Studies on Pyrophosphates. Part III.[1] A New Method for the Synthesis of Nucleotide Coenzymes by Means of Di-n-butylphosphinothioyl Bromide †

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Di-n-butylphosphinothioyl bromide reacts with nucleotides to afford the corresponding nucleoside phosphoric di-n-butylphosphinothioic anhydrides in almost quantitative yields. These mixed anhydrides are stable even in the presence of water, but in the presence of silver salts they react readily with phosphoric acid, pyrophosphoric acid, or nucleotides to give the corresponding nucleoside di- and tri-phosphates, or nucleotide coenzymes in high yields, respectively. In the absence of the phosphates, the mixed anhydrides react with silver nitrate to form the corresponding nucleoside 3′,5′-cyclic phosphates in high yields.

THE synthesis of nucleotide coenzymes involves coupling of a nucleotide with another acid to form an anhydride. Approaches employed previously include the use of carbodi-imide [2] and related coupling reagents,[3] the phosphoromorpholidate method,[4] the phosphoroimidazolidate method,[5] and the use of *S*-ethyl phosphorothioates.[6] Michelson [7] reported the use of diphenyl phosphorochloridate in a method involving controlled nucleophilic displacement of the diphenoxyphosphoryloxy-group of a P^1-diphenyl P^2-nucleoside pyrophosphate by another phosphate. Recently, we have reported a

† Preliminary report, T. Hata, K. Furusawa, and M. Sekine, *J.C.S. Chem. Comm.*, 1975, 196.

[1] Part II, I. Nakagawa and T. Hata, *Bull. Chem. Soc. Japan*, 1973, **46**, 3275.

[2] N. A. Hughes, G. W. Kenner, and A. R. Todd, *J. Chem. Soc.*, 1957, 3733.

[3] G. W. Kenner, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 1958, 546.

[4] J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, 1961, **83**, 649.

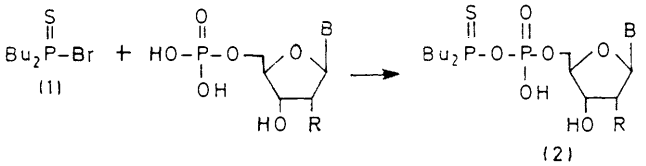
[5] F. Cramer and H. Schaller, *Chem. Ber.*, 1961, **94**, 1634.

[6] A. F. Cook, M. J. Holman, and A. L. Nussbaum, *J. Amer. Chem. Soc.*, 1969, **91**, 1522.

[7] A. M. Michelson, *Biochim. Biophys. Acta*, 1964, **91**, 1.

simple and effective method for the synthesis of nucleotide coenzymes from nucleoside phosphorothioates and the disilver salts of nucleotides without a coupling reagent.[8] We have found that di-n-butylphosphinothioyl bromide (1) reacts with nucleoside 5′-phosphates to give the corresponding nucleoside 5′-phosphoric di-n-butylphosphinothioic anhydrides (2). The products (2) are stable even in the presence of water, but in the presence of silver salts, they react readily with nucleophiles such as inorganic phosphate, inorganic pyrophosphate, and esters of phosphoric acid. We now describe the preparation and reactions of the mixed anhydrides (2) in detail.

When thymidine 5′-phosphate (1 equiv.) was treated with the bromide (1) (2 equiv.) in dry pyridine at room temperature for 3 h, thymidine 5′-phosphoric di-n-butylphosphinothioic anhydride was obtained in almost quantitative yield. In a similar manner, other nucleoside 5′-phosphoric di-n-butylphosphinothioic anhydrides were obtained as shown in Table 1. In each case the



(1) (2)

R = H or OH B = nucleoside base

TABLE 1

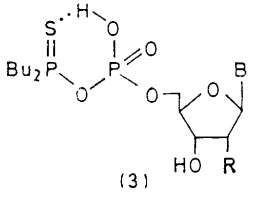
Preparation of nucleoside 5′-phosphoric di-n-butylphosphinothioic anhydrides (2)

Nucleotide	Base	Solvent	Yield (%)
pU	Pyridine	Pyridine	Quant.
pA	MDCA *	Pyridine	Quant.
pG	$(Oct)_3N$ †	Bu^tOH–Bu^n_3N	97
$pAcG(OAc)_2$	Pyridine	Pyridine	Quant.
pC	MDCA *	2-Methylpyridine	Quant.
pT_d	Pyridine	Pyridine	Quant.
pT_dOAc	Pyridine	Pyridine	Quant.
pG_d	$(Oct)_3N$ †	Bu^tOH–Bu^n_3N	95
$pBu^iG_dOBu^i$ ‡	Pyridine	Pyridine	Quant.

