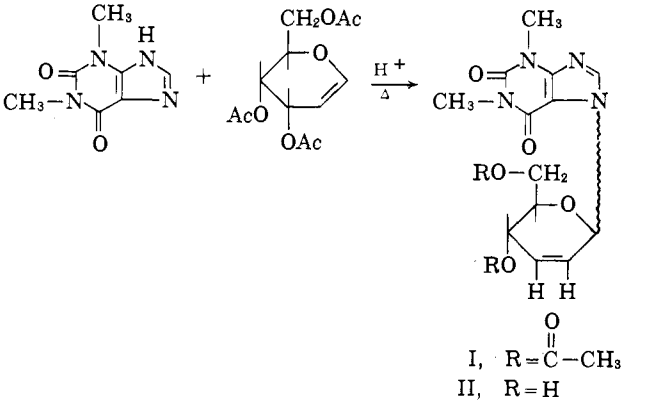
2,3-dihydropyran and 2,3-dihydrofuran.[3,4] However, earlier attempts to employ the use of various glycals under similar reaction conditions as for 2,3-dihydropyran[2] involving the use of ethyl acetate as a solvent did not result in nucleoside formation.

The recent fusion procedures[5] successfully applied to nucleoside syntheses suggested the possibility of the direct reaction of the requisite purine and glycal in the absence of a solvent, since it appeared quite possible that a similar resonance-stabilized carbonium ion at C-1 might be involved in the alkylation process. This reaction now has been shown to occur with 6-chloropurine and 3,4-di-*O*-acetyl-D-arabinal. A suspension of 6.16 g. of 6-chloropurine[6] and 8.80 g. of 3,4-di-*O*-acetyl-D-arabinal[7] was heated to 120° in the presence of 50 mg. of *p*-toluenesulfonic acid in a manner similar to that described[5] for the fusion of 6-chloropurine and 1,3,4-tri-*O*-acetyl-2-deoxy-β-D-ribopyranose. After a similar isolation procedure,[5] 1.02 g. of crystalline product was isolated, m.p. 175–185°. Examination revealed that this material was an anomeric mixture of 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α- and -β-D-ribopyranosyl)purine. Fractional crystallization from absolute ethanol gave 0.9 g. of 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-α-D-ribopyranosyl)purine, m.p. 205–207°, $[\alpha]^{26}D$ +22.5° (*c* 0.75, acetone). *Anal.* Calcd. for $C_{14}H_{15}ClN_4O_5$: C, 47.4; H, 4.23; N, 15.8. Found: C, 47.3; H, 4.26; N, 15.9. The ultraviolet, infrared, and proton magnetic resonance spectra and paper chromatography established the fact that this compound was identical with that anomer prepared[5] by fusion of 6-chloropurine and 1,3,4-tri-*O*-acetyl-2-deoxy-β-D-ribopyranose. The presence of 6-chloro-9-(3′,4′-di-*O*-acetyl-2′-deoxy-β-D-ribopyranosyl)purine[5] in the ethanolic filtrates was also confirmed by paper chromatography in two systems.

In an effort to study the scope of this reaction, 3,4,6-tri-*O*-acetyl-D-glucal[8] (7.5 g.) and theophylline (5.0 g.) were similarly fused *in vacuo* in the presence of 50 mg. of *p*-toluenesulfonic acid. During this process, however, a copious evolution of acetic acid was noted. From the reaction mixture was isolated, after recrystallization from ethanol, 5.0 g. of a crystalline nucleoside (presumably an anomeric mixture, I), m.p. 102–104°, $[\alpha]^{25}D$ +128° (*c* 1.0, ethanol). The ultraviolet absorption spectra, $\lambda_{max}^{pH\,1}$ 273 mμ (ε 7800) and $\lambda_{max}^{pH\,11}$ 231, 273 mμ (ε 4300, 9000), indicated 7-substitution.[9] *Anal.* Calcd. for $C_{17}H_{20}N_4O_7$: C, 52.0; H, 5.10; N, 14.3. Found: C, 51.7; H, 5.24; N, 14.4.

Proton magnetic resonance spectra in deuteriochloroform (TMS internal standard) showed the presence of only two acetylmethyl groups at δ 2.02 and 2.15, respectively. The two vinyl protons occur in the region δ 6.1–6.3.

