, *et al.* (Z. physiol. Chem., **296**, 97 (1954)), details of which will be reported later.

9) L. Zervas, I. Dalaris : Chem. Ber., **89**, 925 (1956).
10) W. E. Cohn, C. E. Carter : J. Am Chem. Soc., **72**, 4273 (1950).
11) J. G. Moffatt, H. G. Khorana : *Ibid.*, **81**, 1265 (1959).
12) C. S. Hanes, F. A. Isherwood : Nature, **164**, 1107 (1949).
13) R. N. Bell : Anal. Chem., **19**, 97 (1947).

The dicyclohexylguanidinium salt of (I) was reacted with (V) in a mixture of tricresol and acetonitrile at 20° for 48 hours, the reaction mixture was treated as usual, and part of the eluate from charcoal was analyzed on ion exchange resin, by which ATP (78%) (76% in enzymic assay),[14] ADP (10%), and AMP (12%) (in molar ratio) were detected by paper electrophoresis. The above eluate was concentrated and poured on a column of Amberlite CG-400 (Cl-form), and the ion exchange resin, after washing with water, was eluted successively with 0.01*N* hydrochloric acid + 0.02*M* sodium chloride, 0.01*N* hydrochloric acid + 0.04*M* sodium chloride and 0.01*N* hydrochloric acid + 0.2*M* sodium chloride, when the barium salt of ATP (in 43% overall yield from (I)) was obtained by treating the ATP fraction eluted with 0.01*N* hydrochloric acid + 0.2*M* sodium chloride in a usual manner (*Anal.* Calcd. for $C_{10}H_{12}O_{13}N_5Ba_2P_3 \cdot 6H_2O$: N, 7.9; P, 10.5. Found: N, 8.08; P, 10.57). To examine the purity of the barium salt, it was converted to the soluble tetrasodium salt with sodium sulfate and the product was subjected to paper electrophoresis and enzymic assay, from which the purity was found to be 94.6~95.2% by the former estimation and 94.2% by the latter.

The new method for the synthesis of ATP thus established was further applied to the syntheses of UTP, CTP, and dATP. Chambers, *et al.*[15] reported the melting point of the dicyclohexylguanidinium salt of CMP-NH_2 (VII) as 180~183° (decomp.), but the present constants were m.p. 198°, $[\alpha]_D^{20}$ +4° (H_2O). The dicyclohexylguanidinium salt of dAMP-NH_2 (VIII) was newly prepared from dAMP. Reaction of the dicyclohexylguanidinium salts of UMP-NH_2[8] (VI), (VII), and (VIII) with bistriethylammonium pyrophosphate (V) in a mixture of tricresol and acetonitrile, and treatment of the reaction mixture as in the case of ATP gave UTP, CTP, and dATP in a good yield. Although their formation was easily recognized from their behavior in paper electrophoresis, the formation of CTP and dATP was further confirmed by analysis of their bases, and total and labile phosphorus.

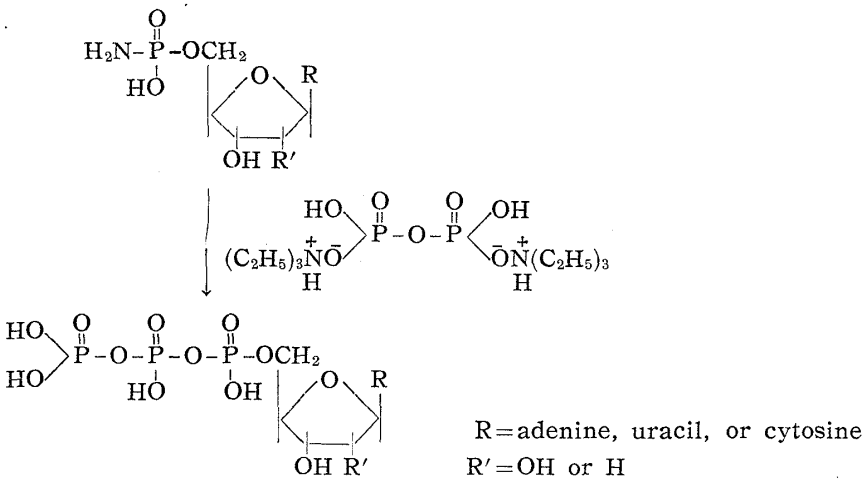

Chart 3.