* MDCA = morpholino-*NN′*-dicyclohexylformamidine. † $(Oct)_3N$ = tri-n-octylamine. ‡ $Bu^iG_dOBu^i$ = 3′-*O*-isobutyryl-N^2-isobutyryldeoxyguanosine 5′-phosphate.

bromide (1) reacted selectively and quantitatively with the phosphate residue of the nucleotide, leaving the hydroxy-groups on the sugar unit and the amino-groups on the nucleoside base unmodified, even when an excess of (1) was used. Dimethylphosphinothioyl bromide and diphenylphosphinothioyl chloride also reacted smoothly with nucleotides to afford the corresponding mixed anhydrides. For example, thymidine 5′-phosphate reacted with either of these reagents under the above condition to give the corresponding mixed anhydride in 96 or 97% yield, respectively. Di-n-butylphosphinothioyl bromide (1) was the preferred starting material because (i) it reacts universally and quantitatively with all the common nucleotides; (ii) the resultant mixed anhydrides (2) are soluble † and more stable than those prepared from the alternative reagents; and (iii) the u.v. spectra of the anhydrides (2) are similar to those of the corresponding nucleotides, since the di-n-butylphosphinothioyl group has no significant absorption in the range 240—310 nm.

In contrast to the Michelson type of mixed anhydride, the mixed anhydrides (2) are stable in aqueous solution, even in the presence of pyridine. For example the free acid adenosine 5′-phosphoric di-n-butylphosphinothioic anhydride (2; R = OH, B = adenin-9-yl) could be precipitated from saturated aqueous solution. The stability of the anhydrides (2) may be explained in terms of the hydrogen-bonded structure (3).[9]



(3)

In order to define further the stability of the anhydrides (2), their hydrolysis under neutral, acidic, and alkaline conditions was studied (Table 2). These results show that the anhydrides are stable under neutral condition, and only slowly degraded under acidic conditions, but are hydrolysed gradually and completely under alkaline conditions to the corresponding nucleotide and di-n-butylphosphinothioic acid. The cytidine derivative (2; R = H, B = cytosin-1-yl) was hydrolysed slightly faster than the others.

An interest in the affinity between sulphur and silver atoms led us to study activation of the mixed anhydrides

TABLE 2

Hydrolysis of nucleoside 5′-phosphoric di-n-butylphosphinothioic anhydrides (BuPspN)

(a) Alkaline conditions (0.2N-NaOH; 25 °C)

	% Hydrolysis after		
	30 min	60 min	120 min
BuPspN ⟶ pN			
BuPspA ⟶ pA	64	83	100
BuPspU ⟶ pU	65	82	100
BuPspG ⟶ pG	61	81	94
BuPspC ⟶ pC	76	93	100
BuPspT ⟶ pT	67	84	100

(b) Acidic and neutral conditions (25 °C)

		% Hydrolysis after	
	Conditions	24 h	48 h
BuPspT ⟶ pT	0.2N-HCl	5	9
BuPspT ⟶ pT	pH 7 *	0	0

* Phosphate buffer (0.2M).

(2) by silver acetate or silver nitrate. The anhydrides (2) were hydrolysed rapidly by addition of silver acetate at room temperature under neutral conditions. We

† For example, the mixed anhydrides (2) containing a thymidine or uridine residue dissolve in tetrahydrofuran.

[8] T. Hata and I. Nakagawa, *J. Amer. Chem. Soc.*, 1970, **92**, 5516.