On this basis and on the analogy of a similar acid-catalyzed reaction of 3,4,6-tri-*O*-acetyl-D-glucal and *p*-nitrophenol as the aglycone,[10] the structure of I is tentatively assigned as 7-(4′,6′-di-*O*-acetyl-2′,3′-didehydro-2′,3′-dideoxy-D-glucopyranosyl)theophylline. Additional evidence for this structure was obtained by mild acid hydrolysis of I which gave theophylline and a carbohydrate residue identified as 4,6-di-*O*-acetyl-2,3-didehydro-2,3-dideoxy-D-*erythro*-hexose (di-*O*-acetylpseudo-D-glucal) by comparison with an authentic sample[11] as judged by paper chromatography in two different solvent systems. No other products were detected in the hydrolysate. Deacetylation of I (1.0 g.) with methanolic ammonia gave 0.52 g. of 7-(2′,3′-didehydro-2′,3′-dideoxy-D-glucopyranosyl)theophylline (II), m.p. 197–198°. *Anal.* Calcd. for $C_{13}H_{16}N_4O_5$: C, 50.6; H, 5.2; N, 18.2. Found: C, 50.3; H, 5.6; N, 17.9. Such unsaturated nucleosides should prove most interesting synthetic intermediates for further work. Additional current interest in 2′,3′-unsaturated nucleosides stems from the fact that such compounds have been postulated as possible biochemical intermediates in the enzymatic conversion of various purine and pyrimidine ribonucleotides to the corresponding deoxyribonucleotides.[12,13] The application of this procedure for the preparation of unusual nucleosides *via* the use of additional glycals and the detailed study of the structure of resulting nucleoside derivatives are problems under active investigation in our laboratory.



(3) L. R. Lewis, F. H. Schneider, and R. K. Robins, *J. Org. Chem.*, **26**, 3837 (1961).

(4) W. A. Bowles, F. H. Schneider, L. R. Lewis, and R. K. Robins, *J. Med. Chem.*, **6**, 471 (1963).

(5) M. J. Robins, W. A. Bowles, and R. K. Robins, *J. Am. Chem. Soc.*, **86**, 1251 (1964), and references cited therein.

(6) A. Bendich, P. J. Russel, Jr., and J. J. Fox, *ibid.*, **76**, 6073 (1954); see also A. G. Beaman and R. K. Robins, *J. Appl. Chem.*, **12**, 432 (1962).

(7) L. Vargha and J. Kuszmann, *Chem. Ber.*, **96**, 411 (1963).

(8) B. Helferich, E. N. Mulcahy, and H. Ziegler, *ibid.*, **87**, 233 (1954).

(9) J. M. Gulland, E. R. Holiday, and T. F. Macrae, *J. Chem. Soc.*, 1639 (1934).

(10) R. J. Ferrier, W. G. Overend, and A. E. Ryan, *ibid.*, 3667 (1962).

(11) E. Fischer, *Chem. Ber.*, **47**, 196 (1914).

(12) P. Reichard, *J. Biol. Chem.*, **237**, 3513 (1962).

(13) A. Larsson, *ibid.*, **238**, 3414 (1963).

DEPARTMENT OF CHEMISTRY
ARIZONA STATE UNIVERSITY
TEMPE, ARIZONA

WILLIAM A. BOWLES
ROLAND K. ROBINS

RECEIVED DECEMBER 13, 1963

The Specific Chemical Synthesis of γ-P^{32} Labeled Adenosine 5′-Triphosphate

Sir:

Nucleoside 5′-triphosphates labeled specifically with P^{32} in the β- or γ-position constitute a valuable tool for the elucidation of the mechanism of many biological reactions. Successful syntheses of these compounds have invariably been enzymatic in nature[1] and frequently are restricted to adenosine polyphosphates by enzyme specificities. As yet, chemical attempts have led only to products with nonspecific labeling.[2,3] We now describe an entirely specific chemical synthesis of ATP-γ-P^{32} which may be extended to any nucleoside 5′-triphosphate and to δ-P^{32} nucleoside 5′-tetraphosphates.

