**Communications to the Editor**

*UDC 577.1:547.857.7'456'118.5*

## A New Synthetic Method for Adenosine 5'-Triphosphate and Other Nucleoside 5'-Triphosphates

There have so far been reported many methods for the chemical synthesis of nucleoside 5'-triphosphates.[1~5] The writers, however, have succeeded in synthesizing the compounds by condensation of pyrophosphoric acid with nucleoside 5'-phosphoramidates, which have recently come to be recognized as important intermediates in the synthesis of nucleotide coenzymes as well as nucleoside 5'-diphosphates.

First, tribenzyl pyrophosphate[6] was left standing with AMP-$NH_2$[7]*1 (I) or AMP-N⟨O⟩ in *o*-chlorophenol at 20° for 2 days and then heated at 60° for 15 minutes, and the reaction mixture was subjected to reductive debenzylation over palladium-charcoal to give ATP. When determined by ion exchange chromatography,[8] the content of ATP in the resulting total nucleotides was only about 12% in molar ratio. On the other hand, a similar reaction between pyrophosphoric acid and (I) scarcely produced any ATP. Therefore, in order to react pyrophosphoric acid in the form of an organic amine salt soluble in the condensation solvent, tetrasodium pyrophosphate was converted into dibarium pyrophosphate, which was then submitted to double decomposition with triethylammonium sulfate to give bis-triethylammonium pyrophosphate (II). Then 22.6 mg. of the 1,3-dicyclohexylguanidinium salt of (I) dissolved in tricresol was allowed to react with 270 mg. of (II) dissolved in a mixture of tricresol and acetonitrile, and the reaction mixture was processed as usual. The product thus obtained, which contained 78% of ATP, 10% of ADP, and 12% of AMP in molar ratio, when determined by ion exchange chromatography, and 76% of ATP, when measured by enzymatic assay,[9]*2 was adsorbed on Amberlite CG-400 (chloride-form), and from the ATP-fraction eluted with 0.01*N* HCl+0.2*M* NaCl, ATP was isolated as its barium salt (overall yield from (I), 43%). To examine the purity of the barium salt, it was converted to the soluble tetrasodium salt and subjected to determination by electrophoresis*3 and enzymatic assay, giving values of 95% of ATP and 5% of ADP in the former and 94% of ATP in the latter (*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).

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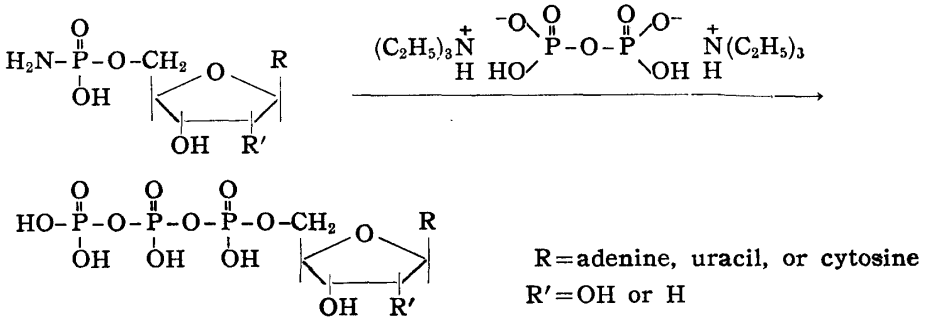
*1 Abbreviations used: AMP, adenosine 5'-phosphate; AMP-$NH_2$, adenosine 5'-phosphoramidate; AMP-N⟨O⟩, adenosine 5'-phosphoromorpholidate; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; CMP-$NH_2$, cytidine 5'-phosphoramidate; CTP, cytidine 5'-triphosphate; UMP-$NH_2$, uridine 5'-phosphoramidate; UTP, uridine 5'-triphosphate; dAMP-$NH_2$, deoxyadenosine 5'-phosphoramidate; dATP, deoxyadenosine 5'-triphosphate.

*2 The enzymatic assay was conducted by members of the Technical Department of Osaka Plant of this company.

*3 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.

1) J. Baddiley, *et al.*: 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) R. W. Chambers, H. G. Khorana: J. Am. Chem. Soc., **80**, 3749(1958).
5) H. G. Khorana, *et al.*: *Ibid.*, **80**, 1141(1958).
6) L. Zervas, I. Dalaris: Ber., **89**, 925(1956).
7) R. W. Chambers, J. G. Moffatt: J. Am. Chem. Soc., **80**, 3752(1958).
8) W. E. Cohn, C. E. Carter: *Ibid.*, **72**, 4273(1950).
9) W. J. Bowen, T. D. Kerwin: J. Biol. Chem., **220**, 9(1956).

The writers also succeeded in synthesizing UTP, CTP, and dATP by allowing (II) to react with UMP-$NH_2$,[7] CMP-$NH_2$ (1,3-dicyclohexylguanidinium salt,[10] m.p. 198° (decomp.), $[\alpha]_D^{20}$ +4° (c=1.0, $H_2O$)), and newly prepared dAMP-$NH_2$ (Rf=0.33, iso-PrOH·$NH_4OH$·$H_2O$=7:1:2).



R=adenine, uracil, or cytosine
R′=OH or H

The writers are grateful to Dr. Satoru Kuwada, Director of the Laboratories, and Dr. Sueo Tatsuoka, head of the department, for their encouragement throughout the present work and for their generosity to permit the publication of the results.

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May 25, 1960.

10) R. W. Chambers, *et al.* : J. Am. Chem. Soc., **82**, 970(1960). They gave m.p. 180~183°(decomp.) for this substance.

*UDC 577.1:547.854.4'456'483'118.5*

## Synthesis of Uridine Diphosphate-Glucuronic Acid

UDPGA*1 is a biochemically important substance which, as the active form of glucuronic acid, plays a major rôle in detoxication and also takes part as a coenzyme in the biosynthesis of polysaccharides. UDPGA has so far been isolated from natural products in very small quantities[1~3] and prepared only from $\alpha$-UDPG by the action of dehydrogenase and DPN,[4] but the chemical synthesis of this substance has not yet been established. Furthermore, as it is not still evidenced whether natural UDPGA takes $\alpha$- or $\beta$-structure stereochemically, an attempt was made for the synthesis of the two isomers.

A solution of 123 mg. (0.128 m.mole) of the dicyclohexylguanidinium salt of UMP-$NH_2$[5]

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

1) I. D. E. Storey, G. J. Dutton : Biochem. J., **59**, 279(1955).
2) E. E. B. Smith, G. T. Mills : Biochim. et Biophys. Acta, **13**, 386(1954).
3) J. Solms, W. Z. Hassid : J. Biol. Chem., **228**, 357(1957).
4) J. L. Strominger, *et al.* : *Ibid.*, **224**, 79(1957).
5) R. W. Chambers, J. G. Moffatt : J. Am. Chem. Soc., **80**, 3752(1958).