[9] R. A. Y. Jones, A. R. Katritzky, and J. Michalski, *Proc. Chem. Soc.*, 1959, 321.

then investigated the reaction of the anhydrides (2) with phosphates in the presence of silver salts. When adenosine 5′-phosphoric di-n-butylphosphinothioic anhydride (2b) (0.1 mmol) was treated with mono(tri-n-butylammonium) dihydrogen phosphate (0.5 mmol) in the presence of silver acetate (0.4 mmol) in dry pyridine † at room temperature for 3 h, adenosine 5′-diphosphate (ADP) was obtained in 82% yield. Similarly, 5′-diphosphates of uridine (UDP), cytidine (CDP), guanosine (GDP), and thymidine (TDP) were obtained in high yields (Table 3).

TABLE 3

Synthesis of nucleoside 5′-diphosphates *

BuPspN	AgOAc (equiv.)	Time (h)	Yield (%)
BuPspA	4	3	82
BuPspU	4	2	77
BuPspG	6	2	76
BuPspC	4	27	86
BuPspT	4	3	86

* In these reactions, 5 equiv. of phosphoric acid was used.

TABLE 4

Synthesis of nucleoside 5′-triphosphates *

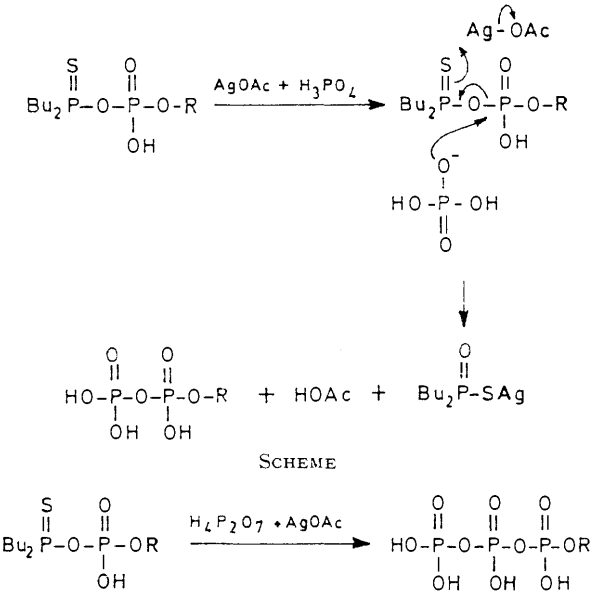
BuPspN	AgOAc (equiv.)	Time (h)	Yield (%)
BuPspA	4	2.5	84
BuPspU	6	1.0	75
BuPspG	6	1.7	87
BuPspC	6	1.5	88
BuPspT	6	2.5	88

* In these reactions, 5 equiv. of pyrophosphoric acid was used.

The reaction seems to proceed by activation of phosphinothioyl group with silver acetate to generate phosphoryl cation, which is attacked by phosphoric acid to form the nucleoside diphosphate (see Scheme).

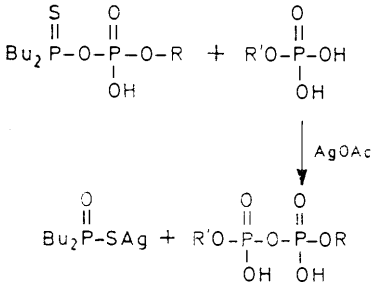
Further, when bis(tri-n-butylammonium) dihydrogen pyrophosphate was employed in place of phosphoric acid,[10] the corresponding nucleoside triphosphates were obtained in high yields ‡ (Table 4).

This reaction was applied to the synthesis of the nucleotide coenzymes uridine diphosphate glucose (UDPG), thymidine diphosphate glucose (TDPG), cytidine diphosphate choline (CDP-choline), flavine adenine dinucleotide (FAD), and nicotinamide adenine dinucleotide (NAD). The coenzymes were obtained in high yields even when two equivalents of the phosphates were employed, except in the case of the NAD synthesis. The results are listed in Table 5.



SCHEME

In addition, adenosine 3′,5′-cyclic phosphate, uridine 3′,5′-cyclic phosphate, and thymidine 3′,5′-cyclic phosphate were obtained in 89, 85, and 86% yields, respectively, when the anhydrides (2) were treated with silver nitrate in tri-n-butylamine–pyridine under reflux.