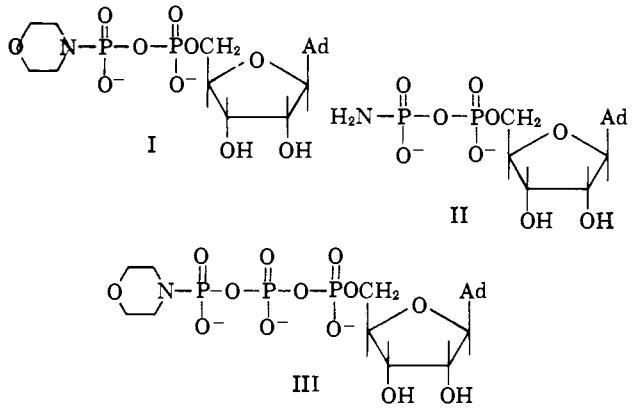
(1) See, *e.g.*, A. Kornberg, S. G. Kornberg, and E. S. Simms, *Biochim. Biophys. Acta*, **20**, 215 (1956); G. Pfleiderer, *ibid.*, **47**, 389 (1961).

(2) J. M. Lowenstein, *Biochem. J.*, **65**, 197 (1957).

(3) A mixed chemical and enzymatic method has been used by R. Tanaka [*J. Biochem.* (Tokyo), **47**, 207 (1960)] for the synthesis of ATP-β-P^{32}.

A solution of dicyclohexylcarbodiimide (5 mmoles) in *t*-butyl alcohol was added dropwise over 9 hr. to a refluxing solution of the morpholine salt of ADP (1.0 mmole) and morpholine (2.4 mmoles) in 50% aqueous *t*-butyl alcohol. After a further 12 hr. under reflux, the mixture was evaporated and partitioned between water and ether. The water phase was separated by ion-exchange chromatography on Dowex-2 (HCO_3^-) using a gradient of triethylammonium bicarbonate giving adenosine 5′-phosphoromorpholidate[4] (19%) and P¹-adenosine-5′ P²-(4-morpholino)pyrophosphate (I, 81%). Chromatographically pure I was isolated as its calcium salt. *Anal.* Calcd. for $C_{14}H_{20}N_6O_{10}P_2Ca\cdot 2H_2O$: C, 29.48; H, 4.24; N, 14.73; P, 10.86. Found: C, 30.36; H, 4.62; N, 14.50; P, 10.68.

As expected, the P–N bond formed involved exclusively the β-phosphorus of the ADP. This follows from the known reactivity of phosphoric acid monoesters, and resistance of phosphoric acid diesters,[4] toward formation of phosphoramidates and could be confirmed by enzymatic means. In a similar reaction in which morpholine was replaced by ammonium hydroxide, the interesting analog P¹-adenosine-5′ P²-aminopyrophosphate (II) was prepared from ADP in 45% yield. Direct extension of the reaction to the synthesis of a terminally activated ATP was also achieved. Thus the reaction of ATP, morpholine, and dicyclohexylcarbodiimide followed by ion-exchange chromatography gave P¹-adenosine-5′ P³-(4-morpholino)triphosphate (III) in 72% yield. *Anal.* Calcd. for $C_{14}H_{20}N_6O_{13}P_3Ca_{1.5}\cdot 4H_2O$: C, 23.84; H, 4.00; N, 11.94; total P:labile P:adenosine, 3.00:2.00:1.00. Found: C, 24.14; H, 3.97; N, 11.06; total P:labile P:adenosine, 2.96:1.94:1.00.



The synthesis of ATP-γ-P^{32} from I utilized the known reactivity of phosphoromorpholidates in pyrophosphate syntheses.[4,5] Thus, the reaction of the 4-morpholine N,N′-dicyclohexylcarboxamidine salt of I (0.1 mmole) with tributylammonium orthophosphate (0.3 mmole containing 2 mc. of P^{32}) in rigorously anhydrous dimethyl sulfoxide (2 ml.) for 45 hr. at 35° gave a mixture of products which were separated by ion-exchange chromatography on DEAE cellulose (HCO_3^-). Minor amounts of unreacted I, AMP, ADP, and AP_4 were cleanly separated from ATP-γ-P^{32} (65%) which was isolated as the chromatographically homogeneous sodium salt with a specific activity of 3 μc. per μmole. Controlled partial degradation of this product (1 μmole) with *E. coli* alkaline phosphatase (40 min. at 35° with 20 μl. of dialyzed enzyme) gave a mixture of adenosine, AMP, ADP, and unreacted ATP, which were separated on a micro-DEAE cellulose (HCO_3^-) column. While the starting material and the unreacted ATP had specific activities of 75,400 c.p.m. per optical density unit at 259 mμ, the AMP contained no isotope and the ADP fractions only 740 c.p.m. per optical density unit. This minor unidentified activity was chromatographically shown not to be due to ADP itself. At any rate, greater than 99% of the P^{32} was located in the γ-position.