Experimental

Synthesis of ATP by Condensation of 1,3-Dicyclohexylguanidinium Salt of Adenosine 5′-phosphoramidate (I) and Tribenzylpyrophosphate (II)——Solutions of 55 mg. of the dicyclohexylguanidinium salt of (I), dried at 100° for 3 hr. *in vacuo*, and 770 mg. of (II) dissolved in 0.3 cc. and 0.5 cc. of *o*-chlorophenol, respectively, were mixed with cooling and allowed to stand at about 5° for 2 days. The reaction mixture was heated at 60° for 15 min. to effect partial debenzylation and, after addition

14) W. J. Bowen, T. D. Kerwin : J. Biol. Chem., **220**, 9 (1956).
15) R. W. Chambers, P. Shapiro, V. Kurkov : J. Am. Chem. Soc., **82**, 970 (1960).

of 5 cc. of H_2O, it was shaken with five 5-cc. portions of Et_2O to remove *o*-chlorophenol. The Et_2O remaining in the aqueous solution was distilled off *in vacuo*, the aqueous solution was shaken in H_2 atmosphere at room temperature for 1.5 hr. in the presence of Pd-C, prepared from 1 cc. of 2% $PdCl_2$ and 0.2 g. of charcoal, and filtered to separate the catalyst (the filtrate scarcely exhibited absorption at 260 mμ). The catalyst was extracted with 50% EtOH containing 2% of conc. NH_4OH, the extract was concentrated *in vacuo* to remove EtOH and NH_3 (total optical density 576), and the residue exhibited ultraviolet-absorbing spots of AMP, ADP, and ATP in paper electrophoresis (0.1*M* acetate buffer of pH 4.15; 400 v./45 cm., 3.5 hr.). On the other hand, the residue was adsorbed on Amberlite CG-400 (Cl-form) and eluted first with 0.01*N* HCl+0.02*M* NaCl and then with 0.01*N* HCl+0.2*M* NaCl. Total optical density of the latter fraction was 70.3, showing that the residue contained 12% of ATP in molar ratio.

Bistriethylammonium Pyrophosphate (V)——(1) Aqueous solutions of 25 g. of Et_3N and 10 g. of pure H_2SO_4 were mixed with stirring and cooling, and the mixture was evaporated to dryness *in vacuo*, giving 22 g. of triethylammonium sulfate. To a suspension in H_2O of 16.4 g. of barium pyrophosphate ground finely in a mortar was added an aqueous solution of the above sulfate, the mixture was stirred for about 3 hr., and the resulting $BaSO_4$ was centrifuged and washed with H_2O. The supernatant was combined with the washing, evaporated to dryness *in vacuo*, and dried over P_2O_5. The yield was quantitative.

(2) A solution of 10.5 g. of $Na_4P_2O_7 \cdot 10H_2O$ dissolved in 200 cc. of H_2O was passed through (at a rate of the space velocity, 4) a column of 500 cc. of Amberlite IR-120 (H-form) and the column was washed with H_2O. The effluent having pH lower than 2 was collected, 13.2 cc. (4 equiv.) of Et_3N was added, and the mixture was evaporated *in vacuo* to dryness at a temperature below 40°. Paper electrophoresis (acetate buffer pH 4.2; 9 v./cm., 2 hr.) of the residue detected a spot corresponding to pyrophosphate (the molybdate-$HClO_4$ spray[12]). *Anal.* Calcd. for $H_4P_2O_7 \cdot 2C_6H_{15}N$: C, 37.92; H, 9.01; N, 7.37; P, 15.92; $H_4P_2O_7$, 46.84. Found : C, 37.91; H, 9.12; N, 6.29; P, 16.32; $H_4P_2O_7$, 46.71, 46.40.