When the silver acetate was used in place of silver nitrate, nucleoside 5′-phosphoric acetic anhydrides (4)

TABLE 5

Synthesis of nucleotide coenzymes R′O·P(:O)(OH)·O·P(:O)(OH)·OR

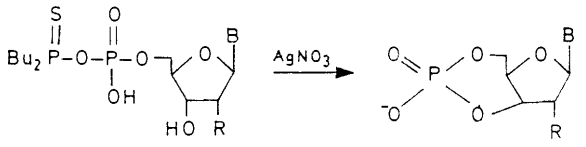
R	R′	Solvent	Time (h)	Product	Yield (%)
Uridine (5′)	Glucose (1)	Pyridine	4	UDPG	78
Thymidine (5′)	Glucose (1)	Pyridine	6	TDPG	80
Cytidine (5′)	Choline	Pyridine–formamide	15	CDP-choline	69
Adenosine (5′)	Riboflavine (5′)	Pyridine–formamide	17	FAD	67
Adenosine (5′)	Nicotinamide nucleoside (5′)	Pyridine–formamide	36	NAD	71

† Silver nitrate can be used in place of silver acetate.

‡ In this case, dismutation of the polyphosphates took place during the desired reaction. The reaction was therefore stopped by addition of water after a relatively short time. Such dismutation reactions have been reported by Moffatt.[11]

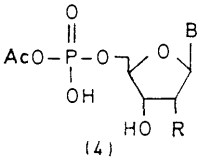
[10] J. G. Moffatt, *Canad. J. Chem.*, 1964, **42**, 599.

[11] D. L. M. Verheyden, W. E. Wehrli, and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1965, **87**, 2257, 2265; W. E. Wehrli and J. G. Moffatt, *ibid.*, p. 3760; A. Adam and J. G. Moffatt, *ibid.*, 1966, **88**, 838; J. R. Reiss and J. G. Moffatt, *J. Org. Chem.*, 1965, **30**, 3381.



B = adenin-9-yl or uracil-1-yl, R = OH
B = thymin-1-yl, R = H

were detected. The mixed anhydrides (4) do not react further with the nucleotide 3′-hydroxy-groups under these conditions.



(4)

EXPERIMENTAL

Paper chromatography was performed by the descending technique with Toyo Roshi No. 51 and 51A papers and the solvent systems isopropyl alcohol–concentrated aqueous ammonia–water (7 : 1 : 2 v/v) (A); ethanol–M-ammonium acetate (pH 7.5; 7 : 3 v/v) (B); n-propyl alcohol–concentrated aqueous ammonia–water (6 : 3 : 1 v/v) (C); and n-butanol–acetic acid–water (5 : 2 : 3 v/v) (D).

Paper electrophoresis was carried out with an apparatus similar to that described by Markham and Smith.[12] The buffer solutions used were 0.2M-phosphate (pH 8.0 or 6.0).

Estimation of the yields of products was most frequently carried out spectrophotometrically after elution of the spots from paper electrophoretograms or paper chromatograms.

The protected nucleotides pT_dOAc,[13] $pBu^iG_dOBu^i$,[14] and $pAcG(OAc)_2$ [15] were prepared according to literature procedures. Dimethylphosphinothioyl bromide [16] and diphenylphosphinothioyl chloride [17] were also prepared by published procedures. Di-n-butylphosphinothioyl bromide (b.p. 143—144° at 6 mmHg) was prepared in 87% yield from tetra-n-butyldiphosphine disulphide [18] by a modification of the procedure of Harwood [16] (see below).

Tetra-n-butyldiphosphine Disulphide.—Magnesium (24.3 g, 1 mol) was covered with dry ether (100 ml) and n-butyl bromide (137 g, 1 mol) was added dropwise. During the addition the mixture was gently refluxed. The refluxing was continued for 1 h and then the mixture was cooled to 0 °C. A solution of thiophosphoryl chloride (53.1 g, 0.313 mol) in dry ether (100 ml) was added slowly. The temperature was maintained between 0 and 5 °C. The mixture was then refluxed for 1 h, poured into ice–water, and acidified with hydrochloric acid, and the ether layer was separated. The aqueous layer was washed with ether (4 × 50 ml). Extracts and washings were combined and concentrated *in vacuo*. The residual precipitate was dissolved in the minimum of hot methanol and kept in a refrigerator. Tetra-n-butyldiphosphine disulphide (53%) was filtered off and dried (P_4O_{10}); m.p. 71.5—72.5°.