The apparent generality, specificity, and good yield of these reactions makes attractive the synthesis of other labeled and unlabeled compounds which will be described shortly.

(6) Financial support for this work from the "Stiftung für Stipendien auf dem Gebiete der Chemie" (Switzerland) is gratefully acknowledged.

CONTRIBUTION NO. 15 D. L. M. VERHEYDEN
SYNTEX INSTITUTE FOR MOLECULAR BIOLOGY W. E. WEHRLI[6]
PALO ALTO, CALIFORNIA J. G. MOFFATT

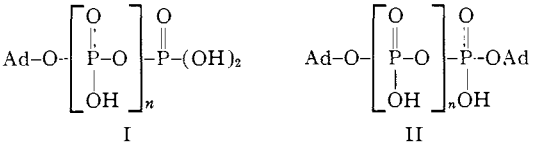
RECEIVED DECEMBER 19, 1963

The Dismutation of Nucleoside 5′-Polyphosphates

Sir:

Several years ago it was reported that the 4-morpholine-N,N′-dicyclohexylcarboxamidine salt of adenosine 5′-triphosphate (ATP) was unstable in anhydrous pyridine at room temperature, and a mixture of the 5′-mono-, di-, tri-, and tetraphosphates was present within a few days.[1] A related degradation of the initially formed nucleoside 5′-triphosphates to the di- and monophosphates during syntheses in pyridine also has been reported by several workers.[2] We have now examined this reaction in detail and are prepared to offer a tentative mechanism.

Ion-exchange chromatography on DEAE-cellulose (HCO_3^-) of the products resulting from storage of a rigorously anhydrous solution of tetrakis(tributylammonium)adenosine 5′-triphosphate (1 mmole) in pyridine (10 ml.) for 3.5 days gave a complex pattern of eight well-resolved peaks. The main products were a homologous series of nucleoside 5′-polyphosphates (I, n = 0, 1, 2, 3, 4, 5, 6) containing from one to seven



phosphate groups.[3] Further chromatography of many of these peaks showed the presence of a second series of compounds, in much smaller amounts (5–10% of the total peak), which has been characterized by analytical and enzymatic means as α,ω-di(adenosine-5′) polyphosphates (II, n = 1, 2, 3, 4, 5, 6). These latter compounds are completely resistant to the action of *E. coli* alkaline phosphatase but are rapidly split by purified venom phosphodiesterase to AMP and an adenosine 5′-polyphosphate which then is cleaved more slowly to AMP and an inorganic polyphosphate. A homologous series of inorganic polyphosphates also is

(1) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 649 (1961).

(2) (a) M. Ikehara and E. Ohtsuka, *Chem. Pharm. Bull.* (Tokyo), **11**, 435 (1963); (b) J. Zemlicka, J. Smrt, and F. Sorm, *Collection Czech. Chem. Commun.*, **28**, 241 (1963).

(3) The following abbreviations are used: AMP, ADP, ATP, AP_4, AP_5, AP_6, and AP_7 for the adenosine 5′-mono-, di-, tri-, tetra-, penta-, hexa-, and heptaphosphates; AP_2A, AP_3A, AP_4A, AP_5A, AP_6A, and AP_7A for the appropriate α,ω-diadenosine 5′-polyphosphates; *e.g.*, AP_5A is P¹,P⁵-di-(adenosine-5′)pentaphosphate.

(4) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 649 (1961).

(5) S. Roseman, J. J. Distler, J. G. Moffatt, and H. G. Khorana, *ibid.*, **83**, 659 (1961); J. G. Moffatt and H. G. Khorana, *ibid.*, **83**, 663 (1961).