Synthesis of ATP by Condensation of 1,3-Dicyclohexylguanidinium Salt of Adenosine 5′-Phosphoramidate (I) and Bistriethylammonium Pyrophosphate (V)——The dicyclohexylguanidinium salt containing one mole of H_2O of crystallization (23.3 mg.) was dried *in vacuo* at 90° for 3 hr. and dissolved in 0.5 cc. of tricresol. On the other hand, 0.27 g. of dry (V) was dissolved in a mixture of 0.6 cc. of tricresol and 0.25 cc. of acetonitrile. These solutions were combined and left standing in a closed vessel at 20° for 43 hr. with occasional shaking. The mixture was then diluted with 0.6 cc. of H_2O and shaken with three 3-cc. portions of $CHCl_3$. The aqueous solution was concentrated *in vacuo* to remove $CHCl_3$, stirred with 0.5 g. of charcoal for 30 min., and filtered, and the filtrate was again stirred with 0.1 g. of charcoal for 20 min. The combined charcoal was washed with a small amount of H_2O and eluted twice with 30 cc. and 20 cc. of 50% EtOH containing 2% of conc. NH_4OH. The eluate containing nucleotides was concentrated to 12 cc. *in vacuo* to eliminate EtOH and NH_3, and 0.5 cc. of the concentrate, after dilution to 50 cc. with H_2O, was subjected to measurement of its ultraviolet-spectrum. The optical density at 260 mμ was 0.38 and the total optical density recovery was 85%. The content of ATP in the eluate, as determined by enzymic assay, was 76%, and the content of the nucleotides, as determined by ion-exchange resin analysis[10] on Amberlite CG-400, was 78% of ATP, 10% of ADP, and 12% of AMP.

Purification of ATP by Ion-exchange Chromatography——The above reaction mixture, concentrated to 12 cc. after removal of NH_3 and EtOH, was poured on a column of 4 cc. of Amberlite CG-400 (Cl-form), the column was washed with a small amount of H_2O and treated first with 0.01*N* HCl+0.02*M* NaCl to elute AMP, AMP-NH_2, and ADP, then with 0.01*N* HCl+0.04*M* NaCl to elute a trace of ADP, and finally with 0.01*N* HCl+0.2*M* NaCl to elute ATP. All the procedures were carried out in a room maintained at 2°.

Isolation of ATP-Ba_2——ATP fraction (240 cc.) obtained as above (containing 48.67 mg. of free ATP as estimated from its total optical density, HCl and a large amount of NaCl) was neutralized with dil. NaOH solution and concentrated to 80 cc. *in vacuo* at a temperature below 30°. A mixture of 2 g. of charcoal added to this concentrate was stirred for 30 min. and filtered. The filtrate was treated again with 0.2 g. of charcoal as above, when the filtrate exhibited no ultraviolet-absorption. The combined charcoal was washed with a small amount of H_2O, eluted three times with 50% EtOH containing 2% of conc. NH_4OH (total 200 cc.), and the eluate was concentrated to about 20 cc. at a temperature below 25°. Paper electrophoresis of the concentrate showed a single ultraviolet-absorbing spot of ATP. The concentrate was further concentrated to 1.7 cc. under the same conditions as above and a cold solution of $(AcO)_2Ba$ (960 mg. of $(AcO)_2Ba \cdot H_2O$ in 5 cc. of H_2O) was added. The resulting precipitate was centrifuged, washed successively with H_2O, EtOH, and Me_2CO, and dried to give 56.0 mg. of a white crystalline powder. Paper electrophoresis of the product exhibited a single ultraviolet-absorbing spot. *Anal.* Calcd. for $C_{10}H_{12}O_{13}N_5Ba_2P_3 \cdot 6H_2O$: N, 7.9; P, 10.5. Found: N, 8.03; P, 10.57.

To a suspension of the product in H_2O an equivalent amount of Na_2SO_4 was added, the resulting

$BaSO_4$ was centrifuged, and the supernatant (a solution of ATP-Na_4) was submitted to determination of ATP, by which the purity was 95% by paper electrophoresis and 94% by enzymic assay. The overall yield of ATP-Ba_2 from the ATP fraction was 67%.

Synthesis of UTP by Condensation of 1,3-Dicyclohexylguanidinium Salt of Uridine 5'-Phosphoramidate (VI) and (V), and Isolation of the Product——After reaction of 108 mg. of the dicyclohexylguanidinium salt (purity, 60%) of (VI) with 770 mg. of (V), as in the case of ATP (20°, 50 hr.), the reaction mixture wsa processed as usual, poured on a column of Dowex-2 (Cl-form, 200~325 mesh), and the resin was treated with 0.01*N* HCl+0.015*M* NaCl to elute UMP, with 0.01*N* HCl+0.1 *M* NaCl to elute UDP(+DUPP), and then with 0.01*N* HCl+0.2 *M* NaCl to wash out UTP. The formation ratio of the nucleotides was UTP, 67.6%; UDP(+DUPP), 13.9%; UMP, 18.5%.