* Compound (1) was hydrolysed very sluggishly to di-n-butylphosphinothioic acid under these conditions.

[12] R. Markham and J. D. Smith, *Biochem. J.*, 1952, **52**, 552.
[13] H. G. Khorana and J. P. Vizsolyi, *J. Amer. Chem. Soc.*, 1961, **83**, 675.
[14] H. Büchi and H. G. Khorana, *J. Mol. Biol.*, 1972, **72**, 251.

Di-n-butylphosphinothioyl Bromide.—To a cooled solution of tetra-n-butyldiphosphine disulphide (28 g, 79 mmol) in carbon tetrachloride (90 ml), a solution of bromine (4.04 ml, 79 mmol) in carbon tetrachloride (130 ml) was added gradually, with the temperature kept below 0 °C. Addition of bromine was continued until the solution was coloured slightly pink. The solvent was then removed and the residual oil distilled *in vacuo* to give di-n-butylphosphinothioyl bromide (b.p. 143—144° at 6 mmHg) in 87% yield.

O-p-Nitrophenylphosphoric Di-n-butylphosphinothioic Anhydride.—To a suspension of *p*-nitrophenyl dihydrogen phosphate (1 mmol) in dry pyridine (10 ml), di-n-butylphosphinothioyl bromide (1) (1.5 mmol) was added with stirring. The solution soon became clear. Stirring was continued for 30 min. After removal of solvent *in vacuo*, tetrahydrofuran was added. Pyridine hydrobromide was precipitated and was filtered off. The filtrate was concentrated and the residue dissolved in aqueous pyridine. The aqueous solution was rapidly washed with ether (or n-hexane) to remove the bromide (1).* The aqueous layer was concentrated; the residue was homogeneous on paper chromatography. The yield was almost quantitatative, estimated spectrophotometrically: $\lambda_{max.}$ (H_2O) 300 nm (ε 10 000) at pH 7. The product was dissolved in aqueous pyridine and stored in a refrigerator as a standard solution after estimation of concentration.

Thymidine 5′-Phosphoric Di-n-butylphosphinothioic Anhydride (2; R = H, B = *thymin-1-yl*).—A solution of thymidine 5′-phosphate (1 mmol) in dry pyridine was rendered anhydrous by repeated evaporation *in vacuo* in the presence of an excess of dry pyridine. Finally, the residual oil was dissolved in dry pyridine (10 ml), the bromide (1) (2 mmol) was added, and the mixture was stirred at room temperature for 2 h. Separation was performed as in the case of *O-p*-nitrophenylphosphoric di-n-butylphosphinothioic anhydride. In this case, the anhydride (2) was isolated from the resulting aqueous solution by addition of morpholino-*NN′*-dicyclohexylformamidine.[19] After removal of solvent, ether was added and the precipitated *amidinium salt* collected, washed with ether, and dried (P_4O_{10}); m.p. 201° (decomp.) (Found: C, 52.65; H, 8.05; N, 9.0. $C_{35}H_{63}N_5P_2SO_9$ requires C, 53.1; H, 7.95; N, 8.85%), P : thymidine ratio 2.07 : 1.

Uridine 5′-Phosphoric Di-n-butylphosphinothioic Anhydride (2; R = OH, B = *uracil-1-yl*).—Uridine 5′-phosphate (1 mmol) was dissolved in dry pyridine. The solution was rendered anhydrous by repeated evaporation *in vacuo* in the presence of an excess of dry pyridine. Finally, the residue was dissolved in dry pyridine (10 ml) and the bromide (1) (2 mmol) was added with stirring. After 2 h, the anhydride (2) was obtained in almost quantitative yield (estimated spectrophotometrically) and isolated as the *mono*(*morpholino*-NN′-*dicyclohexylformamidinium*) salt (by a procedure similar to that described above), m.p. 185° (Found: C, 50.4; H, 7.65; N, 8.95. $C_{34}H_{61}N_5P_2SO_{10}.H_2O$ requires C, 50.3; H, 7.75; N, 8.65%), P : uridine ratio 2.08 : 1.

Adenosine 5′-Phosphoric Di-n-butylphoshinothioic Anhydride (2; R = OH, B = *adenin-9-yl*).—Adenosine 5′-

[15] R. Lohrmann and H. G. Khorana, *J. Amer. Chem. Soc.*, 1964, **86**, 4188.
[16] H. J. Harwood and K. A. Pollart, *J. Org. Chem.*, 1963, **28**, 3430.
[17] L. Maier, *Helv. Chim. Acta*, 1964, **47**, 120.
[18] H. Niebergall and B. Langenfeld, *Chem. Ber.*, 1962, **95**, 64.
[19] G. M. Tener, H. G. Khorana, R. Markham, and E. H. Pol, *J. Amer. Chem. Soc.*, 1958, **80**, 6223.

phosphate (1 mmol) and morpholino-*NN'*-dicyclohexylformamidine (1 mmol) were dissolved in aqueous pyridine. The solution was then concentrated *in vacuo* and the residue was dissolved in dry pyridine. This solution was rendered anhydrous by repeated evaporation *in vacuo* in the presence of an excess of dry pyridine. The residue was finally dissolved in dry pyridine (10 ml) and the bromide (1) (2 mmol) was added dropwise with stirring. A precipitate appeared and redissolved. After 3 h, the mixture was concentrated. The residue was dissolved in water and the aqueous solution was washed with ether to remove the bromide (1). The ether layer was extracted with water. The aqueous layers were combined and concentrated to give compound (2) in almost quantitative yield (estimated spectrophotometrically). The gummy residue was further dissolved in water and the solution was concentrated to a small volume and kept in a refrigerator. The free *acid*, m.p. 170—171° (decomp.), was obtained in 64% yield as a white powder (Found: C, 40.4; H, 6.15; N, 12.8; P, 11.45. $C_{18}H_{31}N_5P_2SO_7$ requires C, 39.95; H, 6.1; N, 12.9; P, 11.45%).

Cytidine 5'-Phosphoric Di-n-butylphosphinothioic Anhydride (2; R = OH, B = *cytosin-1-yl*).—Cytidine 5'-phosphate (0.05 mmol) and morpholino-*NN'*-dicyclohexylformamidine (0.05 mmol) were dissolved in aqueous pyridine. The solution was concentrated *in vacuo* and the residue was dissolved in dry pyridine. The solution was rendered anhydrous by repeated evaporation *in vacuo* in the presence of an excess of dry pyridine and the residue was suspended in 2-methylpyridine (1 ml). Di-n-butylphosphinothioyl bromide (0.15 mmol) was added dropwise with stirring at room temperature. The solution became clear within 10 min. When the reaction was completed,* the 2-methylpyridine was removed *in vacuo*. The residue was treated as for the adenosine analogue. The product was an oil which was homogeneous on paper chromatography and was stored as an aqueous solution in a refrigerator; $\lambda_{max.}$ (H_2O) 271 nm (ε 9 100) at pH 7; P : cytidine ratio 2.02 : 1.

Guanosine 5'-Phosphoric Di-n-butylphosphinothioic Anhydride (2; R = OH, B = *guanin-9-yl*).—Well-ground guanosine 5'-phosphate (0.05 mmol) was suspended in methanol. n-Octylamine (0.05 mmol) was then added with stirring. Pasty material was formed gradually. The solvent was evaporated off and the residue was treated with dry pyridine, then concentrated to dryness. This was repeated three times for complete removal of moisture. The residue was dissolved in t-butyl alcohol (1.2 ml) and the bromide (1) (0.12 mmol) was added. Tri-n-butylamine (0.2 mmol) was then added with stirring, which was continued at room temperature for 24 h. The mixture was concentrated *in vacuo* to remove t-butyl alcohol and water was then added. The aqueous solution was washed with ether to remove the bromide (1).

The product (2), an oil, was homogeneous on paper chromatography, and was stored as an aqueous solution in a refrigerator; $\lambda_{max.}$ (H_2O) 252 nm (ε 13 700) at pH 7; P : guanosine ratio 2.02 : 1.