1,3-Dicyclohexylguanidinium Salt of Cytidine 5'-Phosphoramidate (VII)——A solution of 2 g. of pure CMP and 6.5 g. of DCC dissolved in a mixture of 15 cc. of 2*N* NH_4OH, 10 cc. of $HCONH_2$, and 40 cc. of *tert*-BuOH was heated ina closed tube at 80° for 11 hr. *tert*-BuOH was distilled off, the remaining liquid was shaken with Et_2O, and evaporated at about 40°. Me_2CO was added to the residue to give 3.5 g. of a crude product, 700 mg. of which was purified by recrystallization from 3.5 cc. of Me_2CO plus 5.5 cc. of MeOH to colorless needles, m.p. 198°(decomp.). *Anal.* Calcd. for $C_{22}H_{40}O_7N_7P$: C, 48.42; H, 7.39; N, 17.97; P, 5.68. Found: C, 48.27; H, 7.41; N, 17.01; P, 5.52.

Synthesis of CTP by Condensation of the Dicyclohexylguanidinium Salt of (VII) and (V)——A solution of 30 mg. of the dicyclohexylguanidinium salt of (VII) and 300 mg. of (V) dissolved in a mixture of 0.7 cc. of tricresol and 0.3 cc. of acetonitrile was kept at 20° for 43 hr. The reaction mixture was diluted with 7 cc. of H_2O and washed with 3 cc. of $CHCl_3$. $CHCl_3$ remaining in the aqueous layer was distilled off *in vacuo*. Paper electrophoresis (pH 5; acetate buffer; 11 v./cm., 2 hr.) of the residual solution indicated the formation of CTP and CNP in a ratio of 2:3.

Isolation of CTP-Na_4——The above reaction mixture was adsorbed on 1 g. of charcoal, the charcoal was washed with H_2O, and extracted twice with 30 cc. and 20 cc. of 50% MeOH containing 2% of conc. NH_4OH. MeOH and NH_3 in the extract were removed *in vacuo*, the remaining solution was diluted to 20 cc. with H_2O (pH 8.5), and adsorbed on 3 cc. of Amberlite CG-400 (Cl-form). The resin was washed with H_2O and treated first with 0.002*N* HCl, then with 0.01*N* HCl, and finally with 0.01*N* HCl+0.05*M* NaCl to elute CMP, CDP (or DCPP), and CTP, respectively.

The CTP fraction was adjusted to pH 7.5 with NaOH and adsorbed on 500 mg. of charcoal (the Butch method). The charcoal was washed with H_2O and eluted twice with 15 cc. of 50% MeOH containing 2% of conc. NH_4OH, and the eluate was evaporated to dryness *in vacuo* to give a white crystalline powder. Paper electrophoresis of the product confirmed that it is CTP-Na_4 (R_{CMP}*6≒1.93).

1,3-Dicyclohexylguanidinium Salt of Deoxyadenosine 5'-Phosphoramidate (VIII)——To a solution of 40 mg. of $(NH_4)_2SO_4$ dissolved in 8 cc. of H_2O 128 mg. of dAMP-Ba was added and the resulting $BaSO_4$ was centrifuged and washed with H_2O. The supernatant was combined with the washing, evaporated to dryness *in vacuo*, and the remaining dAMP-NH_4 was dissolved in a mixture of 0.75 cc. of 2*N* NH_4OH and 0.5 cc. of $HCONH_2$. To the solution a solution of 0.3 g. of DCC in 2 cc. of *tert*-BuOH was added and the mixture was heated in a closed vessel at 80° for 9 hr. with stirring with a magnetic stirrer. The resulting urea derivative was filtered, washed with a small amount of H_2O, the filtrate was combined with the washing, concentrated *in vacuo* to remove *tert*-BuOH from it. The concentrate was diluted with an appropriate amount of H_2O and shaken three times with the same volume of Et_2O. The aqueous layer, after concentration to less than 1 cc., was mixed with 6 cc. of Me_2CO, and the mixture was allowed to stand overnight with cooling, separating 129 mg. of a hygroscopic, pale yellow powder. Paper electrophoresis (pH 7.5; phosphate buffer; 11 v./cm., 3 hr.) and paper partition chromatography (iso-PrOH-H_2O-conc. NH_4OH=7:2:1, 20 hr. ascending method) of the product exhibited R_{dAMP}*6≒0.60 and 2.90, respectively.

Synthesis of dATP by Condensation of Dicyclohexylguanidinium Salt of (VIII) and (V), and Isolation of the Product——A solution of 43 mg. of the dicyclohexylguanidinium salt of (VIII) and 560 mg. of (V) dissolved in a mixture of 2.3 cc. of tricresol and 0.54 cc. of acetonitrile, with stirring, was left standing at room temperature for 48 hr., mixed with 12 cc. of H_2O, and extracted with three 8-cc. portions of $CHCl_3$. The aqueous layer was concentrated to remove $CHCl_3$ and the nucleotides in the solution were adsorbed first on 1 g. and then on 0.3 g. of charcoal. The combined charcoal was washed with H_2O, eluted with 50% EtOH containing 2% of conc. NH_4OH, and the eluate was concentrated *in vacuo* to eliminate NH_3 and EtOH. Paper electrophoresis (pH 4.2; acetate buffer) of the eluate gave an ultraviolet-absorbing spot at the site corresponding to ATP. The eluate (5 cc.) [total optical density 95 (pH 7), 90 (pH 2)] was adjusted to pH 8 with NH_4OH and poured on a column of 3 cc. of Dowex-1 (formate-form, X-8, 200~400 mesh). The column was washed with 75 cc. of H_2O and successively eluted with 0.1*M*, 0.4*M*, 0.8*M*, and 1.0*M* ammonium formate buffer (pH 3.5) whereupon dATP was washed out in the last fraction. This fraction was subjected to analysis to deter-

*6 Ratio of the migration distance of the sample divided by that of CMP.

mine deoxyribose from the standard curve (diphenylamine method), total phosphate (Allen method), labile phosphate (H_3PO_4 liberated on action of adenylpyrophosphatase prepared from potato was determined), and H_3PO_4; adenine : deoxyribose : total phosphate : labile phosphate : inorg. phosphate = 1 : .087 : 3.2 : 2.14 : 0 (Calcd. 1 : 1 : 3 : 2 : 0). Paper electrophoresis of the dATP fraction exhibited a single ultraviolet-absorbing spot.

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Summary

Reaction of the dicyclohexylguanidinium salt of adenosine 5′-phosphoramidate (I) with tribenzyl pyrophosphate (II) in *o*-chlorophenol, and reductive debenzylation of the reaction mixture resulted in the formation of adenosine 5′-triphosphate (ATP). The guanidinium salt was reacted with bis-triethylammonium pyrophosphate (V) in a mixture of tricresol and acetonitrile, and ATP was isolated as its barium salt from the reaction mixture by ion-exchange chromatography. The overall yield of ATP from (I) was 43%.

Reaction of the dicyclohexylguanidinium phosphoramidates of uridine, cytidine, and desoxyadenosine with (V) also gave uridine, cytidine, and desoxyadenosine 5′-triphosphates in a good yield.

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37. Mikio Honjo, Yoshiyasu Furukawa, Kin-ichi Imai, Hiroki Moriyama, and Kuniyoshi Tanaka : Synthesis of Uridine Diphosphate-Glucuronic Acid.*1

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Since UDPGA*3 was discovered[1~4] in the liver as a factor promoting the formation of conjugated glucuronic acid, it has been found by many workers that this substance is a biochemically important compound, which participates not only in detoxication as the active form of glucuronic acid but also in the synthesis of various polysaccharides. Up to now, UDPGA has been isolated in very small quantities from the liver of rabbits[4] and guinea pigs,[5] or from mung bean seedlings,[6] or prepared enzymically from UDPG and

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*1 Brief communication published in this Bulletin, 8, 750 (1960).

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*3 Abbreviations used : UDPGA, uridine diphosphate glucuronic acid; UDPG, uridine diphosphate glucose; DPN, diphosphopyridine nucleotide; UTP, uridine 5′-triphosphate; GA-1-P, glucuronic acid 1-phosphate; UMP-NH_2, uridine 5′-phosphoramidate; UMP, uridine 5′-phosphate; DUPP, P^1,P^2-diuridine 5′-pyrophosphate; UDP, uridine 5′-diphosphate; DCC, dicyclohexylcarbodiimide.

1) G. J. Dutton, I. D. E. Storey : Biochem. J., **48**, xxix (1951).
2) *Idem* : *Ibid.*, **53**, xxxvii (1953).
3) *Idem* : *Ibid.*, **57**, 275 (1954).
4) *Idem* : *Ibid.*, **57**, 279 (1954).
5) E. E. B. Smith, G. T. Mills : Biochim. et Biophys. Acta, **13**, 386 (1954).
6) J. Solms, W. Z. Hassid : J. Biol. Chem., **228**, 357 (1957).