Preparation of Nucleoside 5'-Diphosphates. General Procedure.—The nucleoside 5'-phosphoric di-n-butylphosphinothioic anhydride (0.1 mmol) and mono(tri-n-butylammonium) dihydrogen phosphate (0.5 mmol) were dissolved in dry pyridine. The solution was rendered anhydrous by repeated evaporation *in vacuo* in the presence of an excess of dry pyridine. Finally, the residue was dissolved in dry pyridine (1 ml) and silver acetate (0.4—0.6 mmol) was added in one portion with stirring at room temperature. After a few hours (see Table 3), water was added. Hydrogen sulphide was bubbled into the mixture. The precipitate was filtered off and the filtrate was concentrated and applied to a column of DEAE cellulose (HCO_3^- form) (40 × 1.7 cm). Elution was performed with a linear gradient of 0.005M-triethylammonium hydrogen carbonate (TEHC) (1 l) to 0.1M-TEHC (1 l). The fractions containing nucleoside 5'-diphosphate were collected and concentrated *in vacuo* below 30 °C. Then methanol was added and evaporated off for complete removal of the triethylammonium hydrogen carbonate. The residual oil was homogeneous on paper chromatography. Data are given in Table 3.

Preparation of Nucleoside 5'-Triphosphates. General Procedure.—The pyridinium salt of pyrophosphoric acid (0.5 mmol) prepared from $Na_4P_2O_7$ by using Dowex 50W-X2 resin (pyridinium form), was suspended in methanol and dissolved by addition of tri-n-butylamine. After removal of methanol by evaporation, the nucleoside 5'-phosphoric di-n-butylphosphinothioic anhydride (0.1 mmol) was added. The mixture was rendered anhydrous by repeated evaporation *in vacuo* in the presence of an excess of dry pyridine. The residue was dissolved in dry pyridine (1 ml) and silver acetate (0.4—0.6 mmol) was added in one portion with stirring. Isolation was performed by use of DEAE cellulose column as in the case of nucleoside diphosphates. The elution was performed by a linear gradient of 0.005M-TEHC (1 l) to 0.14M-TEHC (1 l). Data are given in Table 4.

Uridine Diphosphate Glucose (UDPG).—A mixture of mono(tri-n-butylammonium) D-glucose 1-phosphate (0.1 mmol) and compound (2; R = OH, B = *uracil-1-yl*) (0.05 mmol) was dissolved in dry pyridine. The solution was rendered anhydrous by repeated evaporation *in vacuo* in the presence of an excess of dry pyridine. The residue was dissolved in dry pyridine (1 ml) and silver acetate (0.2 mmol) was added in one portion with stirring. After 4 h, water was added and hydrogen sulphide was bubbled into the mixture. Silver sulphide precipitated was filtered off and the filtrate was concentrated and applied to a column of Dowex 1 × 2 (Cl^- form) (8 × 1.5 cm). The column was washed with water and then with 0.003N-HCl in 0.01M-LiCl for elution of uridine 5'-phosphate. Uridine diphosphate glucose (dilithium salt) was obtained in 78% yield by elution with 0.003N-HCl in 0.06M-LiCl; $\lambda_{max.}$ (H_2O) 262 nm (ε 10 000) at pH 7; P : uridine ratio 2.05 : 1.

Thymidine Diphosphate Glucose (*TDPG*).—Mono(tri-n-butylammonium) 1-phosphate (0.1 mmol) was treated with compound (2; R = H, B = thymin-1-yl) (0.05 mmol) in the presence of silver acetate (0.2 mmol) at room temperature for 6 h. TDPG was obtained in 80% yield as described in the above experiment: $\lambda_{max.}$ (H_2O) 267 nm (ε 9 600) at pH 7; P : thymidine ratio 1.89 : 1.

Cytidine Diphosphate Choline (*CDP-choline*).—Compound (2; R = H, B = cytosin-1-yl) (0.05 mmol) and the mono-(tri-n-butylammonium) salt of choline phosphate (0.1 mmol) were dissolved in formamide (0.2 ml). The solution was rendered anhydrous by repeated evaporation *in vacuo* by addition of dry pyridine. Finally, dry pyridine (0.8 ml) was added. Silver nitrate (0.25 mmol) was then added and the mixture was stirred at 55 °C overnight. After addition of water, hydrogen sulphide was bubbled into the mixture.

* The reaction was monitored by t.l.c. on cellulose (Avicel) developed with isopropyl alcohol–concentrated ammonia–water (7 : 1 : 2 v/v).

Silver sulphide precipitated was filtered off. The filtrate was concentrated *in vacuo*. CDP-choline was obtained in 69% yield by electophoretic separation; $\lambda_{max.}$ (H_2O) 271 nm (ε 9 100) at pH 7; mobility on paper electrophoresis relative to cytidine 5′-phosphate, 0.47 (at pH 8), 0.65 (at pH 6) (phosphate buffer); R_F values 0.12 (A) and 0.29 (B).

Flavine Adenine Dinucleotide (*FAD*).—Compound (2; R = H, B = adenin-9-yl) (0.05 mmol) and the mono-(tri-n-octylammonium) salt of flavin mononucleotide (0.1 mmol) were dissolved in formamide (0.2 ml). Pyridine was added and evaporated off. This was repeated three times for complete removal of moisture. The residue was dissolved again in dry pyridine (0.7 ml). Silver nitrate (0.25 mmol) was added in one portion and the mixture was stirred at room temperature for 17 h. Separation was performed as in the case of CDP-choline. FAD was obtained in 67% yield and the structure was confirmed spectrophotometrically; ε = 37 000 at 260 nm (pH 7); $\varepsilon_{260}/\varepsilon_{375}$ 2.82, $\varepsilon_{375}/\varepsilon_{450}$ 0.90, $\varepsilon_{260}/\varepsilon_{450}$ 3.44; mobility on paper electrophoresis relative to adenosine 5′-phosphate, 0.45 (at pH 8; phosphate buffer).

Nicotinamide Adenine Dinucleotide (*NAD*).—Compound (2; R = H, B = adenin-9-yl) (0.1 mmol) and nicotinamide mononucleotide (0.05 mmol) were dissolved in formamide (0.2 ml). Pyridine was added and evaporated off. This was repeated three times for complete removal of moisture. To the residue, dry pyridine (0.4 ml) was added. Silver nitrate (0.4 mmol) was then added in one portion and the mixture was stirred at room temperature for 36 h. NAD was obtained in 71% yield and separated as in the case of CDP-choline; $\lambda_{max.}$ (H_2O) 259 nm (ε 17 800) at pH 7; mobility on paper electrophoresis relative to adenosine 5′-phosphate, 0.36 (at pH 8; phosphate buffer), 0.53 (at pH 6; phosphate buffer); R_F 0.18 (D); P : NAD ratio 2.10 : 1.

Adenosine 3′,5′-Cyclic Phosphate (*3′,5′-Cyclic AMP*).—Silver nitrate (0.5 mmol) and tri-n-butylamine (0.75 mmol) were dissolved in dry pyridine (20 ml) and boiled under reflux. To the boiling solution, a solution of compound (2; R = OH, B = adenin-9-yl) (0.05 mmol) in dry pyridine (10 ml) was added dropwise slowly within 30 min. After boiling for 2 h, the solution was treated as in the case of CDP-choline. 3′,5′-Cyclic AMP was obtained in 89% yield; $\lambda_{max.}$ (H_2O) 258 nm (ε 14 650); mobility on paper electrophoresis relative to adenosine 5′-phosphate, 0.54 (at pH 8; phosphate buffer); R_F 0.41 (A).

Uridine 3′,5′-Cyclic Phosphate (*3′,5′-Cyclic UMP*).—3′,5′-Cyclic UMP was prepared in 85% yield from compound (2; R = OH, B = uracil-1-yl) (0.05 mmol) by the procedure used for 3′,5′-cyclic AMP; $\lambda_{max.}$ (H_2O) 260 nm (ε 10 000) at pH 7; R_F 0.35 (A).

Thymidine 3′,5′-Cyclic Phosphate (*3′,5′-Cyclic TMP*).—3′,5′-Cyclic TMP was prepared in 86% yield from compound (2; R = H, B = thymin-1-yl) (0.05 mmol) by the procedure used for 3′,5′-cyclic AMP; $\lambda_{max.}$ (H_2O) 265 nm (ε 9 600) at pH 7.

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